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Biological Activities and Phytochemical Analysis of Some Selected Medicinal plants

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بسم الله الرحمن الرحيم

قال تعالي:

(هُوَ الَّذِي أَنزَلَ مِنَ السَّمَاء مَاء لَّكُم مِّنْهُ شَرَابٌ وَمِنْهُ شَجَرٌ فِيهِ تُسِيمُون (10) يُنبِتُ لَكُم بِهِ الزَّرْعَ وَالزَّيْتُونَ وَالنَّخِيلَ وَالأَعْنَابَ وَمِن كُلِّ الثَّمَرَاتِ إِنَّ فِي ذَلِكَ لآيَةً لِّقَوْمٍ يَتَفَكَّرُونَ (11)).

صدق الله العظيم

سورة النحل الآيات (10-11).

DEDICATION

To my mother, heavens of love

To my father, land of patience

To my sisters, lake of joy

To my brothers, wind of eagerness

To my teachers, rivers of giving

To my friends, flowers of happiness

To my fiancée, fragrance of hope

AHMED SAEED ALI KABBASHI (2022).

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I would like to conclude by conveying my respect to the Nation and to the Almighty.

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ABSTRACT

This study aimed to investigate the biological activities and phytochemical properties of some selected medicinal plants viz. *Mangifera indica* (L.), *Rosmarinus officinalis* (L.) and *Tinospora bakis* (A Rich.) Miers). However, bioassay-guided plant extraction, isolation protocols, and supporting analytical and biological methodologies were used. Moreover, structural identification of compounds present in ethyl acetate fractions was performed by mass spectrometry (MS) and nuclear magnetic resonance (NMR). The results showed that extracts, fractions and compounds, from the medicinal plants investigated, were highly active as antimicrobial, antiprotozoal, molluscicidal and anti-oxidant activities. The *M. indica* extract showed the lowest MIC (0.78 mg/ml) while the isolated compound Methyl gallate showed the lowest MIC (15.62 µg/ml). The *M. indica* extract highest antiprotozoal activity was found against *Entamoeba histolytica* and *Giardia lamblia* (IC_{50}= 3 µg/ml). On the other hand *M. indica* extract highest molluscicidal activity was estimated to be (IC_{50}= 0.3 µg/ml). Among the plants used in this *R. officinalis* and *M. indica* extract showed high antioxidant activity; IC_{50} = 0.00225 ± 0.001 and 0.0083 ± 0.0001 mg/ml, respectively and Methyl gallate compound showed strong activity (IC_{50}= 0.03 ± 0.01 mg/ml). Moreover, the studied extracts and fractions showed no toxic effect. The phytochemical investigation in this study showed presence of phenols, tannins, flavonoids, alkaloids, triterpenes, steroids, coumarins and saponins. While, anthraquinone was only *R. officinalis* extract. The thin layer chromatography (TLC) showed anthraquinone and flavonoids three spot compounds were for both *R. officinalis* and *M. indica* extract. On the hand quantitative analysis confirm presence of phenolic, flavonoid and tannin with highest amount characterizing of *M. indica* extract. 66 identified compounds were detected through GC-MS technique for all extracts of *M. indica*, *R. officinalis* and *T. bakis*, and all the compounds had been reported to have biological activities. Extra exploration of *M. indica* and *T. bakis* through NMR machine showed presence of 17 different compounds, twelve of which showed antimicrobial activity and one out of seventeen with high antioxidant activity. In conclusion: this important a novel scientific work with the promising potential of scientific docking antimicrobial, antiprotozoal, molluscicidal and anti-oxidant activities, it needs to be continued for more experimental investigation, especially for the newly discovered compounds.

مستخلص البحث

هدفت هذه الدراسة إلى معرفة الأنشطة البيولوجية والخصائص الكيميائية النباتية لبعض النباتات الطبية المختارة وهي نبات المانجو *M. indica*، أكليل الجبل *R. officinalis* وعرق الحجر *T. bakis*. تم استخلاص النباتات المختارة في الدراسة بطرق الاستخلاص والعزل المعروفة، واستخدمت لها العديد من الطرق التحليلية والبيولوجية. وعلاوة على ذلك، تم إجراء التحديد الهيكلي للمركبات المعزولة من مجزئيات خلات الإيثيل بواسطة مطياف الكتلة (MS) والرنين المغناطيسي النووي (NMR). أظهرت نتائج الدراسة أن المستخلصات، الجزئيات والمركبات المعزولة من النباتات الطبية المختاره لها فعالية عالية ضد الميكروبات والطفيليات والرخويات والأكسدة. و أظهر مستخلص المانجو أدني تركيز تثبيط للميكروبات (0.78 مجم / مل) وكذلك أظهر مركب الميثايل جاليت المعزول من نبات المانجو أدنى تركيز تثبيط للميكروبات (15.62 ميكروجرام / مل). و وجد أن مستخلص نبات المانجو أعطى أعلى نشاط مضاد للأوليات ضد طفيلي الأنتاميبا هوستيلتكا والقارديا لامبيليا (3 ميكروغرام / مل). ومن ناحية أخرى، أعطت فعالية مستخلص نبات المانجو نشاط عالي ضد الرخويات 0.3 ميكروغرام / مل. أظهرت المستخلصات والجزئيات من نباتي اكليل الجبل والمانجو نشاطًا عاليًا لمضادات الأكسدة وبلغت أعلي نسبة 50%تركيز مثبط (0.00225 = 0.001 ± و 0.0083 ± 0.0001 مجم / مل، على التوالي). بالإضافة إلى ذلك، أظهر مركب الميثايل جاليت المعزول من نبات المانجو نشاطًا قويًا وبلغت نسبة تركيزة المثبط 50% وهي (0.01 ± 0.03 مجم / مل). وعلاوة على ذلك، لم تظهر المستخلصات والجزئيات المدروسة أي تأثير سام. أظهرت نتائج المسح الكيميائي وجود الفينولات، التانين (العفص)، الفلافونيدات، القلويدات، التيربين، الاستيرويدات، الكومارين والصابونين. بينما، الأنثراكينون وجد فقط في مستخلص اكليل الجبل. كذلك، كشفت نتائج تحليل كروماتوغرافيا الطبقة الرقيقة (TLC) وجود الأنثراكينون والوفلافونويدات بتراكيز عالية في مستخلصي المانجو واكليل الجبل. و من ناحية أخري أظهر التحليل الكمي وجود تراكيز عالية من الفينولات والفلافونويدات والتانينات في مستخلص المانجو. وأظهر التحليل عن طريق GC-MS وجود 66 مركبًا تم تحديدها من جميع المستخلصات المستهدفة فى هذه الدراسة لها أنشطة بيولوجية متعدده. تم عزل سبعة عشرة (17) مركبا مختلفا من مجزئات خلات الإيثيل من نباتي المانجو وعرق الحجر، منها أثنا عشر مركبا أظهرت نشاطًاً مضادًا للميكروبات وواحدًا من أصل سبعة عشر به نشاط عالي كمضاد للأكسدة. في الختام، هذا عمل جديد ومهم مع الإمكانات الواعدة للدراسة العلمية لأنشطة مضادات الميكروبات والأوالي ومبيدات الرخويات ومضادات الأكسدة. ومع ذلك، يحتاج المزيد من الدراسات والتحقيقات التجريبية، خاصة بالنسبة للمركبات الجديدة.

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List of Abbreviations

Abbreviation	Full name
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
DPPH	2,2-diphenyl-1-picrylhydrazyl
AcOH	acetic acid
Me_2CO	Acetone
CAN	Acetonitrile
A	Alpha
ATCC	American Type Culture Collection
BSLT	Brine Shrimp Lethality Test
^{13}C-NMR	Carbon 13 Nuclear Magnetic Rezones
$CHCl_3$	Chloroform
CFU	Colony forming unit
CC	Column Chromatography
CON	Control
COSY	Correlation Spectroscopy
CV	Crystal violet
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DEPT	Distortion less Enhancement by Polarization Transfer
DMEM	Dulbecco modified Eagle's medium
ED	Effective dose
ESI^+	Electrospray positive mode
EtOH	Ethanol
EtOAc	ethyl acetate
EDTA	Ethylene diamine tetra acetate
FBS	Fetal Bovine Serum
FC	Folin-Ciocalteau
GC-MS	Gas chromatography – Mass Spectrometer
GPS	Global Positioning System
G -ve	Gram negative
G +ve	Gram positive
IC_{50}	Half maximal inhibitory concentration
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
HPLC	High Performance Liquid Chromatography
HCL	Hydrochloric Acid
^1HNMR	Hydrogen Nuclear Magnetic Rezones
ISC-MAP	International Standard for Sustainable Wield Collection of Medicinal Plants.
ME	Maceration
MS	Mass Spectrometer
LD_{50}	lethal dose
MAPTMRI	Medicinal an Aromatic Plants and Traditional Medicine Research Institute
MeOH	Methanol
MIC	Minimum inhibitory concentration

Abbreviation	Full name
MHB	Mueller Hinton Broth
MHA	Muller Hinton Agar
NCTC	National Collection of Type Culture
NIST	National Institute Standard and Technology
NA	Not applicable
NMR	Nuclear magnetic resonance spectroscopy
NOESY	Nuclear Overhauser effect spectroscopy
1D	One dimensional
PE	Petroleum Ether
PAL	Phenylalanine Ammonia Lyse
PBS	Phosphate buffer saline
KOAc	Potassium acetate
K_2CO3	Potassium carbonate
KOH	Potassium Hydroxide
PG	Propyl Gallate
R_f	Retardation factor or retention factor
RP-C18	Reverse Phase C18
RPMI	Roswell Park Memorial Institute medium
Si	Silica
NaOAc	Sodium Acetate
$NaHCO_3$	Sodium Bicarbonate
NaCl	Sodium Chloride
NaoH	Sodium Hydroxide
Na_2SO_4	Sodium sulfate
SD	Standard deviation
H_2SO_4	Sulphuric Acid
Syn.	Synonym
TLC	Thin Layer Chromatography
TFC	Total flavonoid content
TPC	Total phenolic content
TTC	Total tannin content
TCM	Traditional Chinese Medicine
2D	Two dimensional
UV	Ultraviolet spectroscopy
WHO	World Health Organization

List of Symbols

Full name	Abbreviation
%	Percent
δ	Chemical Shift Value
µg	Microgram
µl	Micro Liter
Cm	Centimeter
G	Gram
H	Hours
Kg	Kilogram
Mg	Milligram
Min	Minutes
Ml	Milliliter
mM	Milli /mole
Nm	Nanometer
ºC	Degree Centigrade
Ppm	Parts Per Million
Rpm	Revolutions Per Minute
v/v	Volume By Volume
v/w	Volume By Weight
w/w	Weight By Weight
µg/l	Microgram Per Liter
µmol	Micro Mole
µm	Micro Meter
Ft	Feet
N	Number of Replicates
MHz	Mega Hertz
Hz	Hertz
m/z	Mass/ Charge Ratio
[M]+	Molecular Ion Peak
Nm	Nanometer

CHAPTER ONE

INTRODUCTION

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INTRODUCTION

1.1 Introduction

The history of drug discovery and development is firmly set in the study of natural therapies used to treat human ailments over centuries (Habtemariam, 2019). Since antiquity, mankind has depended on plants for their fundamental needs, in particular for medicines in the treatment of various diseases (Goswami and Ram, 2017). Founded on observations and folklore, medicinal plant remedies were the first, and for a long time, the only medicines available to mankind (Pandey, 2020). Leaves, barks and roots of higher plants were used as medicines in the form of crude preparations such as teas, tinctures, poultices and powders (Parkash *et al*., 2018).

Natural products were found in various natural sources, plants, microbes and animals (Yu *et al*., 2018). They can be an entire organism (a plant, an animal or a microorganism), a part of an organism (leaves or flowers of a plant, an isolated animal organ), extract of an organism or part of an organism and an exudate or pure compounds (alkaloids, coumarins, flavonoids, lignans, steroids and terpenoids) isolated from plants, animals or microorganisms (Nwonu *et al*., 2019). However, in practice, the term natural product refers to secondary metabolites (Tittikpina *et al*., 2016, Lajayer *et al*., 2017). Nowadays, natural products are an integral part of human health care system because, there is accepted fear of toxicity and side effects of chemical drugs (Thomford *et al.*, 2018, Goudie, 2018).

There is also a realization that natural drugs are safer and allopathic drugs are often ineffective (Jamshidi-Kia *et al.*, 2018). In the last few decades there has been an exponential growth in the field of herbal medicine (Siddiqui and Sanna, 2016), and is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Jamshidi-Kia *et al*., 2018). Medicinal natural products are very frequently used in Sudan and also are widely consumed in Africa and all over the world. About 80% of the populations in African countries are dependent on traditional medicine for their primary health care (Karar and Kuhnert, 2017). Sustainability of the use of medicinal plants is important concern, and the demand is increasing in Africa as the population grows and pressure on their resources will become greater than ever. Interest in plant derived medicines has also increased in the developed countries between the pharmaceutical companies (Kala *et al*., 2006). In contrast, due to their minor side effects, the medicinal plants are widely used to treat many human diseases (Chintamunnee and Mahomoodally, 2012). Traditional medicine in Sudan has roots in Islamic and West African medicine (Abdallah, 2016a). It is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (Abdollaah *et al.*, 2019). People in many areas of the country depend on herbal medicines, which are an integral part of the health care system (Karar and Kuhnert, 2017). There is wide experience with the use of herbs in medical treatment (Ozioma and Chinwe, 2019), and many families specialize in herbal medicines and this knowledge is passed on from one generation to another (Junsongduang *et al*., 2020). Patients travel from

the capital to rural regions to consult herbalists, especially for difficult diseases (Kpobi *et al*., 2019). Medicinal plants are still invaluable source of safe, less toxic, lower price (cheap), available and reliable natural resources of drugs all over the world (Roy *et al*., 2018).

Sudan represents one of the largest African countries and characterized by rich flora described by many botanists (Khider and Hubbe, 2018, Abdelkreem and Ibrahim, 2016). Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases (Abdallah, 2017). People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves (Osman, 2016). In Sudan, 90% of Sudan population depends mainly on traditional medicine, since admission to hospitals and obtaining modern synthetic drugs is limited and a high percentage of the population are nomads (Abubakar, 2017).Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Elhadi *et al*., 2013, Dahab *et al*., 2010).

Recently, the interest in medicinal plants is growing, since many plant species have been recognized to have medicinal benefits and positive impact on human health, such as anti-inflammatory, antibacterial, hypolidemic, anti-carcinogenic, anti-oxidant and many others (Salehi *et al*., 2019). Today, most of the modern drugs (synthetic or semi-synthetic) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine (Dwivedi and Singh, 2020). Atropine, Digoxin, Ephedrine, Morphine, Quinine, Reserpine and Tubocurarine are few examples of medicines invented from the knowledges of the traditional medicine (Saad *et al*., 2017).

Medicinal plants represent a rich source of antimicrobial and anti-parasitic agents. A wide range of medicinal plant parts are used for extracts as raw drugs and they possess varied medicinal properties. The secondary metabolites of plants were found to be sources of various phytochemicals that could be directly used as intermediates for the production of new drugs (Hussain *et al*., 2012). Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times (Foroughi *et al*., 2016). Plants are the largest drug stores ever known on earth, by producing endless bioactive chemical compounds which have direct effects on animal and human health (David *et al*., 2015). According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Jamshidi-Kia *et al*., 2018, Wangkheirakpam, 2018). Researches on Pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on Ayurvedic medicinal plants and many of the major pharmaceutical corporations have renewed their strategies in favour of natural products drug discovery (Chattopadhyay *et al*., 2015). Numerous drugs have entered the international pharmacopoeia through the study of ethno pharmacology and traditional medicine (Adhikari and Paul, 2018).

The use of plants and herbs for medicinal purposes spread overall the world. Therefore, a high consumption of medicinal plants is clearly observed in the developing Islamic and non-Islamic countries (Fahmi, 2017). Consequently, treatments exist for a variety of diseases, both epidemic and endemic (Iliffe, 2017). To face

these diseases, people have tapped the environmental resources, e.g. plants, minerals and animal products for the management of their health. In this respect, the Sudanese have a large body of curative methods, techniques and recipes (Sheikhaldin, 2014). Though not yet investigated systematically or in depth, there are clues in literature about the bioactivity of medicinal plants and their chemical constituents. Sudanese medicinal plants have been reported to exert antimicrobial activity against viruses, bacteria, and protozoa (ElBashir, 2016).

Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities (Gargouri *et al.*, 2017). They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Dhama *et al*., 2016).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Chavan *et al.*, 2015). In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Pott *et al*., 2019). Today more than 30,000 diseases are clinically described, and less than one third of these can be treated symptomatically and only a few can be cured. Consequently, there exists a strong interest in getting access to new therapeutic agents which is a major driving force for advanced drug discovery strategies (Lidani *et al*., 2019).

Phytochemicals compounds are bioactive chemicals of plant origin are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; barks, leaves, stems, roots, flowers, fruits and seeds, etc. i.e. any part of the plant may contain active components (Kusari *et al*., 2012). This study was chosen to reveal the biological guided once, cytotoxicity and chemical analysis of candidate plants that are used by Sudanese in traditional medicine.

1.2 Problem of study

The last two decades have witnessed a dramatic rise in the incidence of life threatening bacterial and fungi infections. It is a major health problem with its frequency increasing every day in most countries, and also, the increase in resistance to many commercially synthetic antimicrobial agents by microorganisms with time. However, current medication is not readily accessible to many rural populations and many traditional plants have not been scientifically validated. hence the need of searching for new antimicrobial agents (Namivandi-Zangeneh *et al.*, 2019).

Infections caused by protozoan and schistosomiasis parasites affect more than billion people worldwide causing a serious health problem, mainly in developing countries (Omarova *et al.*, 2018). Amoebiasis and giardiasis are among the main neglected parasitic diseases in Latin America (Roca-Mézquita *et al.*, 2016), also schistosomiasis is one of most devastating parasitic diseases in tropical countries. The disease mostly affects children and young women (Adenowo *et al.*, 2015), because, vaccinations do not work in most

instances and the parasites have sometimes become resistant to the available synthetic therapeutics, hence it is important to search for alternative sources of anti-parasitic drugs.

Natural antioxidants are perceived to be safe, less toxic and beneficial for human health. However, natural antioxidants are very expensive and have been not widely commercialized. Recently, the demand for natural antioxidants has increased due to consumer concerns about the safety of synthetic antioxidants (Augustyniak *et al*., 2010). There had been an explosive interest in studying antioxidants of some fruits due to their health promoting properties. Moreover, the need to replace synthetic antioxidants with natural and properly safe ones, together with the interest of food industry and preventive medicine in the development of bioactive naturally-occurring antioxidants, has fostered research on the screening of plant sources.

Plants produce a high diversity of secondary metabolites with interesting biological activities, such as anti-microbial, anti-parasitic, anti-schistosoma and antioxidant activities. Therefore, there is a need to search for better sources of compounds that will be better leads to an effective medication. Therefore, it is important to explore these avenues in search for safe and alternative compounds that can act as leads for drugs.

1.3 Objectives of the study

1.3.1 General Objective

The aim of the present study was to evaluate the biological activities, cytotoxicity and phytochemical analysis as well as isolation of the bioactive compounds of some selected medicinal plants.

1.3.2 Specific Objectives:

1. To investigate *in-vitro* the biological guided activity of crude extracts, fractions and isolated compounds of some medicinal plants and to compare their activity with reference drugs.
2. To investigate the cytotoxicity against normal cell line by using Micro-culture tetrazolium test (MTT-assay) and brine shrimps (*Artemia salina* L.) lethality bioassay of some medicinal plants.
3. To determine qualitative and quantitative chemicals analysis of the selected medicinal plants.
4. To use biological guided isolation protocol for bioactive compounds isolation, and structure elucidations of isolated compounds by modern spectroscopic methods.

1.4 Scope of the study

There are some important tasks to be carried out in order to achieve the objectives of the study, and therefore five important scopes have been identified:

1. The extractions were done from selected medicinal plants using ethanol (80%) as solvent.
2. Bioassay of the crude extracts, fractions and isolated compounds will be screened for antimicrobial, antiprotozoal, anti-schistosoma, and antioxidant activities.
3. Chemical analysis of qualitative and quantitative analysis of the crude extracts.
4. The cytotoxicity against normal cell line by using Micro-culture tetrazolium test (MTT-assay), and brine shrimps (*Artemia salina* L.) lethality bioassay of crude extracts.

5. Isolation of the compounds from most active fractions will be by chromatography techniques; thin layer chromatography (TLC), high performance liquid chromatography (HPLC), column chromatography (CC). And the identification by spectra techniques; mass spectroscopy (MS) and nuclear magnetic resonance (NMR).

1.5 Significance of the study

In Sudan antibiotic resistance has lead to significant morbidity and mortality which is a drawback to economic development (Geta, 2019, Tom, 2016). Antibiotics are expensive to poor communities in developing countries and most diseases are resistant to antibiotics have become resistant to almost all available drugs (Chokshi *et al.*, 2019, Zaman *et al*., 2017). The World Health Organization advocates for traditional medicine as a safer remedy for ailments of microbial and non-microbial origin (Antinozzi, 2020). Most modern medicines were discovered through study of plants which were used traditionally to treat specific illnesses (Chao *et al*., 2017). In addition, very few medicinal plants have been analyzed chemically and their bioactive constituents are yet to be validated (Pengelly, 2020). As a result, knowledge from traditional medicine can be very essential in the development of cheap and effective antibiotics. The worldwide use of natural products including medicinal plants has become more important in primary health care especially in developing countries (Mustafa *et al*., 2017, Howes *et al*., 2020). Phytochemicals are relatively safe and abundantly available from dietary sources (Chang *et al.*, 2019), hence research into new alternative for such disease is warranted.

1.6 Layout of the study

Encompassing all the above mentioned objectives, this thesis has been divided into five chapters as described below.

Chapter one provides an introduction to medicinal plants and secondary metabolites. Chapter tow presents a thorough literature review on natural products, infection diseases and a review of literature on secondary metabolites. The basis of the selection of some selected medicinal plants for biological activities is also provided in this chapter. Chapter three outlines all the experimental methods, procedures, techniques and materials used for biological activities, cytotoxicity assays and phytochemical analysis as well as the isolation and characterization of major compounds from plants.

Chapter four shows the results obtained from the study and discussion the biological activities, cytotoxicity and phytochemical analysis of the plants. Chapter five is a summation of the conclusions and recommendations drawn from the outcomes of the study.

1.7 Research hypotheses

The hypothesis is that extracts, fractions and compounds from selected medicinal plants materials namely: *Mangifera indica* L. (Family: Anacardiaceae), *Rosmarinus officinalis* L. (Family: Lamiaceae), and *Tinospora bakis* (A. Rich) Miers (Family: Menispermaceae) were a source of highly effective biological guided antimicrobial, antiprotozoal, anti-schistosoma, and antioxidant activities.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to man for his life and supply the three important necessities of life food, clothing and shelter- and a host of other useful products. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today there are many effective means of ensuring health-care (Lewis and Elvin-Lewis, 2003). The human being appears to be afflicted with more diseases than any other animal species (Halliwell and Whiteman, 2004). There can be little doubt then very early, sought to alleviate sufferings from injury and disease by taking advantage of growing plants. In the past, almost all the medicines used were from the plants and the plant has been man's only chemist for ages. Today, a vast store of knowledge concerning therapeutic properties of different plants has accumulated (Lewis and Elvin-Lewis, 2003).

2.2 Medicinal Plants

Plants are the largest drug stores ever known on Earth, by producing endless bioactive chemical compounds which have direct effects on animal and human health (Chang and Wasser, 2012, Abdallah, 2011). Today, most of the modern drugs (Winborn and Kerrigan, 2019) are initially produced from natural products such as medicinal plants prescribed in the ancient traditional medicine (Lahlou, 2013, Pan *et al*., 2013). Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are few examples of medicines invented from the knowledges of the traditional medicine (Ansari and Inamdar, 2010, Lahlou, 2013). Although, there is wide experience among the people of Sudan in the employment of medicinal plants as an integral part of the health care system, this experience passed from one generation to another without documentation (Baer *et al*., 2013, Eyong, 2007).

Medicinal plants play an important role in the lives of rural people by curing common ailments especially in developing countries of the world (Hussain *et al*., 2013). Medicinal plants have a promising future because there are about half million plants around the world, and most of their medical benefits have not been investigated yet, and their medical activities could be decisive in the treatment of present or future studies (Rasool Hassan, 2012). However more than 80% of the world population in developing countries uses plant derived medicines and about half of the population in industrialized countries also using traditional medicines practices to meet their healthcare needs *(*Eddouks *et al*., 2002, Organization, 2002). In Sudan, people dependent on medicinal plants for health care is estimated at over 90% of the population, these plants and derived products play an important role in the primary health care of Sudan (Ibrahim and El-Khateeb, 2013).

2.2.1 Medicinal Plants in Africa

Medicinal plants are an integral part of the African healthcare system since time immemorial. Interest in traditional medicine can be explained by the fact that it is a fundamental part of the culture of the people who use it and also due to the economic challenge: on one side, the pharmaceutical drugs are not accessible to the poor and on the other side, the richness and diversity of the fauna and flora of Africa are an inexhaustible source of therapies for panoply of ailments (Sawadogo *et al*., 2012).

The demand for medicinal plants is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever. Interest in plant-derived medicines has also increased in developed countries among the pharmaceutical companies (Hostettmann *et al*., 2000). In contrast, due to their minor side effects, the medicinal plants are widely used to treat many human diseases (Başgel and Erdemoğlu, 2006).

2.2.2 Sudanese Medicinal Plants

Sudan is the largest country in Africa with a diverse flora. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases. Sudanese traditional medicine is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (Mohamed *et al*., 2010). Sudanese medicinal plants have been reported to exert antimicrobial activity against viruses, bacteria, and protozoa (El-Tahir *et al*., 1999, Hussein *et al*., 2000, Elegami *et al*., 2001, Ali *et al*., 2002, Kabbashi *et al*., 2014, Kabbashi *et al*., 2015b, Garbi *et al*., 2014). As infections with worms or molluscs represent a common affliction in that area, medicinal plants have been considered for treatment of these infections (Koko *et al*., 2000, Koko *et al*., 2005, Koko *et al*., 2008b). Immunomodulatory properties of Sudanese medicinal plants have also been observed (Koko *et al*., 2008b).

2.3 Natural Products

Natural products play a highly significant role in the drug discovery and development of processed (Newman and Cragg, 2012). They not only serve as drugs or templates drugs directly, but in many instances lead to the discovery of novel mechanisms of action that provide a better understanding of the targets and pathways involved in the disease process (Löscher *et al*., 2013). They have additionally been used as starting templates in the synthesis of combinatorial libraries (Lam, 2007). Natural products are products from various natural sources, plants, microbes and animals (Gershenzon and Dudareva, 2007; Adam, 2017). However, in practice, the term natural product refers to secondary metabolites (Hastings *et al*., 2012)).

2.3.1 Ethno-botany and Traditional Sources of Natural Products

Historically, mankind has been intrigued by the power and potential of plants in nature (Steffen *et al*., 2007). Ancient texts attest to the knowledge passed down through generations on the beneficial effects of plants. Wisdom was gained on how to extract ingredients as foods, medicine, and mood enhancers long before knew how the chemicals themselves worked (Lewis and Elvin-Lewis, 2003). At the time of the Renaissance and through the Reformation period, the tools were found to explore the chemistry of the

natural ingredients. Today, we embrace the science of ethno-botany, which is the scientific study of the relationships that exist between people and plants (Cooper and Deakin, 2016). The discipline requires a variety of skills: botany (the identification and preservation of plant specimens); anthropology, to understand the cultural concepts around the perception of plants; and natural products chemistry (Martin, 2010). As sources of health products, the majority of the world continues to heavily rely on herbal remedies for their primary health care (Petersen, 2003). Ancient civilizations of both China and India have provided a wealth of knowledge on the use of traditional medicines (Sheng-Ji, 2001). Traditional Chinese Medicine (TCM) has been used for thousands of years (Petersen, 2003) and the basis and philosophy of TCM is not well understood in Western practice (Yu *et al*., 2006). Many TCM formulas are mixtures of herbs and plants, used in combination and prepared as teas (Omri *et al*., 2012).

2.3.2 Sources of Natural Products

With respect to the sources of natural products from plants, first, attention needs to be paid to the correct identification of the species (Cragg and Newman, 2013). Second, due to variability in the growing conditions, it is important to keep in mind that the same identified species found in different geographical locations might yield different metabolites or ratios of metabolites (Bérdy, 2005). Finally, the selection of the plant part is very important: flowers, fruiting bodies, berries, bark, seeds, or stems. Metabolites vary within the whole plant as fresh or dried material, or from the roots or the aerial parts (Salgueiro *et al*., 2010). Thus, the botanical extracts are complex mixtures of multiple compounds. For chemical analysis, standard reference may be difficult to generate or obtain, and also, the stability of chemicals in the extracts may sometimes be difficult to control (Folashade *et al*., 2012).

2.3.3 Natural Products in Medicine

The use of natural products, especially plants, for healing is as ancient and universal as medicine itself. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian. Even now, continuous traditions of natural product therapy exist throughout the third world, especially in East and South Asia (Sarker and Nahar, 2012). According to the World Health Organization, 3.4 billion people in the developing world depend on plant based traditional medicines. This represents about 88 % of the world's inhabitants, who rely mainly on traditional medicine for their primary health care (WHO, 2017). In China alone, 7295 plant species are utilized as medicinal agents (Nobili *et al*., 2009). Over the last century, a number of top selling drugs have been developed from natural products (Newman and Cragg, 2012). Apart from natural product derived modern medicine, natural products are also used directly in the "natural" pharmaceutical industry that is growing rapidly in Europe and North America, as well as in traditional medicine programmers being incorporated into the primary health care systems of Mexico (Cordell and Colvard, 2012).

2.3.4 Drug Discovery and Natural Products

Although drug discovery may be considered a recent concept that evolved from modern science during the 20th century, in reality the concept of drug discovery dates back many centuries, and has its origins in nature (Drews, 2000). Time and time again, humans have turned to Mother Nature for cures, and discovered unique drug molecules (Ashburn and Thor, 2004). Thus, the term natural product has become almost synonymous with the concept of drug discovery (Ganesan, 2008).

2.3.5 Plants as Drugs Source

All plants produce chemical constituents, part of their normal metabolic activities (Davies, 2010). These chemical components can be divided into primary metabolites, such as sugars, amino acids, nucleotides and fats, found in all plants, and secondary metabolites which have no obvious function in a plant "s primary metabolism as well as in growth, photosynthesis, or other primary functions of the plant cell. They may possess an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses, or to be responsible for the chemical defense of the plant against microorganisms, insects and higher predators (Seigler, 2012, Cseke *et al*., 2016). Secondary metabolites can be classified based on their chemical structure (e.g., having rings, with or without sugar moieties), composition (containing nitrogen or not), the pathway by which they are biosynthesized or their solubility in various solvents (Kabera *et al*., 2014, Dai and Mumper, 2010). A simple classification includes three main groups: (1) Terpenoids made through mevalonate pathway, composed almost entirely of carbon and hydrogen, (Cseke *et al*., 2016, Brahmkshatriya and Brahmkshatriya, 2013). Phenolics (made from simple sugars, containing benzene rings, (2) hydrogen, and oxygen, Nitrogen-containing compounds or containing sulphuric parts (Chinou, 2008), as shown in Figure (2.1).

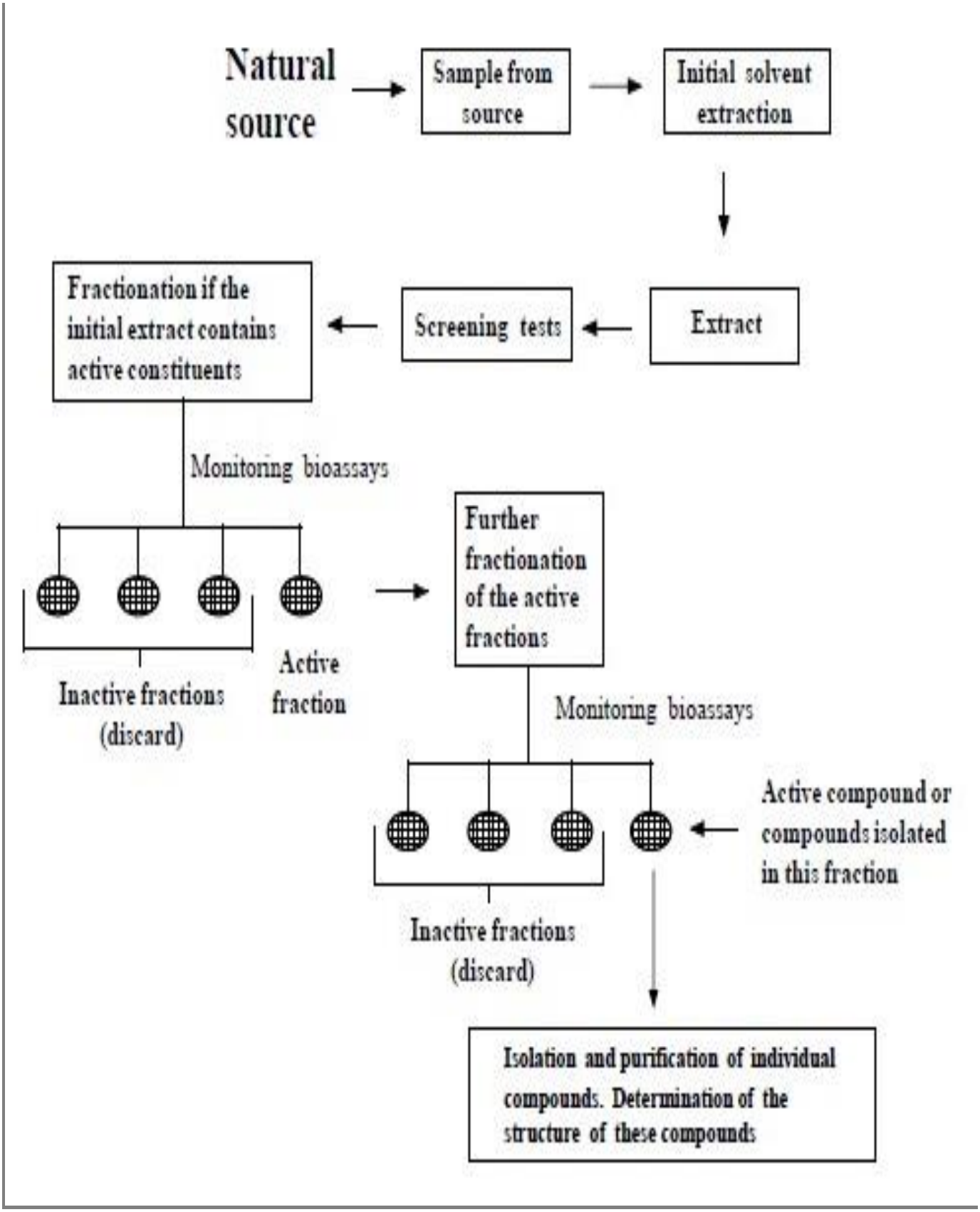


Figure (2.1): General approach to the isolation of active compounds from a natural source (Sarker and Nahar, 2012).

2.4 Biological Studies

2.4.1 Antimicrobial Activity

Infectious diseases caused by bacteria and fungi affect millions of people worldwide (Rodrigues and Nosanchuk, 2020). Throughout the history of mankind, infectious diseases have remained a major cause of death and disability (Friedrich, 2017). Today, infectious diseases account for one-third of all deaths in the world and the World Health Organization estimates that nearly 50,000 people die each day throughout the world (Pink, 2016, Holmes *et al.*, 2017). The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind (Jouda *et al.*, 2015, Lewies *et al.*, 2019). Different antibiotics exercise their inhibitory activity on different pathogenic organisms, hence the development and spread of resistance to currently available antibiotics is a worldwide concern (WHO, 2017). The situation has further been complicated by the rapid development of multi-drug resistance by the microorganisms to the antimicrobial agents available (De Waele *et al*., 2018). However, in addition to the production of intrinsic antimicrobial compounds (Hernando-Amado *et al*., 2016), some medicinal plants also produce multidrug resistance inhibitors which enhance the activities of antibiotics against multidrug resistant bacteria pathogens (Ibrahim *et al.*, 2017).

A. Antimicrobial Activity of Medicinal Plants

The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments (Dhanavade *et al.*, 2011). In recent years, a number of studies have been conducted in various countries to prove such efficiency (Nascimento *et al.*, 2000). Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants (Arguelles-Arias *et al.*, 2009). These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning (Evans, 2009b). While 25-50 % of current pharmaceuticals derived from plants none are used as antimicrobials (Bozin *et al.*, 2007). Traditional healers have long used plants to prevent or cure the infectious condition (Bahmani *et al*., 2014). Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavonoids which have been found *in-vitro* to have antimicrobial properties (Sher, 2009).

B. Antimicrobial Activity of Some Sudanese Medicinal Plants

Bakheit and Omer (2021) evaluated the antimicrobial activity of *Boswellia papyrifera* essential oil against clinical bacterial pathogens. The essential oil of *B. papyrifera* (300 μg) showed the highest *in-vitro* antimicrobial activity against *S. aureus*, a moderate activity against *P. aeruginosa*, and a weak activity against *E. coli*. It was not effective against *K. pneumoniae* and *Proteus vulgaris*. Also it showed an antimicrobial activity stronger than that of Tetracycline for *E.coli*, and it had a synergistic effect with Amoxicillin against *S. aureus*. Moglad *et al*. (2020) evaluated the antimicrobial and wound healing activities of *Loranthus acacia*, *Cassia obtusifolia* and *Cymbopogon proximus* plants. The results showed that all tested plants have various antimicrobial and wound healing activities. Out of these plants, *L. acacia*

exhibited a significant result for antimicrobial activities counter to all Gram-positive, Gram-negative bacteria and wound healing activities in comparing with the reference drug.

Hamadnalla and Jack (2019) tested antibacterial activity of *Solenostemma argel* a medicinal plant used in folk medicine in Sudan for cure of many diseases. Five solvents with grade polarity were used to extract *S. argel* (petroleum ether, chloroform, ethyl acetate, ethanol and distilled water). Four extracts except petroleum ether showed clear inhibitory action against both bacteria. Elrofaei *et al*. (2018) investigated the *in-vitro* antibacterial activity of the petroleum ether, methanol and chloroform extracts of *Citrullus colocynthis*, *Grewia tenax*, *Mentha longifolia*, *Senna obtusifolia* and *Zingiber officinale* against five standards bacterial strain. The petroleum ether and chloroform extracts were inactive compared to methanol extracts. The maximum antibacterial activity against the test organisms was found in methanol extract. Methanol extract of *Citrullus colocynthis* had maximum inhibitory activity (32 mm) against *Escherichia coli*. The MIC (minimum inhibitory concentration) of extracts was observed using well diffusion method. Amongst Gram negative bacteria *Escherichia coli* being inhibited at <3.12 mg/ml by *C. colocynthis* root methanolic extract.

Hassan *et al*. (2017) tested antimicrobial activity of the rhizome essential oil of *Zingiber officinale* (family: Zingiberaceae) against six standard bacteria. The rhizome essential oil of *Z. officinale* dissolved in methanol (1:10), showed high activity against the Gram positive *S. aureus* (23 mm), *B. subtilis* (20 mm), Gram negative *E. coli* (21 mm), *P. vulgaris* (20 mm) and *P. aeruginosa* (27 mm). It also showed moderate activity against the Gram negative *K. pneumoniae* (15 mm), *C. albicans* (16 mm) and no activity against *Aspergillus niger*. Abdallah (2016b) investigated *in-vitro* antibacterial activity of leaf extracts of Water, Butanol, Ethyl acetate and Chloroform of *Moringa oleifera*. Ethyl acetate extract recorded the highest active extract against four microorganisms namely, *Staphylococcus epidermidis* ATCC 49461 (16.0±0.5 mm), *Staphylococcus aureus* ATCC 25923 (13.6±0.3 mm), *Pseudomonas aeruginosa* ATCC 27853 (13.3±0.3 mm) and *Bacillus cereus* ATCC 10876 (10.2±0.7 mm), respectively. Butanol extract was active only against *Staphylococcus epidermidis* (14.0±0.0 mm) and *Staphylococcus aureus* (10.3±0.3 mm). Water extract was active only against *Staphylococcus epidermidis* (12.3±0.6 mm). Chloroform extract showed antibacterial activity against *Staphylococcus aureus* (11.0±0.5 mm).

Abd-Ulgadir *et al.* (2015) evaluated antimicrobial potential of leaves and seeds methanolic extracts of the medicinal plants *Hibiscus sabdariffa* and *Ricinus communis* against standard microbial strains. Leaves from methanolic extracts of *H. sabdariffa* was found to be more active against Gram-positive bacteria (*Bacillus subitilis* NCTC: 8236 and *Staphylococcus aureus* ATCC: 25923), as well as Gram-negative bacteria (*Escherichia coli* ATCC: 25922, *Pseudomonas aeruginosa* ATCC: 27853, *Klebsiella pneumoniae* ATCC: 53657 and *Proteus vulgaris* ATCC: 6380) than leaves methanolic extracts of *R. communis*. The leaves methanolic extracts of *H. sabdariffa* gave an intermediate antifungal activity against two reference fungal strains (*Candida albicans* ATCC: 7596 and *Aspergillus niger* ATCC: 9765). The

seeds methanolic extracts of *R. communis* and *H. sabdariffa* did not show any activity against both bacterial and fungal reference strains examined. Mosa *et al*. (2014) evaluated the phytochemical constituents and antimicrobial activities of some Sudanese medicinal plants (*Combretum hartmannianum* leaves (Habiel), *Hydnora abyssinica* rhizome (Tartous), *Sesbania leptocarpa* leaves (Surib) and *Ficus vasta* leaves (Gom'aiz)). All the screened plants (concentration 100 mg/ml) exhibit positive effects against tested bacterial strains except *S. leptocarpa* has negative effects against *Staphylococcus aureus* bacteria. The most prominent activity against tested bacteria is showed by *H. abyssinica* and *F. vasta* ethanolic extracts. *S. leptocarpa* (100mg/ml) has no antifungal activity against the tested fungal strains. *H. abyssinica*, *C. hartamannianum*, and *F. vasta* show promising antifungal activity.

Ismail *et al.* (2013) investigated the antibacterial activity of petroleum ether and methanol extracts of two Sudanese medicinal plants *Vigna coerulea* Bak and *Aloe vera* were tested against three species of bacteria, *Bacillus subti*lis, *Escherichia coli* and *Staphylococcus aureus*. Results revealed the methanol extracts of two medicinal plants were effective on bacterial strains, while the crude plants were weakly effective. Moglad *et al*. (2012) investigated the *in-vitro* antibacterial and antifungal activity of *Cadaba farinose* (Family: Capparaceae), *Solanum nigrum* (Family: Solanaceae), *Senna occidentalis* (Family: Caesalpiniaceae) and *Maerua oblongifolia* (Family: Capparaceae). All methanol extracts exhibited inhibitory effects against all tested organisms with zones of inhibition ranging from 11-25 mm except the methanol extract of both parts of *Maerua oblongifolia* were active against *Escherichia coli* and *Aspergillus niger*. The results obtained from plants extracts were compared to some of the commercially used drugs. The Minimum Inhibitory Concentrations (MICs) of the most active extracts of these plants against standard bacteria and fungi were also determined and found that MICs a ranging between concentration 2.5-5 mg/ml^{-1}. Saadabi and Moglad (2011) screened for *in-vitro* activity against some Gram-positive and Gram-negative pathogenic bacteria. The extracts at concentration of 0.1 ml/cup showed varying degrees of inhibitory activity against the tested organisms. Extracts from *Withania somnifera* showed the highest activity, followed by *Datura stramonium*, while *Zygophyllum portulacoides* demonstrated the least activity when compared to 40 µg/ml Ampicillin control antibiotic. The bacteria tested differed significantly in their susceptibility to plant extracts, with complete inhibition in case of *Staphylococcus aureus* and *Bacillus subtilis*. EL-Kamali and EL-Amir (2010) performed the antibacterial screening of the ethanol extracts of the selected plants. All the eight ethanolic extracts showed good activity against four tested bacteria. The activity of the *Cymbopogon schoenathus spp*. *proximus* aerial parts, *Cymbopogon nervatus* inflorescence and *Cassia occidentalis* seed extracts were more pronounced.

C. Antimicrobial Activity and Bioassay

Infectious diseases caused by bacteria, fungi, viruses and other pathogenic parasites are still a major threat to public health, despite the tremendous progress in human medicine. Antimicrobial activity of natural extracts and pure compounds can be detected by observing the growth response of various microorganisms

to samples that are placed in contact with them. Several methods for detecting antimicrobial activity are available, but since they are not equally sensitive or not based upon the same principle, results will be profoundly influenced by the method (Cos *et al*., 2006). The antibacterial and antifungal test methods are classified into two main groups: diffusion and dilution methods (Das *et al*., 2010).

D. Agar Diffusion Method

A very simple way of determining the susceptibility of a microorganism to an antimicrobial agent is to use a microbe-seeded agar plate and to allow the agent to diffuse into the agar medium, which is known as the Kirby-Bauer technique (De Beer and Whitlock, 2009). This test is less expensive but less reliable than the dilution method; however, it provides qualitative susceptibility information. In the diffusion technique, the organism to be tested is exposed on an agar medium, either seeded in the agar, by flooding or spreading, to a diffusion gradient of the chemotherapeutic drug arising from reservoir which may be impregnated filter paper disc, ditch cut in the medium and filled with (agar containing) the drug, or the ditch may be filled directly with drug solution, or the solutions of the drugs may be placed in circular holes cut in the inoculated agar medium (Jorgensen and Turnidge, 2015).

E. Dilution Method

The aim of broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimum inhibitory concentration MIC) that, under defined test condition, inhibits the visible growth of the microorganisms being investigated. MIC values are used to determine susceptibility of bacteria and fungi to drugs and also to evaluate the activity of new antimicrobial agents. The most commonly used method in the dilution techniques is the broth dilution technique, described by Balouiri *et al*. (2016). In the agar dilution method, the medium is inoculated with the test microorganism and the samples to be tested are mixed with the inoculated medium. The material is inoculated and the growth of the microorganism is viewed and compared with control culture which does not contain the tested sample. The experiment is repeated at various dilutions of the test sample in the culture medium and the highest dilution at which the sample just prevents the growth of the microorganism (MIC) is determined (Trias *et al*., 2008).

F. Factors affecting the *in-vitro* Antimicrobial Activity

The diffusion process may be altered by a variety of factors including; the content of drug at the source, the density of the gel, the speed of diffusion, and the ionic concentration of the medium (Kuo and Ma, 2001). The zone of inhibition that results is dependent upon both the infusibility of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculums can influence to a marked degree the size of inhibitory zones (Jorgensen and Turnidge, 2015). The pH of the environment also affects antimicrobial activity *in vitro*. Some drugs are more active at acid pH (e.g. nitrofurantoin) others at alkaline pH (e.g. aminoglycosides, sulphonamides) (Yang *et al*., 2014). The components of the medium significantly influence the results of the tests e.g. sodium polyanethol

sulphonate and other anionic detergents inhibit aminoglycosides. Serum proteins bind Penicillin's in varying degrees, ranging from 40% for methicillin to 98% for Dicloxacillin (Stead, 2000).

G. General characteristics of standard microorganisms under study

G.1 *Bacillus subtilis*

It is one of Gram-positive rods that grow aerobically on nutrient agar and form resistant end spores. It is one of the commonest saprophytes found as contaminants in foods, clinical specimens and laboratory cultures. It is a facultative thermophile, capable of growth over the range 12-55˚C. *B. subtilis* spores are exceptionally heat resistant. *B. subtilis* grows well on ordinary media, forming large colonies that are circular or irregular, grey-yellow granular and difficult to emulsify. Its positive Voges Proskauer reaction and its production of acid from glucose, xylose, sucrose and mannitol (Vijayakumar *et al*., 2013), as shown in Figure (2.2).

G.2 *Staphylococcus aureus*

It is a Gram positive; aerobic and also grows anaerobically but less well. Temperature range for growth is 10-42°C with an optimum of 35- 37°C. It grows in blood agar and produces yellow to cream or occasionally white 1-2 mm in diameter colonies, in MacConkey agar produces smaller colonies (0.1- 0.5mm). *Staphylococcus aureus* ferment mannitol (give yellow colour), it is coagulase, catalase and DNase positive. *Staphylococcus aureus* causes boils, styes, pustules, imprtigo, infection of wound, ulcer and burns, osteomyelities, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. It is carried in the nose of 40% or more of healthy people (Osman, 2005), as shown in Figure (2.3).

G.3 *Escherichia coli*

Gram negative rod, coliform, motile, aerobic and facultative anaerobic. *Escherichia coli* is catalase positive, oxidase negative, attack sugars fermentatively; gas normal produces, ferment lactose with production of acid and gas, produces indole, give positives methyl red reaction and negative Vogues-Proskaour and decomposes urea. The organism gives colorless colonies 2-3 mm in diameter in 18 hours in nutrient agar and red large colonies in MacConkey agar and may be hemolytic on blood agar. *E.coli* commonly causes the urinary tract infections and diarrhea in infant and travelers. Also it causes meningitis, septicemia as well as sepsis in operating wounds and abscess (Hemraj *et al.*, 2013), as shown in Figure (2.4).

G.4 *Pseudomonas aeruginosa*

It is motile and Gram-negative rod shaped, obligate aerobe, and grow readily on many types of media at 37-42˚C sometimes producing sweet or grape-like odour due to 2-amino acetophenone production. It forms smooth round colonies with fluorescent greenish colour (pyoverdin fluorescent pigment). It is oxidase positive; produce acid from carbohydrate due to oxidation not fermentation, catalase positive, and citrate positive. It is pathogenic only when introduced into areas devoid of normal defenses. It can cause purulent infections of wounds, burns, external ear, urinary tract and contamination of traumatic lesions in the eye leads to opthlamitis (Parsek and Greenberg, 2000), as shown in Figure (2.5).

G.5 *Candida albicans*

Budding yeast produces pseudo hyphal both in culture and in tissue appears as a Gram–positive. On Sabouraud agar at room temperature, incubation gives cream-colored colonies with a yeasty odour. Subculture of the organism in 0.5 ml of Human serum incubated at 37 ˚C for 1.5-2 showed the formats of Germ-tube by some of the yeast cells. I.e. Germ tube test (G.T.T) positive. It ferments glucose and maltose, producing both acid and gas and does not attack lactose. It is opportunistic fungi, is a member of the normal flora of the mucous membranes in the respiratory, gastrointestinal and female genital tract. In such location it may gain dominance and be associated with pathologic conditions (Mayer *et al.*, 2013), as shown in Figure (2.6).

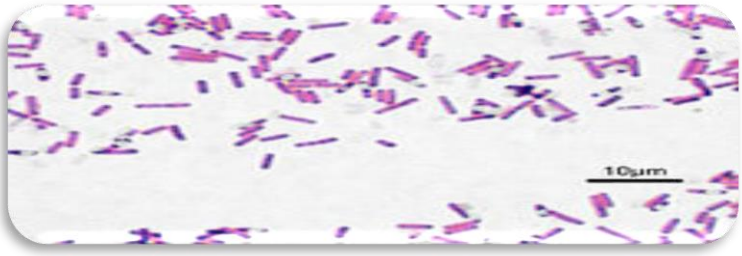


Figure (2.2): ***Bacillus subtilis*** (Oren and Garrity, 2021)

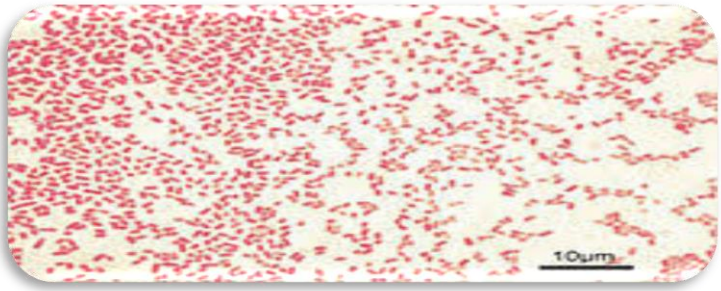


Figure (2.3): ***Staphylococcus aureus*** (Yan, 2015).

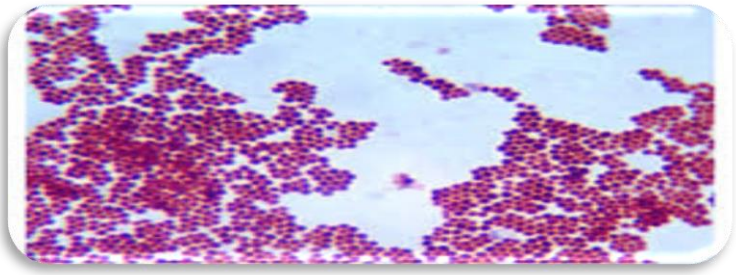


Figure (2.4): ***Escherichia coli*** (Gillen and Augusta, 2018)

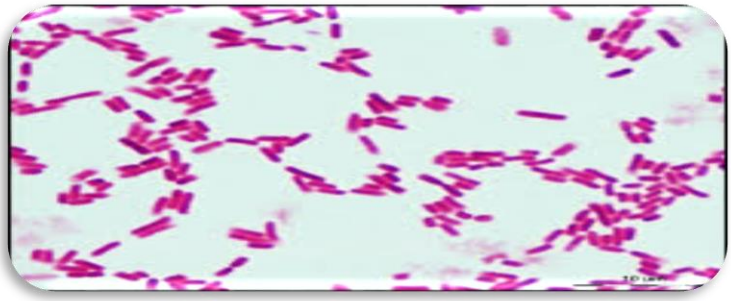


Figure (2.5): ***Pseudomonas aeruginosa*** (Lail, 2006).

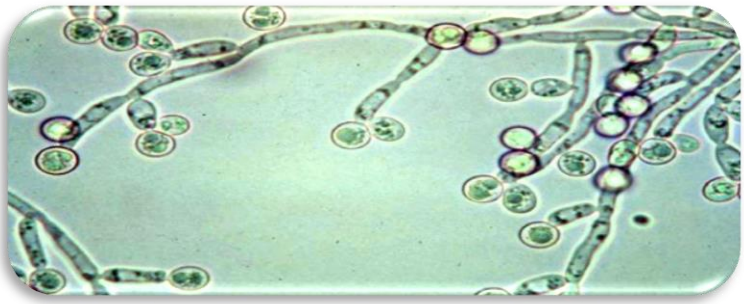


Figure (2.6): ***Candida albicans*** (Staniszewska *et al*., 2013).

H. Antibiotics used for antimicrobial activity

H.1 Ampicillin

Ampicillin is one of antibiotics in penicillin group of drug. It is used to treat a wide variety of bacterial infections. It works by stopping the growth of bacteria. Its chemical formula is ($C_{16}H_{19}N_3O_4S$) and has molecular weight of 349.41 g/mol. Ampicillin is a beta-lactam antibiotic that is part of amino penicillin family and it is active against gram-positive and gram-negative bacteria. Ampicillin sodium salt is semi synthetic penicillin derived from the basic nucleus, 6-aminopenicillanic acid. The chemical structure of Ampicillin is schematically, as shown in Figure (2.7).

H.2 Gentamicin

Gentamicin ($C_{21}H_{43}N_5O_7$) antibiotics are 2-DOS-based 4, 6-disubstituted pseudosaccharides and are produced for clinical use as a mixture comprising Gentamicins C1, C1a, C2a, C2, and C2b. Structurally, the compounds are very similar to each other, the only difference being the methylation pattern on the purpurosamine ring, Figure (2.8). It is established in clinical use and despite its significant toxicity remains valuable to treat severe Gram-negative bacterial infections. All the members have similar bactericidal properties (Reva, 2019).

H.3 Clotrimazole

Clotrimazole ($C_{22}H_{17}C_lN_2$) is a weak base that is freely soluble in methanol but practically insoluble in water (Jøraholmen, 2012). The azoles are a group of synthetic antifungal agents with a broad spectrum of activity based on the imidazole nucleus, Figure (2.9). Their mechanism of action involves the inhibition of a fungal enzyme (14α-demethylase) responsible for converting lane sterol to ergo sterol, which is the main sterol in the fungal cell membrane. The subsequent reduction of ergo sterol alters the fluidity of the membrane, interfering with the action of membrane-associated enzymes. The net effect is an inhibition of replication. Azoles also inhibit the transformation of candida yeast cells into hyphae – the invasive and pathogenic form of the parasite (Rang, 2007).

H.4 Nystatin

Nystatin ($C_{47}H_{75}NO_{17}$) binds to the fungal membrane ergosterol with up to 10 times more affinity than to the human membrane sterols (i.e. cholesterol) providing selective binding and thus antifungal activity (Kodedová and Sychrová, 2015) with minimal effects if any on host cells, (Figure 2.10). Therefore, nystatin can be used at a dose that is low enough to be bound to candida cell membranes but not to mammalian cells, making this agent non-toxic to human while still retaining its antifungal activity. In suspension, nystatin (especially the form for oral use) is not absorbed in the gut and therefore has no systemic effects or side effects. It is found to be the most established antifungal agent that is effective in the treatment of superficial fungal infection caused by *Candida sp* (Carrillo-Muñoz *et al*., 2013).

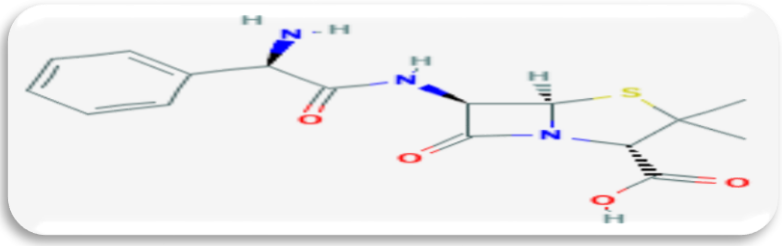


Figure (2.7): Chemical Structure of Ampicillin (Abdelrahman *et al*., 2015).

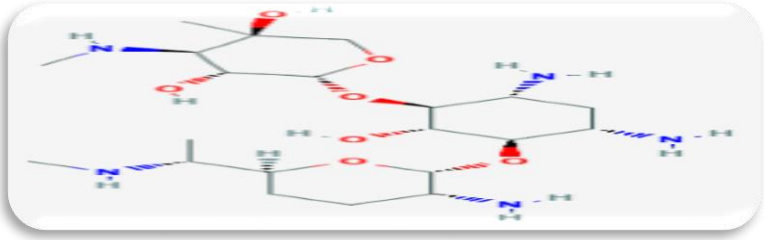


Figure (2.8): Chemical Structure of Gentamicin (Mohammed *et al*., 2016b).

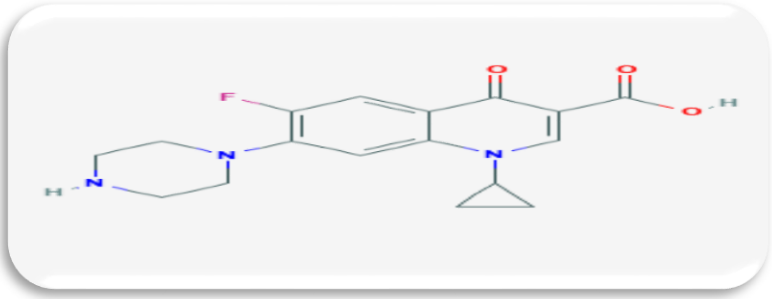


Figure (2.9): Chemical Structure of Clotrimazole (Mohammed *et al*., 2016b**).**

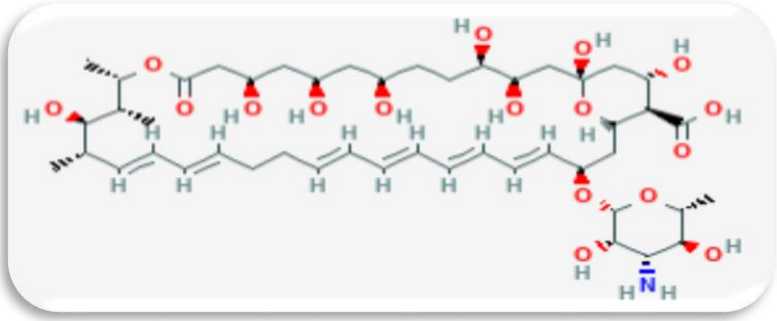


Figure (2.10): Chemical Structure of Nystatin (Hemmateenejad *et al*., 2014).

2.4.2 Antiprotozoal Activity

Intestinal parasites of humans are important health problems of most communities, especially in tropical and subtropical areas (Siddig *et al*., 2017). Parasitic infections have high prevalence rates throughout the world and it is estimated that more than three billion people are infected with intestinal parasites in the world (Wang et al., 2018b). Studies on human parasitic infections have demonstrated a common relationship between parasitic infections and lower socioeconomic status of the region (Faria *et al*., 2017). Protozoa are the cause of a number of major diseases which spread massive misery and death, mainly

throughout the tropical world (Organization, 2010). For centuries, man has been confronted with pathogenic microorganisms (Morens *et al*., 2004), and of them protozoa form a major group and stand the first place for developing human diseases. Protozoal attacks are common in different parts of the world population and account for the major cause of mortality in developing countries (Tempone *et al*., 2005, Hotez and Kamath, 2009).

A. Antiprotozoal activity of Medicinal Plants

Medicinal plants have been used throughout the world for the treatment of tropical diseases such as malaria, leishmaniosis, leprosy, etc. (Alilio *et al*., 2004). The discovery of antimalarial compounds from natural sources (quinine from *Cinchona spp.*, and artemisinin from *Artemisia annua*) indicates a great potential of such traditional plants which are in human use since centuries, as a new source of new medicines for malaria, and for other tropical diseases (Oliveira *et al*., 2009). Sudan is an African country which has tremendously suffered from all the above mentioned parasitical diseases. Sudan is very rich in medicinal plants which have been used by local people for the treatment of diseases such as schistosomiasis, malaria and leishmaniasis (Abdalla and Koko, 2018).

B. Antiprotozoal activity of Some Sudanese Medicinal Plants

Mahmoud *et al*. (2020) performed a survey of medicinal plants of Sudan and selected 62 ones that are being used in Sudanese traditional medicine. This resulted in a library of 235 fractions, which was tested *in-vitro* against *Plasmodium falciparum* (erythrocytic stages), *Trypanosoma brucei* rhodesiense (bloodstream forms), *Trypanosoma cruzi* (intracellular amastigotes), and *Leishmania donovani* (axenic amastigotes). Active fractions were also tested for cytotoxicity. Of the 235 fractions, 125 showed growth inhibitory activity >80% at 10 mg/ml, and >50% at 2 mg/ml against at least one of the protozoan parasites. *Plasmodium falciparum* was the most sensitive of the parasites, followed by *T. b. rhodesiense* and *L. donovani*. Only few hits were identified for *T. cruzi*, and these were not selective.

Abdelwahid *et al*. (2019) evaluated synthesized quinoline-4-carboxylic acids, confirm their chemical structures, and their anti-leishmanial activity. The IC_{50} for each compound was determined and manipulated statistically. Among these compounds, Q1 (2-methylquinoline-4-carboxylic acid) was found to be the most active in terms of IC_{50}.

Najumudin *et al*. (2018) investigated the *in-vitro* antigiardial activity of ethanolic extracts of *Solennostemma argel*, *Trigonella foenum* graecum and *Menthax piperita* in comparison with metronidazole. *Trigonella foenum* graecum, *Menthax piperita* and *Solennostemma argel* exhibited 98, 92 and 65% mortality respectively within 96 h at concentration of 5000 ppm (5 mg/ ml). This was compared to Metronidazole which gave 100% inhibition at concentration of 0.3125 ppm at the same time. The ethanolic extracts of *Trigonella foenum* graecum, *Solennostemma argel* and *Menthax piperita* had potent antigiardial activity. Kabbashi *et al*. (2017) determined *in-vitro* antiprotozoal activities of some selected Sudanese medicinal plants. All plants examined gave 100% inhibition at a concentration 500 µg/ml after

96 h; comparable with Metronidazole which gave 95% inhibition at concentration 312.5 µg/ml at the same time against *Entamoeba histolytica* and *Giardia lamblia.* Shayoub *et al*. (2016) evaluated anti-malarial activity (*Plasmodium falciparum*) of *Khaya senegalensis* extract of methanol. The methanol of *K. senegalensis* extract gave 81% mortality in 72h, at concentration 500 ppm; this was compared with Artemether which gave 85% inhibition at the same time.

Kabbashi *et al*. (2015a) evaluated antigiardial activity (*Giardia lamblia*), antioxidant (DPPH assay) and cytotoxicity (MTT assay) of ethanol extract of *A. nilotica* leaves. The ethanolic *A. nilotica* leaves extract which exhibited 100% mortality within 96 h, at a concentration 500 µg/ml; compared with Metronidazole which gave 96% inhibition at concentration 312.5 µg/ml at the same time. Kabbashi *et al*. (2014) investigated the amoebicidal activities of *Adansonia digitata* leave and *Cucurbita maxima* (seeds) extracted by ethanol. Ethanolic extracts of *A. digitatat* leave exhibit 100% inhibition against *E. histolytica* at concentration 500 µg/ml after 72 h, this was compared with Metronidazole powder which gave 75% inhibition at concentration 312.5 µg/ml at the same time. Ethanolic extracts of *C. maxima* exhibit 100% inhibition at concentration 500 µg/ml after 96 h; this was compared with Metronidazole powder which gave 80% inhibition at concentration 312.5 µg/ml at the same time.

Elhadi *et al*. (2013) evaluated antigiardial activity of *Cucurbita maxima* D., *Cucurbita pepo* L. and *Lagenaria siceraria*. The highest activity against *Giardia lamblia*, with respect to time, was obtained from *C. maxima* seeds petroleum ether extract which exhibited 100% mortality within 48 giving IC_{50} of 548.80 ppm (with a concentrations of 1000 and 500 ppm) followed by *L. siceraria* petroleum ether extract which exhibited 100% mortality within 72 hours with IC_{50} of 95.65 ppm whereas Metronidazole, a pure compound, (positive control) showed 100% mortality within 96 hours. On the other hand the lowest antigiardial activity was recorded by *C. pepo* petroleum ether extract (83.67% mortality with 500 ppm concentration within 96 hours) giving IC_{50} of 60671.32 ppm whereas *C. maxima* and *L. siceraria* methanol extract exhibited 100 % mortality within 96 hours with IC_{50} 35.6ppm and 120 hours with IC_{50} 8.9ppm respectively (with 1000ppm concentration). The best result was obtained by *C. maxima* petroleum ether extract at 250 ppm (up to 100% mortality within 72 hours) with IC_{50} 1 ppm. Eltayeb and Ibrahim (2012) tested antileishmanial activity of three organic solvent extracts and water residue of the plants: *Acacia nilotica* (Family: Mimosaceae) (husk), *Ambrosia miratima* (Family: Astraceae) (aerial shoot) and *Azadarichta indica* (Family: Meliaceae) (leaves) *in-vitro* against *Leishmania donovani* promastigotes. The study revealed that the extracts of *A. nilotica* and *A. miratima* have effectious antileishmanial activity at concentrations (IC_{50}) less than 8 µg/ml, while the extracts of *A. indica* lack antileishmanial activity.

Dahab *et al*. (2011) examined whole plants of *Xanthium brasilicum* and *Argemone mexicana* extracted by methanol, chloroform and water and then prepared in different concentrations for their trichomonacidal activities *in-vitro*. Methanolic extracts of *X. brasilicum* exhibit 100% mortality at concentration 500 µg/ mL after 192 hours, this was compared with Metronidazole powder which gave 98.5% mortality at

concentration 312.5 µg/mL at the same time, while the chloroform extracts gave mortality 100 % at 1000 µg/mL after 216 hours, meanwhile, the Water extracts gave 100% mortality at 1000 µg/ml after 192 hours. Water extract of *Argemone mexicana* gave 100% mortality 1000 µg/ml after 192 hours, while the chloroform and methanol extracts 1000 µg/ml gave 100 % mortality after 216 and 192 hours respectively. Nour *et al*. (2010) tested dichloromethane extract prepared from aerial parts of *Ageratum conyzoides* L. (Family: Asteraceae), a plant commonly used in folk medicine for a number of illnesses including sleeping sickness, was recently found to exhibit a prominent activity (IC_{50} = 0.78 g/mL) against bloodstream forms of *Trypanosoma brucei* rhodesiense, the etiologic agent of East African Human Trypanosomiasis (East African Sleeping Sickness). This extract also exhibited noticeable activities against *Leishmania donovani* (Kala Azar, IC_{50} = 3.4 g/mL) as well as *Plasmodium falciparum* (Malaria tropica, IC_{50} = 8.0 g/mL).

C. Protozoal Under Study

C.1 *Entamoeba histolytica*

Entamoeba histolytica Figure (2.11) is a pathogenic amoeba found throughout the world, especially common in the developing world, in area with low socioeconomic status and poor hygiene (Thompson and Smith, 2011). The parasite causes invasive disease in over 50 million people and approximately 100,000 deaths per year are reported, making it one of the leading causes of parasitic death in man (Fletcher *et al.*, 2012). Infection caused by *E. histolytica* can lead to asymptomatic colonization, amoebic colitis or extra-intestinal disseminated disease (Shirley and Moonah, 2016). Infection with *E. histolytica* has been estimated to be as high as 50% in some developing countries as South and Central America, Africa and Asia (Shirley *et al*., 2018). Factors as illiteracy, poverty, low socio-economic standards including bad sanitation, improper water supply, and overcrowding contribute positively to the increased rates of transmission of the parasite and disease (Mehraj *et al*., 2008). The infection usually prevails in two extremes of age: the children and the old individuals (Wang *et al*., 2018a). *Entamoeba histolytica* exists in two forms: the motile and invasive trophozoite and an infective cyst. The trophozoites measure 10-50 micro meter in diameter and contain a single nucleus whereas; the cysts are 10-15 micrometer in diameter and contains four nuclei when matured. Cysts are resistant to acidification, chlorination and desiccation, and capable of surviving in a moist environment for several weeks. Cysts are spread via the ingestion of faecally contaminated food or water (Menkir and Ayenew, 2013).

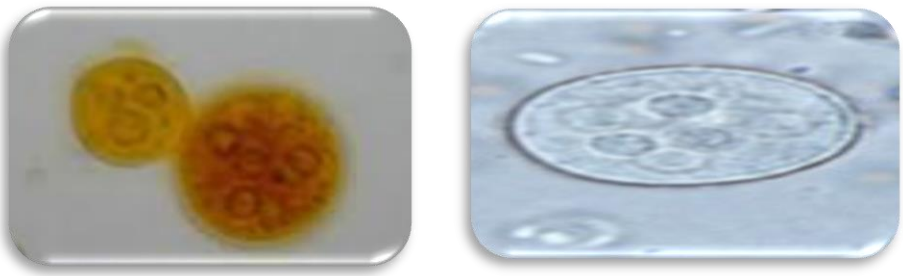


Figure (2.11): *Entamoeba histolytica* trophozoites and cyst (Alam, 2018).

C.2 *Giardia lamblia*

Giardia lamblia Figure (2.12) is a major cause of diarrhea in humans (Esteghamati *et al*., 2019), is a flagellate protozoan with worldwide distribution causes significant gastrointestinal diseases in a wide variety of vertebrates (Khabnadideh *et al*., 2007). *G. lamblia* is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries (Yusuf *et al*., 2007). *G. lamblia* is considered to be one of the leading causative agents of diarrhea in both children and adults. Giardiasis (disease caused by *Giardia lamblia*) occurs worldwide and may infect up to a third of the population in developing countries. Approximately about 200 million of people in the world are with clinically manifested giardiasis, with 500,000 new cases per year (Ahmed, 2016). Giardia exists in two forms: the trophozoite and the cyst forms. The trophozoite is bilaterally symmetrical; each structure being paired, roughly pear shaped. Body length ranges from 9 to 21µm and width from 5 to 15 µm (Ahmed, 2016). The anterior portion of the ventral surface is modified to form a sucking disk, which serves for attachment of organism, two nuclei lie within sucking disk. Two curved rods known as median bodies, axonemes which divide the body into two-halves throughout most of its length. Trophozoite has characteristic falling leaf motility observed in wet preparation (House, 2012). The cyst is oval and a range from 8 to 17µm by 7to 10 µm, mature cyst contains four nuclei, median bodies and longitudinal fibers. The cyst characterized by clear zone between the cytoplasm and the cell wall (Anandakumar and Thajuddin, 2013).

Figure (2.12): *Giardia lamblia* cyst and trophozoite (Ahmed, 2016)**.**

D. Antibiotics used for antiprotozoal

D.1 Metronidazole (Flagyl)

Metronidazole ($C_6H_9N_3O_3$) (Figure 2.13) was introduced in the mid-1950s by Rhone-Poulenc under the brand name Flagyl. It was the first drug of the group that is now called nitroimidazoles. Flagyl was first introduced as a drug in the treatment of trichomonas vaginalis, a sexually transmitted disease, and it revolutionized as therapy for that condition. In 1964, a dentist noted that patients with gingivitis treated with Flagyl for *Trichomonas vaginalis* were cured and the second major indication was then established. Flagyl was also found useful in the treatment of protozoan parasite *Giardia lamblia* and in the treatment of *Entamoeba histolytica* during the late 1960s and 1970s. In the early 1970s, it was found that Flagyl was very active against the obligate anaerobes of which the two best-known families are bacteroides and clostridia. Flagyl is regarded as the gold standard for treating these infections. The exquisite anaerobic

activity of this drug makes it exceedingly effective against anaerobic bacteria. Metronidazole exerts its effect on bacteria by inhibiting microbial RNA synthesis. The drug is active against almost all strict anaerobes including bacteroides, eubacterium, tusobacterium, and peptostreptococcus species. The drug is indicated in the treatment of acute necrotizing ulcerative gingivitis and for moderate to severe odontogenic infections, frequently in combination with penicillin's (Musher *et al*., 2007, Ellison, 2009).

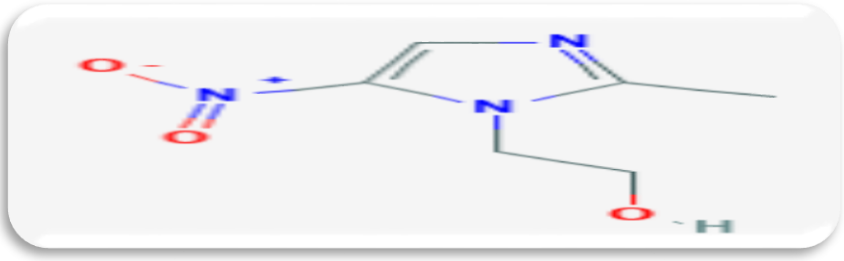


Figure (2.13): Chemical Structure of Metronidazole (Klimenko *et al*., 2019).

2.4.3 Anti-Schistosoma activity

Schistosomiasis is the second most prevalent parasitic disease in the world ranked only after malaria regarding the number of people infected and those at risk. Schistosomiasis is an important parasitic disease in the tropics with strong impact on the socio- economic development of affected regions (Mari *et al*., 2017). The control is mainly with chemotherapy (Mäder *et al.*, 2018). Schistosomiasis is the most common parasite disease transmitted through contact with fresh water (Gaspard *et al*., 2020). It is endemic in more than 70 Low income countries where it occurs in rural areas and the fringes of cities (Amin and Abubaker, 2017). Over 650 million people globally are at risk of infection, with more than 200 million people infected. Of these, 120 million are estimated to have symptoms, with 20 million people experiencing serious consequences (La Hoz *et al.*, 2019), with more than 90% of the cases occurring in Africa (Organization, 2014). The annual number of death resulting from schistosomiasis in sub-Saharan Africa may be as high as 200,000 (Abudho *et al*., 2018, Mari *et al.*, 2017). The disease possesses a threat to more than 600 million in more than 76 countries (Alabi *et al*., 2018). According to previous estimates, the disease causes annual loss of 1.7 - 4.5 million disability- adjusted life years (Finkelstein *et al*., 2008). Most of the present schistosomiasis burden is concentrated in sub-Saharan Africa with the highest prevalence and infection intensity usually found in school-age children, adolescents and young adults (Hotez and Kamath, 2009). Thus Schistosomiasis negatively has impacts on school performance and the debilitation caused by untreated infections undermines social and economic development in heavily affected areas (Makaula *et al*., 2014).

A. Molluscicidal Activity of Medicinal Plants

Many community-based programmes depend on Praziquantel for treating patients with schistosomiasis and other fluke infections. There is however, loss of Praziquantel efficacy (King *et al*., 2000), and its inaccessibility (Mwonga, 2014), drug resistance and high cost requiring hard currency (Sturrock *et al*.,

2011), which set back helminthes control efforts. There is the need to screen local plants as other alternative source of Schistosoma chemotherapeutic agents. This claim is due to the frequent use of plants by most people in the third world countries in primary health care (Adamu *et al*., 2006). Plants are known to offer excellent perspective for the discovery of new therapeutic products (Lahlou, 2007), whose subsequent development may lead to discovery of a safe and therapeutically effective form of useful drugs (Adamu *et al*., 2006). In several trials using different plants, promising anti-Schistosoma compounds were reported (Mantawy *et al*., 2011, de Moraes *et al*., 2011).

B. Molluscicidal Activity of Some Sudanese Medicinal Plants

Eisa *et al*. (2019) performed to study the molluscicidal activity of aqueous and ethanolic extracts of *Khaya grandifoliola* bark against fresh water snails. The results showed that the aqueous extracts of *Khaya grandifoliola* barks possess100% molluscicidal property at concentration of 1 g/l.

Abdelmageed *et al*. (2018) investigated the molluscicidal activity of the herb *Pulicaria crispa* against the freshwater snails *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni* parasites that cause intestinal schistosomiasis. Results revealed that leaves were the most potent against the snails (having the least LC_{50} and LC_{90} values) for all exposure periods (24, 48, and 96 hours), followed by flowers, and finally the stems. With regard to the effect of exposure time, it was evident that the prolongation of exposure period resulted in more potency of plant leaves against the snails as shown by the decreased LC_{50} and LC_{90} values for 96 hours exposure period, followed by 48 hours, and finally 24 hours. During the first five hours of exposure, snails showed sluggish activity, meanwhile dead snail remained inside their shells with blood around the shells. Abdelsalam *et al*. (2016) investigated the molluscicidal activity of *Acacia seyal* bark methanolic extract against the snail *Biomphlaria pfeiffer* with various concentrations. *A. seyal* bark methanolic extract exhibited 100% mortality within 24 h, only at a concentration of 1000 ppm; and exhibited 100% mortality within 48 h, at 1000, 500, 250, 125 and 62.5 ppm. The IC_{50} of bark of *A. seyal* at 24 h and 48 h was 80.79 and 34.33 ppm, respectively.

Alsadeg *et al*. (2015) investigated the the effect of the methanol stem bark extract of *A. senegal* against *F. gigantica*. The methanol extract exhibited significant antihelminthic activity with 100% mortality at 1000 and 500 ppm 6 and 12 hours after parasite exposure to the extract. The lower concentration (250 ppm) gave 100% mortality 24 hours.

Ahmed *et al*. (2014) evaluated the molluscicidal activity of aqueous leaf extract of *Solenostemma argel* (Del Hayne) plant against adults of *Biomphalaria pfeifferi* snails under laboratory conditions. The values of the lethal concentration LD_{50} and LD_{95} were found to be 0.103 and 0.187 ppm respectively. Bashir and El Shafie (2013) Jatropha seed oil concentrations of 5%, 10%, 15% and 20% were tested for biological activity against the third nymphal instar of the desert locust, *S. gregaria*. All tested concentrations caused significant ($p< 0.05$) mortality in the experimental insects ranged from 22.4% to 59.2% after 7 days of application. The LD50 values for treated nymphs at 48, 72, and 96 hrs. were 3.12%, 6.57% and 9.85%

respectively. Oil concentration of 10% has resulted in a delay of the development time from the 5th to 6th nymphal instar by 5 days, where treated nymphs had completed development in 16.50 days compared with only 11.33 days in the untreated control group and 12.00 days in the group treated with hexane. The same concentration (10%) also significantly reduced the per cent of egg hatch. The concentration of 5% caused a significant, 50% more, antifeedant effect on the treated nymphs as compared to the untreated control group.

Osman *et al*. (2012) presented work on seeds of *Peganum harmala* were extracted by distilled water and methanol. The extracts were tested as anti-cercaria *in vitro* which were collected from the natural infected snails with *schistosoma mansoni* cercaria. All concentrations of 250 ppm up to 1000 ppm showed 100% mortality rate during the first half an hour of exposure for all used extracts. serial dilution concentrations of both water and methanolic extracts (125/67/35 and 15ppm) were tested and found to exhibit different mortality rates with an LCR 50R 291.27, 111.05,64.74 and47.95 ppm for the water extract and 97.92,53.49 and 26.95 methanolic extract successfully for the diluted concentrations of (125, 62, 31 and 15 ppm). On the other hand the methanolic extract showed a100% mortality rates after 3 hours of exposure for the whole concentrations.

Rawi *et al*. (2011) work on the evaluation of toxicological, biological and physiological effects of water suspension, cold water and boiled water extracts of *Agave filifera* whole plant, *Ammi majus* flowers and leaves, and *Canna indica* flowers and leaves comparing with the effect of different sulphate compounds. The sub lethal doses (LC_{10}) of copper sulphate and water suspension of the tested plants reduced the total protein and total lipid contents of the hemolymph of *B. alexandrina* snails. EL-Kamali *et al*. (2010) evaluated the molluscicidal activity of the volatile oils of *Cymbopogon nervatus* and *Boswellia papyrifera* against the snails *Biomphalaria pfeifferi* and *Bulinus truncatus* under laboratory conditions. It is evident from the present results that *C. nervatus* (LD_{50} against *B. pfeifferi* = 213.099 ppm; LD_{50} against *B. truncates* = 237.33 ppm) and *B. papyrifera* (LD_{50} against *B. pfeifferi* = 213.31 ppm; LD_{50} against *B. truncates* = 311.05 ppm) essential oils are potential molluscicidal agents.

C. Schistosoma under study

C.1 *Biomphalaria pfeifferi*

Schistosomiasis, one of the most common neglected tropical diseases (NTDs), is caused by parasitic trematode worms of the genus Schistosoma (da Paixão Siqueira *et al*., 2017). Three medically important species known to infect humans are *Schistosoma mansoni* and *S. japonicum*, which cause intestinal schistosomiasis, and *S. haematobium*, which causes urogenital schistosomiasis (Sady *et al*., 2015). Almost 240 million people are infected worldwide and about 700 million people are at risk of this infection (Platt *et al*., 2016). The genus *Biomphalaria* is well known because of the role some species have in the transmission of schistosomiasis (Perera De Puga, 1996, Cong, 1998), which is considered to be one of the most important tropical diseases in the world (Stothard *et al*., 2009). Throughout sub-Saharan

Africa, *Biomphalaria pfeifferi* snails are freshwater intermediate hosts for *Schistosoma mansoni* blood flukes, which cause intestinal schistosomiasis. *Schistosomiasis mansoni* is a debilitating and sometimes fatal disease that affects many individuals in Africa and Brazil (Song *et al*., 2016). Currently available diagnostic methods are not sensitive for patients with low parasite load which leads to underreported cases (Kayuni *et al*., 2019). The selection of target diagnostic antigen candidates is a promising tool for the development of a new and more sensitive assay (Cai *et al*., 2017). *S. mansoni* migrate through the bloodstream, rest in the portal vein where they mature into adult worms, pair, and migrate into the mesenteric veins (Ogongo *et al*., 2018). They might remain in the mesenteric veins for years and produce a large amount of eggs, which deposit in the liver, intestines, and other organs. The eggs are associated with an intense, granulomatous response from the host (Costain *et al*., 2018).

The colonic complications of *S. mansoni* infection are related to the deposition of viable eggs in the terminal venules of the colonic mucosa (Schwartz and Fallon, 2018). The descending colon is principally involveendoscopic features including oedema, hyperaemia, punctuate haemorrhage, granularity, and focal shallow ulcers. In chronic infection, there might be multiple, inflammatory pseudopolyps, largely in the rectum and sigmoid colon, which are composed of ova and marked granulomatous inflammatory reaction products. These areas also show mucosal thickening and luminal narrowing (Lauwers *et al*., 2018).

D. Antibiotic used

D.1 Praziquantel

Chemotherapy is considered as a general strategy for control of schistosomiasis. In this regard praziquantel ($C_{19}H_{24}N_2O_2$) (Figure 2.14) that kills the larval and adult worms is the drug of choice in treatment of schistosomiasis (Thétiot-Laurent *et al*., 2013). But the limited access to praziquantel continues to be the major constraint to controlling the disease (Tchuenté *et al*., 2017). On other hand the recent reports on praziquantel elucidate its fail to stop reinfection as a result of development of drug resistant schistosoma, beside it induces the hemorrhages in the lung tissues of the host as well as abdominal pain and diarrhea by long term application of the drug (Aly, 2012).

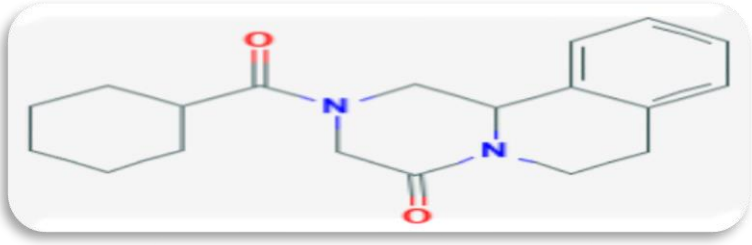


Figure (2.14): Chemical Structure of Praziquantel

2.4.4 Antioxidant Activity

Antioxidants are a class of chemical substances naturally found in our food which can prevents or reduce the oxidative stress of the physiological system. The body is constantly producing free radicals due to regular use of oxygen. These free radicals are responsible for the cell damage in the body and contribute to various kinds of health problems, such as heart disease, diabetes, macular degeneration, and cancer. Antioxidants being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals (Lobo *et al*., 2010, Sen *et al*., 2010, Nimse and Pal, 2015, Engwa, 2018). Antioxidants compounds are important in the prevention of human diseases. And may function as free radical scavengers, complexing agents for pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Gülçin *et al*., 2010, Fiedor and Burda, 2014, Kasote *et al*., 2015). Antioxidants are often used in oils and fatty foods to retard their autoxidation; therefore, the importance of search for natural antioxidants has greatly increased in the recent years (Parsaeimehr *et al*., 2010).

A. Antioxidant Activity of Medicinal Plants

Medicinal plants used in the traditional medicine are well-known significant sources of natural antioxidants. Medicinal plants-derived natural antioxidants, which are in the form of raw extracts and/or chemical constituents, are very efficient to block the process of oxidation by neutralizing free radicals (Tungmunnithum *et al*., 2018). It is also commonly accepted that medicines taken from plant products are safer than their synthetic counterparts; however, the toxicity profile of most medicinal plants have not been comprehensively assessed (Rosenbaum *et al*., 2012)**.**

A wide range of diversity of naturally occurring antioxidants are found in medicinal plants which are different in their composition, physical and chemical properties and site of action. Among these, phenolics and flavonoids are reported powerful anti-oxidants and have been consistently protective through scavenging numerous diverse reactive oxygen species, including hydroxyl radical, peroxyl radical, hypochlorous acids, superoxide anion, and peroxynitrite in various *in-vitro* cellular model (de Oliveira *et al*., 2018). Similarly, antioxidant activity of polyphenols in cardiovascular diseases, hepatoprotective, antiocarcinogenic, antimicrobial, antiviral, and anti-inflammatory effects are well investigated (Bhatt *et al*., 2013).

B. Antioxidant Activity of Some Sudanese Medicinal Plants

Hussein and Hamad (2021) determined the preliminary phytochemical component, the antioxidant and antibacterial activity of the leaves and bark extracts of *Ziziphus spina-christi* (L.) (Rhamnaceae) against two clinical isolates (*Staphylococcus aureus* and *Escherichia coli* species) using the standard method of analysis. The result of antibacterial activity showed that the bark ethanolic extract exhibit a higher zone of inhibition against all the clinical isolates; *Escherichia coli* species showed zones of inhibition of 22 mm followed by *Staphylococcus aureus* 15 mm. The antioxidant activity of the leaves and bark extracts was evaluated using the standard 2, 2 diphenyl-1- picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the

leaves water and ethanolic extracts was 30 ± 0.05 and 91 ± 0.02 respectively and the antioxidant activity of the bark water and ethanolic extracts was 44 ± 0.03 and 70 ± 0.02 respectively.

Hagr *et al*. (2019) examined phytochemical analysis of *A. squmosa* ethanolic extracts and investigating the chemical constituent's and to assess it potential antibacterial and antioxidant activities of Sudanese *A. squmosa* seeds oil. The results showed that Alkaloids, Flavonoid, Carbohydrate, Saponins, Triterpene, Streol, Tannins and phenolic compounds were present in the *A. squmosa* seeds ethanolic extracts where GC-MS analysis detected Twenty-one components in the *A. squmosa* seeds oil. Five of them are major namely, Hexadecanoic acid (18.48), Heptadecanoic acid Methyl margarate (1.32%), 9,1 2-octadecadienoic acid (Z, Z) (19.29%), 9 - octadecenoic acid (Z) (34.01%), Methyl stearate (17.12%), Cis-11-Eicosenoic acid (1.14%) and Eicosanoic acid (3.28%.). The DPPH assay, showed moderate antioxidant potential (50 ± 0.09 compared with standard 93± 0.01; the antibacterial showed high inhibitory effect against *Pseudomonas aeruginosa* (16 mm), *Bacillus subtilis* (15 mm), *Candida albicans* (15 mm) moderate against *Escherichia coli* (13mm) and *Candida albicans*.

Adam *et al*. (2018) determined antioxidant activity by measuring the radical scavenging effects against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and N,N-dimethyl-p-phenylendiamine (DMPD), metal-chelation capacity, ferric-reducing (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) methods using ELISA microtiter assays. *Geranium favosum* followed by *Kalanchoe glaucescens*, *Malva parviflora*, *Aizoon canariense* and *Coleus barbatus*, respectively, possessed the highest antioxidant activity among the studied plants. *Chrozophora oblongifolia* and *K. glaucescens* exerted considerable cytotoxicity against CCRF-CEM leukemia cells.

Awadelkarim *et al*. (2017) investigated the antioxidant activity of *Solenostemma argel*, *Commiphora myrrha* and *Vernonia amygdaline*. The highest chelating ability was showed by the petroleum ether extract with 60%.

Mohamed *et al*. (2016) investigated the antioxidant and phytochemical screening of secondary metabolites of a combination of *Solanum dubium* L. powdered seeds and Sudanese honey. The water extract of the seeds and honey combination showed the highest radical scavenging activity (RSA) 91±0.04%, according to the DPPH assay and the control propyl gallate gave RSA 93±0.01%.

Khiralla *et al*. (2015) evaluate the total phenolic content and total antioxidant capacity of ethyl acetate extracts of 21 endophytic fungi isolated from five Sudanese medicinal plants: *Calotropis procera*, *Catharanthus roseus*, *Euphorbia prostrate*, *Vernonia amygdalina* and *Trigonella foenum-graecum*. Among the endophytes, endophytic fungus *Aspergillus sp*. from *Trigonella foenum-graecum* seeds demonstrated the highest both total phenolic content in term of gallic acid equivalent [(89.9 ± 7.1) mg GAE/g] and antioxidant activity for 1,1-diphenyl2-picrylhydrazyl radical scavenging assay [IC_{50}: (18.0 ± 0.1) mg/mL]. A high positive linear correlation (R2 = 0.999 1) was found between total antioxidant capacity and total phenolic content of endophytic fungi isolated from *Vernonia amygdalina*.

Osman *et al*. (2014) screened the crude extracts of some Sudanese medicinal plants; *Combretum hartmannianum* (leaves), *Hydnora abyssinica* (rhizome), *Striga hermonthica* (whole plant), *Ficus vasta* (leaves), *Guiera senegalensis* (leaves) to determine their active chemical constituents using conventional chemical tests (precipitation and color reagents) where applicable. All the tested plants showed the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and sterols.

Elnour *et al*. (2013) investigated the antioxidant and cytotoxic activities of six Sudanese medicinal plants commonly used as anti-inflammatory. All the extracts showed strong anti-DPPH with IC_{50} values 0.49, 4.74, 9.91, 2.14, 17.19 and 4.24 μg/ml respectively, the inhibition percentage of propyl gallate levels was found 88 % at concentrations 0.5 mM. The inhibition percentage of radical scavenging activity against iron metal chelating 38.44, 26.88, 8.28, 17.05, 15.23, 27.73 % for all of them at concentration 50 μg/ml respectively.

Abdallah *et al*. (2012) screened for antimicrobial, antioxidant and some active phytochemical compounds. The extract showed the presence of saponins, terpenoids, phenolic compounds and alkaloids. The methanolic extract of the fruits extracted from *B. aegyptiaca* exhibited a strong antioxidant activity in the DPPH assay and a potent capacity in preventing linoleic acid oxidation. Methanol extracts, particularly at concentration of 100 mg/ml was found to be active against all bacterial and fungal strains and it was comparable to standard antibiotics Gentamicin and Amphotericin B.

Idris *et al*. (2011) evaluated physicochemical properties of antioxidant activities and phenolic contents of seven types of Sudanese honeys derived from flowers of *Acacia nilotica, Acacia seyal, Azadirachta indica, Cucurbita maxima, Balanites aegyptiaca,* and two *Ziziphus spina christi* plant species. The moisture content was in the range from 16.2 to 21.3, g/100 g honey, ash content from 0.121 to 1.205 g/100 g honey, nitrogen from 0.032 to 0.046 g/100 g honey while the corresponding protein content was 0.200 to 0.286 g/100 g honey. Total phenolic content varied from 4.44 to 201.08 mg/100 g honey as gallic acid equivalent. The values of the antioxidant activities were in a range from 3177 to 6247 μg for the lC_{50}.

Fawole *et al*. (2010) investigated medicinal and therapeutic potentials of traditional medicinal plants. Plant extracts were evaluated for anti-inflammatory activity and other pharmacological properties such as anticholinesterase and antioxidant activities. Phytochemical analysis of total phenolic contents, condensed tannins, gallotannins and flavonoids in the aqueous methanol extracts of the medicinal plants were also carried out. At the screening assay concentration (0.25 μg/μl), 13 extracts showed good COX-1 inhibitory activity (>50%), while good activity was observed in 15 extracts against COX-2 enzyme. All the extracts of *Crinum moorei* (bulbs) showed good inhibition against both COX-1 and COX-2 enzymes. Though not significantly different (P = 0.05), the highest COX-1 percentage inhibition (100%) was shown by Aloe ferox leaf PE and *Colocasia antiquorum* tuber DCM extracts, while *Colocasia antiquorum* tuber PE extract exhibited the highest (92.7%) percentage inhibition against COX-2. *Crinum moorei* bulb DCM extract

showed the lowest EC_{50} value (2.9 µg/ml) in the AChE assay. In addition, good to moderate bioactivities were observed in some extracts of *Aloe ferox* (leaves), *Crinum moorei* (bulbs) and *Pycnostachys reticulata* (leaves) in all the assays.

C. Free radicals:

Free radicals Figure (2.15) are essentials to any biochemical progression and signify a fundamental part of aerobic life and metabolism. It may be defined as any molecular variety with an unpaired electron in an outer atomic orbit which is capable of autonomous survival and the presence of such unpaired electron may result in definite familiar properties shared by most radicals (Halliwell, 2011). Most of the radicals are extremely reactive and are unstable and can donate or accept an electron from other neighbouring molecule, for that reason acting as oxidants or reductants (Bhattacharya, 2015). In general, free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds (Kumar and Pandey, 2015). They may be either reactive oxygen species (ROS) i.e. oxygen derived or reactive nitrogen species (RNS) i.e. nitrogen derived (Kapoor *et al*., 2019). Oxygen derived molecules include O_2 - (superoxide), ROO (peroxyl), HO_2 (hydroperoxyl), HO (hydroxyl), RO (alkoxyl) as free radical and H_2O_2 oxygen as non-radical. Nitrogen derived free radical mostly involves NO (nitric oxide), NO_2 (nitrogen dioxide), N_2O_3 (dinitrogen trioxide) and ONOO (peroxy nitrate) (Devasagayam, and Kesavan, 1996). Most of the diseases are chiefly linked to oxidative stress caused by free radicals (Augusto and Miyamoto, 2011).

Free radicals are concerned in the development of a wide range of chaos in humans, such as cell death, cardiovascular diseases, tissue damage, ischemic heart diseases, cancer, atherosclerosis, central nervous system injury, neural disorders, reperfusion injuries of many tissues, inflammation, obesity, gastritis, arthritis, and ageing (Caicedo *et al*., 2018). The human body showed complicated antioxidant defensive mechanisms in the form of natural enzymatic and non-enzymatic processes which neutralize the detrimental effects of free radicals and other oxidants (Nimse and Pal, 2015).

Recently it has been reported that naturally obtained antioxidants from plants defend from noxious and detrimental effects of free radicals and showed broad range of pharmacological consequences such as antimutagenic, antimicrobial, antiallergic, anticarcinogenic, antioxidant free radical scavenging actions and antidiabetic (Rahman, 2012, Petruk *et al*., 2018).

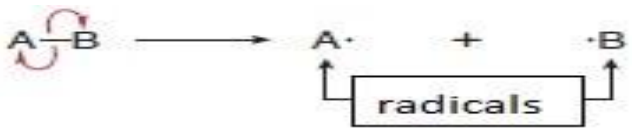


Figure (2.15): Free radicals (Severino *et al*., 2009).

D. DPPH Scavenging Activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picrylhydrazyl; DPPH) Figure (2.16) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The

delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. According to Alam *et al*. (2013) the sample extract (0.2 ml) is diluted with methanol and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\%\ \text{inhibition of } \mathbf{DPPH} \text{ radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100$$

Where A_{br} is the absorbance before reaction and $\boldsymbol{A_{ar}}$ is the absorbance after reaction has taken place.

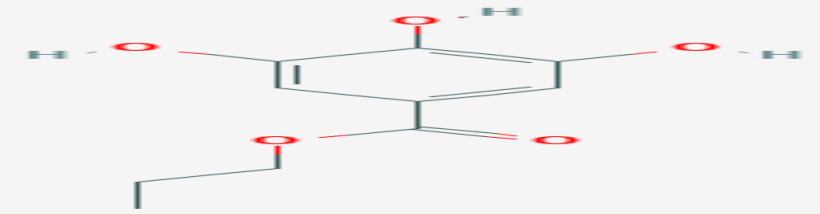


Figure (2.16): Structure of stable DPPH free radical (Severino *et al*., 2009).

E. Standard for antioxidant activity

E.1 Propyl gallate

Propyl gallate ($C_{10}H_{12}O_5$) (PG, propyl 3, 4, 5-trihydroxybenzoate) Figure (2.17) is used as antioxidant in processed food, cosmetics and food packing materials in order to prevent rancidity and spoilage. According to the US Food and Drug Administration list, PG is also used to preserve and stabilize medicinal preparations (Bouaziz *et al*., 2010). It is an allowed additive in the European Union and in many others countries. Because of its prevalent usage, the potential toxicity of PG has been investigated *in-vivo* (Carocho *et al*., 2014, Additives and Food, 2014) and *in-vitro* to assess various toxicological properties, i.e. mutagenicity (Fowler *et al*., 2012) and cytogenetic effects (Han *et al*., 2010). Although PG has a low toxicity, it has various effects on tissue and cell functions.

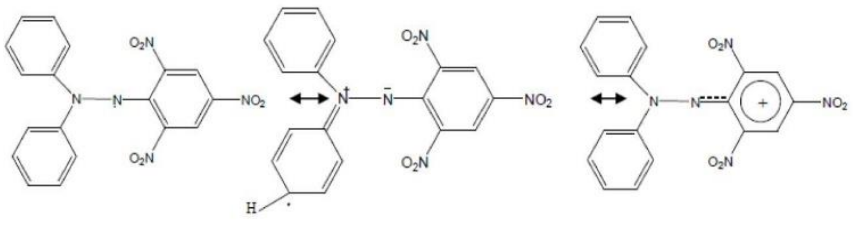


Figure (2.17): Chemical Structure of Propyl gallate (Nguyen *et al*., 2021).

2.5 Toxicity Studies

Medicinal plants are rich in active phytochemical compounds with various biological activities. Researchers are highly interested in studying plants with the aim of isolating novel active drugs to replace synthetic drugs present in the market (Süntar, 2019). The availability of these plants constituents provides

a source of natural drugs for modern medicine (Hosseinzadeh *et al*., 2015). Hence, cytotoxic level of medicinal plants must also be evaluated against host cells. The safety of plants as a potential therapeutically agents must be ascertained and the side effects should be acceptable to the host. Bioactive compounds with no or less toxic effect to the host are the good candidates for formulation of drugs (Nemudzivhadi and Masoko, 2014). The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant. Various assays are used for the research of potential toxicity of herbal extracts based on different biological models, such as *in vivo* assays on laboratory animals. However, recent studies employed efforts for alternative biological assays that include species of *Artemia salina, Artemia franciscana, Artemia urmiana* and *Thamnocephalus platyurus*. These toxicity tests are considered a useful tool for preliminary assessment of toxicity (Daniel and Ibok, 2019).

2.5.1 Toxicity of Medicinal Plants

Although plant extracts have been used in the treatment of diseases over centuries, recent scientific research has shown the presence of potentially toxic and mutagenic substances in some useful medicinal plants (Mensah *et al*., 2019). It has been demonstrated that several potential side effects such as allergic reactions, cramps, diarrhea, fever, gastrointestinal disturbances, headaches, hematuria and vomiting may be experienced when administering some types of traditional medicine (Bernstein *et al*., 2020). The use of medicinal plants can have deleterious effects on health has been reported (Mounanga *et al*., 2015). Indeed, of about 1,500,000 plants investigated, most of them contain toxic substances (Alexander *et al*., 2019) like some secondary metabolites. Plants produce a variety of secondary metabolites that are sub-divided by chemists into several classes based on their molecular structure. Among secondary metabolites are saponins, terpenoids, cyanogenic, tannins, toxic amino acids, glycosides, alkaloids (Vardhan and Shukla, 2017). It has been shown that the toxicity of a given plant depends on various factors, including the strength of secondary metabolites, the quantity consumed, the time of expo-sure, different parts of the plant (root, oil, leaves, stem bark and seeds), individual body chemistry, climate and soil, and genetic differences within the species (Ifeoma and Oluwakanyinsola, 2013). This said clearly shows that medicinal plants should be used with precautions and toxicology studies conducted to increase the knowledge on the plant or plants preparation given to populations (Kahraman *et al*., 2020).

2.5.2 Toxicity of Sudanese Medicinal Plants

Elnour *et al*. (2018) investigated the prostate cancer, antioxidant and Cytotoxicity activities of some Sudanese medicinal plants. The extract *A. Arabica* gum and leaves has shown very high activity against-PC3 and the extract *C. cajan* leaves and *T. Foenum graecum* L and *A. mellifera* leaves is shown none active antiPC3with IC_{50} values 39.4, 64.9, > 100,> 100 and > 100µg/ml respectively. All the extract revealed Cytotoxicity activity against Vero cell line were found of no toxic and the inhibition percentage with (87.3,

71.7, 66.6) (-48.03,-46.2, 3.7) respectively. The extract shown very high activity against DPPH (above 80%) *A. mellifera* leaves 87.64µg/ml.

Elnour *et al*. (2017) investigated the anticancer, antioxidant and Cytotoxicity activities of four Sudanese Medicinal Plants commonly used as anti-inflammatory and anti-tumor. The extract of *Prosopis juliflora* (SW.) DC has shown very high activity against-PC3 and *Hibiscus sabdariffa* has shown very high activity against-PC3 the *Halexylon salicornietum* and *Sonchus oleraceus* L extracts showed none active anti- PC3 with IC_{50} values 30.1, 94.7, >100, and >100 µg/ml respectively. All the extracts revealed cytotoxicity activity against Vero cell line except last concentration in extract of *Sonchus oleraceus* L, and the inhibition percentage with (90.56, 87.12, 86.24) (82.78, 82.31, 77.38) (75.21, 59.49, 41.24) (74.93, 73.78, 71.13) respectively. The extracts of *Hibiscus sabdariffa, Halexylon salicornietum* and *Sonchus oleraceus* L. are revealed low active against Chemiluminescence assay, *Prosopis juliflora* (SW) DC is revealed high active against Chemiluminescence assay with IC_{50} values 166.6, 189.5, >176.2 and 75.4 µg/ml respectively.

Kabbashi *et al*. (2016) investigated the *in-vitro* antigiardial activity and cytotoxicity (MTT assay) of ethanol extract of *A. nilotica* subsp. nilotica (bark). *A. nilotica* bark ethanolic extract exhibited 100% mortality within 96 h, at a concentration of 500 ppm; this was compared with Metronidazole which gave 96% inhibition at the concentration of 312.5 µg/ml at the same time. In addition cytotoxicity (MTT-assay) verified the safety of the examined extract with an IC_{50} less than 100 µg/ml.

Saeed *et al*. (2015) aimed to identify plant extracts and isolated compounds thereof with activity towards otherwise drug-resistant tumor cells. The results of the resazurin assay on different tumors showed that *Lawsonia inermis, Trigonella foenum-graecum* and *Ambrosia maritma* were the most active crude extracts. Ambrosin was selected as one active principle of *A. maritima* for microarray-based expression profiling. Genes from various functional groups (transcriptional regulators, signal transduction, membrane transporters, cytoskeleton organization, chaperones, immune system development and DNA repair) were significantly correlated with response of tumor cell lines to ambrosin.

Hassan *et al*. (2014) work evaluated the antioxidant, antiangiogenic and cytotoxic activities of six Sudanese medicinal plants which have been traditionally used to treat neoplasia. The results indicated that out of 6 plants tested, 4 plants (*Nicotiana glauca*, *Tephrosia apollinea*, *Combretum hartmannianum* and *Tamarix nilotica*) exhibited remarkable anti-angiogenic activity by inhibiting the sprouting of microvessels more than 60%. However, the most potent antiangiogenic effect was recorded by ethanol extract of *T. apollinea* (94.62%). In addition, the plants exhibited significant antiproliferative effects against human breast (MCF-7) and colon (HCT 116) cancer cells while being non-cytotoxic to the tested normal cells. The IC_{50} values determined for *C. hartmannianum, N. gluaca* and *T. apollinea* against MCF-7 cells were 8.48, 10.78 and 29.36 µg/ml, respectively. Whereas, the IC_{50} values estimated for *N. gluaca, T. apollinea* and *C. hartmannianum* against HCT 116 cells were 5.4, 20.2 and 27.2 µg/ml, respectively. These results were more or less equal to the standard reference drugs, tamoxifen (IC_{50} = 6.67 µg/ml) and 5-fluorouracil (IC_{50} =

3.9 µg/ml) tested against MCF-7 and HCT 116, respectively. Extracts of *C. hartmannianum* bark and *N. glauca* leaves demonstrated potent antioxidant effect with IC_{50}s range from 9.4–22.4 and 13.4–30 µg/ml, respectively. Extracts of *N. glauca* leaves and *T. apollinea* aerial parts demonstrated high amount of flavonoids range from 57.6–88.1 and 10.7–78 mg quercetin equivalent/g, respectively.

Elnour *et al*. (2013) investigated antioxidant and cytotoxic activities of six Sudanese medicinal plants commonly used as anti-inflammatory. All the above extracts showed strong anti-DPPH with IC_{50} values 0.49, 4.74, 9.91, 2.14, 17.19 and 4.24 µg/ml respectively, the inhibition percentage of propyl galate levels was found 88 % at concentrations 0.5 mM. The inhibition percentage of radical scavenging activity against iron metal chelating 38.44, 26.88, 8.28, 17.05, 15.23, 27.73 % for all of them at concentration 50 µg/ml respectively. None of the above mentioned extracts revealed cytotoxic activity against Vero cell line.

Gadir (2012) presented study, ethanolic extracts of some medicinal plants screened for their cytotoxicity using brine shrimp lethality test. Out of the 25 plants tested, *Azadrichta indica* (w.p.) LC_{50} 45 ppm, (L) LC_{50} 21; *Aristolochia bracteolatea* (w.p.) with LC_{50}. 50 ppm; (Sd.) LC_{50} 185; (Aristolochic acid, LC_{50} 19 ppm; while *Savadoora persica*, (L.), (Salvadoraceae) and *Ocimum basilicum*; (Labiatae); show no toxicity. The present study supports the previous that brine shrimp bioassay is simple, reliable, and convenient method for assessment of bioactivity of medicinal plants and leads support for their use in traditional medicine.

2.5.3 Toxicity and Bioassay

A. Cytotoxicity Study using Brine Shrimp Lethality Test (BSLT)

During the past 30 years, the Brine Shrimp Assay has been widely used to test the toxicity of a great variety of plant products. Brine shrimp (*A. salina*) is most extensively studied of the *Artemia* species, estimated to represent over 90% of the studies in which *Artemia* is used as an experimental test organism (Hamidi *et al*., 2014), Figure (2.18). The Brine Shrimp Toxicity Assay was proposed and developed by Michael *et al*. (1956) and later adapted by Vanhaecke *et al*. (1981), Meyer *et al*. (1982b), and Sleet and Brendel (1983). In the current study, Brine Shrimp and cell line were used as testing organism to evaluate the cytotoxic effect of the plant extracts.

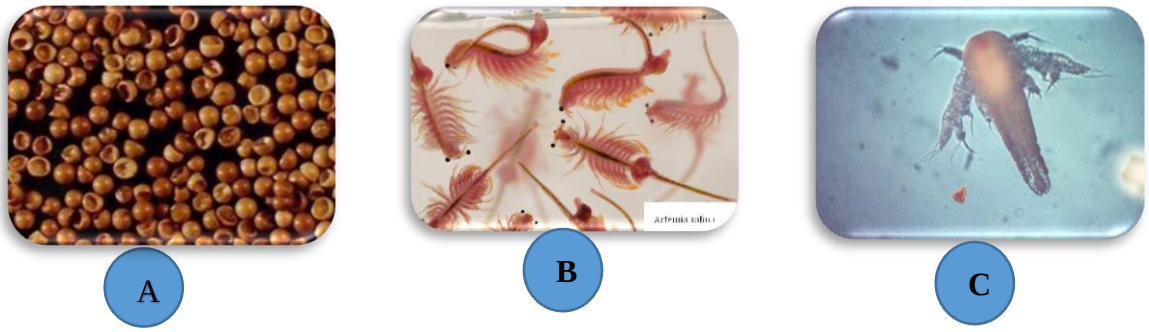


Figure (2.18): (A): Cysts of *Artemia*, (B): Hatched *Artemia nauplius* and (C): Adult *Artemia salina*.

A.1 Testing organism

Scientific name: *Artemia salina*

Common name: Brine shrimp

A.2 Scientific classification

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Branchiopoda

Order: Anostraca

Family: Artemiidae

Genus: *Artemia*

Species: *A. salina*

A.3 Brine Shrimp lethality Description

The technique is economic and utilizes small amount of test material (Pisutthanan *et al*., 2013). Since its introduction, this *in vivo* test has been successively employed for bioassay-guide fractionation of active cytotoxic and antitumor agents (Hamidi *et al*., 2014). Additionally, several studies demonstrated that there is a good correlation between the results for the lethal concentration that kills 50% of the exposed population (LC_{50}) obtained with the Brine Shrimp Lethality Assay using *A. salina* and the results of the Acute Oral Toxicity Assay in Mice Assay in Mice (Lawi *et al*., 2018). This assay was developed by Meyer *et al*. (1982b) and is widely used as a simple, reliable and cheaper prescreens method to determine the cytotoxicity of crude plant extract and pure natural compounds, especially antitumor compounds from the natural source (Hullatti and Murthy, 2010). Bioactive compounds are often toxic to shrimp larvae (*Artemia salina*); therefore, Brine Shrimp Lethality Assay is in use to monitor different chemicals' *in-vivo* lethality to shrimp larvae (Ohikhena *et al*., 2016, Lalisan Jeda *et al*., 2014).

The general toxic activity was considered weak when the LD_{50} values of crude extracts and pure substances were between 500 and 1000 µg/ml, moderate when the LD_{50} was between 100and 500 µg/ml, and designated as strong when the LD_{50} ranged from 0 to 100 µg/ml but those with <20 µg/ml were considered to be very active (Padmaja *et al*., 2002)., while Meyer *et al*. (1982b) considered the LD_{50} values > 1000 µg/ml as non-toxic.

B. Cytotoxicity study using MTT-assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) Figure (2.19) assay has become the gold standard for determination of cell viability and proliferation since its development by Mosmann in the 1980′s . This assay measures cell viability in terms of reductive activity as enzymatic conversion of the tetrazolium compound to water insoluble formazan crystals by dehydrogenases occurring in the mitochondria of living cells although reducing agents and enzymes located in other organelles such

as the endoplasmic reticulum are also involved (Lü *et al*., 2012, Stockert *et al*., 2012). The increased sensitivity of the assay and its potential as a miniaturised high-throughput assay made it a breakthrough in cell enumeration technology by replacing the radioactive isotope based ^{3}H-thymidine incorporation assay. Initially, the method involved no wash steps, but called for the solubilisation of the formazan crystals in acid-isopropanol, a time-consuming procedure (Mosmann, 1983). However several modifications, including the addition of DMF to solubilise the formazan in aqueous medium or removing excess dye with gentle aspiration and washing with PBS followed by solubilising the formazan crystals in DMSO (Van Rensburg *et al*., 1997) improved the simplicity and sensitivity of this assay. Several tetrazolium-based assays, such as the XTT (Scudiero *et al*., 1988), MTS (Khabar *et al*., 1996) and WST (Peskin and Winterbourn, 2000) assays, in which water soluble formazan products are generated, eliminating the need for washing and solvent solubilisation steps, have been developed but have not replaced the well-established MTT assay.

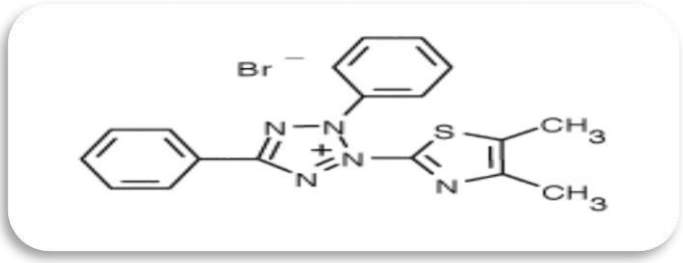


Figure (2.19): Chemical Structure of MTT (Lin *et al*., 2019).

2.6 Extraction process and phytochemical analyses

2.6.1 Extraction process

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plants and animals tissues using selective solvents through standard procedures. The purpose of standardized extraction procedures for crude drugs from medicinal plant parts is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent (Hijazi *et al*., 2015). The extract obtained may be used as medicinal agents in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsule (Handa, 2008). The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, deoctition, hot extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave assist extraction, ultrasound extraction (sonication), super critical fluid extraction, and phytonic extraction with the hydro fluorocarbon solvents. For aromatic plants, hydro distillation techniques (water distillation, steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction can be employed (Sahoo, 2012). Some of the new extraction methods for aromatic plants are head space trapping, solid phase micro extraction, protoplast extraction, microdistillation, thermomicro distillation and molecular distillation (Handa, 2008). The basic parameters enhancing the quality of an extracts are plant

part used as starting material, solvent used for extraction and the extraction procedure (Pandey and Tripathi, 2014).

Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing. In order to obtain bioactive compounds from plants, the existing classical techniques are: (1) Soxhlet extraction, (2) Maceration and (2) Hydro distillation.

Extraction efficiency of any conventional method mainly depends on the choice of solvents (Azmir *et al*., 2013). The polarity of the targeted compound is the most important factor for solvent choice. Molecular affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and financial feasibility should also be considered in selection of solvent for bioactive compound extraction (Shuai and Luterbacher, 2016). Some examples of bioactive compound extracted using different solvents are given in Table (2.1).

Table (2.1): Example of some extracted bioactive compounds by different solvents (adapted from (Cowan, 1999):

Solvents						
Water	**Ethanol**	**Methanol**	**Chloroform**	**Dichloromethane**	**Ether**	**Acetone**
Anthocyanins	Tannins	Anthocyanin	Terpenoids	Terpenoids	Alkaloids	Flavonoids
Tannins	Polyphenols	Terpenoids	Flavonoids		Terpenoids	
Saponins	Flavonol	Saponins				
Terpenoids	Terpenoids	Tannins				
	Alkaloids	Flavones				
		Polyphenols				

2.6.2 Phytochemicals analyses

Phytochemicals Table (2.2) of plants extracts depends on nature of the plant material, its origin, degree of processing, moisture content and particle size. The variation of different extraction methodology that will affect quantity and secondary metabolite composition of an extract, depends upon type and time of extraction, temperature, nature of solvent, solvent concentration and Polarity (Ncube *et al*., 2008). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the procedure of extraction. The solvent should be nontoxic and should not interfere with the bioassay. The choice will also depend upon the targeted compounds to be extracted (Azmir *et al*., 2013).

Table (2.2): Structural features and activities of various phytochemicals from plants (Bhattacharya *et al*., 2018, Gulfraz *et al*., 2011**)**:

Phytochemicals	Structural features	Example (s)	Activities
Phenols and Polyphenols	C3 side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antioxidant, antimicrobial, anthelmintic and antidiarrheal activities.
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antioxidant, antimicrobial, anthelmintic, antidiarrheal, wound healing, antiviral and anti-tumor activities.
Flavonoids	Phenolic structure, containing 15 carbon atoms arranged in a C6–C3–C6 configuration	Abyssinone, Chrysin, Quercetin, Rutin	Antioxidant, antimicrobial, antidiarrheal, anti-inflammatory, antiallergenic, anti-viral and anti-(heart diseases) activities.
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial activity.
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine,	Antimicrobial, anthelmintic and antidiarrheal activities.
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin	Antimicrobial, antidiarrheal and anti-(lipid peroxidation) activities.
Saponins	Amphipathic glycosides	Vina ginsenosides-R5 and -R6	Antidiarrheal, anti-diabetic, anti-fungal, antioxidant, anticancer, anti-inflammatory and antimicrobial activities.

A. Primary and Secondary Metabolites

Metabolites are intermediates in metabolic processes in nature and are usually small molecules: A primary metabolite is directly involved in normal growth, development, and reproduction, for example, fermentation products (ethanol, acetic acid, citric and lactic acid) and cell constituents (lipids, vitamins, and polysaccharides) (Rose, 2012). A secondary metabolite is not directly involved in those processes and usually has a function but is not that important for the organism (antibiotics, pigments, and carotenoids) (Luckner, 2013).

B. Role and classification of secondary metabolites

Secondary metabolites are organic compounds that do not play any role in the normal growth and development of plants. The production of secondary metabolites normally emanates from the maximum level during the transition from active growth to stationary phase (Tiwari and Rana, 2015). The organism that produces this secondary metabolite normally grows without their production, thus, the secondary metabolism in the organism may not be significant, yet, is pivotal for short survival of the organism (Bills and Gloer, 2017). There are numerous protective roles that these secondary metabolites provide, for example, they act as free radical scavenging, antioxidants, UV-light absorbing, and antiproliferation agents (Nowruzi *et al*., 2020). They also protect plants and defend them from harmful pathogens, microorganisms

such as bacteria, fungi and viruses (López-Ráez *et al*., 2017). This was supported by the fact that some plants manufacture these chemicals as part of their defence system. For instance, phytoalexins are produced by plants when they are attacked by bacteria and fungi, hence, their antibacterial and antifungal properties (Reichling, 2018).

There are various classes of secondary metabolites present in plants; they include phenolics, flavonoids, steroids, terpenoids and alkaloids. The different categories in which the secondary metabolites belong to share a distinct structure, their derivatives are made up of structural units or some composed of complex molecules that are compiled by large numbers of simple molecules (Bartwal *et al*., 2013). The following sections reflect a brief account on some of the classes known.

B.1 Phenolic compounds

Phenolics are known to be the largest category of phytochemicals and cosmopolitan across the plant kingdom with about 10.000 structures being identified (Kang *et al*., 2019). Some of the phenolics are soluble in organic solvents and water which are known as carboxylic acid and glycosides; while others are large, insoluble polymers (Ruesgas-Ramón *et al*., 2017). Phenolic are aromatic compounds with hydroxyl substitutions, having a phenolic parent compound, but most are more complex polyphenolic compounds classified by the number of carbon atoms in the basic skeleton. Only plants and microorganisms are capable of synthesizing the aromatic nucleus. Plant phenolic arise from two main biosynthetic pathways: (1) via shikimic acid (benzoic acid derivatives, lignans, coumarins, etc.) and through acetate, leading to polyketides which upon cyclization can produce xanthones and quinines (Vermerris and Nicholson, 2007).

All phenols (including vitamin E) inhibit oxidation donating a hydrogen atom from the OH group to a radical which form a new resonance-stabilized radical (Fuhrman *et al*., 2000). This new radical does not participate in chain propagation, but rather terminates the chain and halts the oxidation process.

Phenolic acids have various biological and pharmacological effects, although their roles in plants is still unknown, and they have been connected with diverse functions, including protein synthesis and enzyme activity (Quideau *et al*., 2011). They were found to play an important role in the natural host defense mechanism of plants against infectious diseases and inhibit multiplication of plant pathogenic bacteria, viruses, and fungi. The phenolic phytochemicals play a significant role as defense compounds; manifest several properties that are beneficial to humans. Their antioxidant properties are one of the most important properties that determine their roles as protecting agents against free radical mediated disease processes (Soto-Vaca *et al*., 2012).

B.2 Flavonoids compounds

Flavonoids constitute one of the largest and popular group of phytochemicals (Somani *et al*., 2015). They are consisting of units derived from both shikimic acid and polyketide pathways (Stocker-Wörgötter, 2008). Until now, more than 4000 flavonoids have been identified and the number is constantly growing due to

the great structural diversity arising from the various hydroxylation, metoxylation, glycosylation, and acylation patterns (Medi and Jasprica, 2008). Most frequently encountered groups of flavonoid aglycones include flavones, flavonols, anthocyanidins, isoflavones, flavanones, dihydroflavonols, biflavonoids, chalcones, and aurones (Corradini *et al*., 2011). Flavonoid aglycones possess the chemical properties of phenolics, and thus they are slightly acidic.

Flavonoids have important roles in plant physiology and are components of the diet of numerous herbivores and omnivores, including humans. This group of compounds exhibit an extraordinary array of biochemical and pharmacological activities in mammalian systems, such as anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, antimicrobial, and antiviral (Ginwala *et al*., 2019, Wang *et al*., 2019, Kong *et al*., 2003) With the increased popularity and use of herbal medicines containing flavonoids, the question of composition determination and standardization arises. To compare biological activities of plant extracts, it is important to analyze the chemical constituents using the appropriate analytical tools (Waksmundzka-Hajnos *et al*., 2008).

B.3 Tannins compounds

Tannins are polyphenols sometimes called plant polyphenols (Okuda and Ito, 2011), although originally the name tannin was given to the plant extracts exhibiting astringency, without knowing their chemical structures. The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former (Shahat and Marzouk, 2013), binding to proteins, basic compounds, pigments, large-molecular compounds and metallic ions, and also anti-oxidant activities, etc. Tannin-rich plant materials have an important traditional medicine (Guanghou, 2004), for ages they have been used in the external treatment of skin and mucosa diseases such as inflammations of different etiologies, haemorrhages or haemorrhoidal disease, mostly because of the observed anti-inflammatory, wound healing and antibacterial activity (Piwowarski *et al*., 2011).

2.6.3 Methods of isolation, purification and identification of phytochemicals

Plant extracts contains various type of bioactive compounds having different polarities, their separation still remains a big challenge for the process of isolation, purification, identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds using different separation techniques such as TLC, HPTLC, paper chromatography, column chromatography, Gas chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity (Sasidharan *et al*., 2011).

A. Chromatography techniques

Chromatography is a technique where the molecules are separated based on their size, shape and charge (Krull *et al*., 1997). During chromatography analytic in solvent and move through solid phase that acts as a sieving material, as molecule proceeds further through molecular sieve it gets separated. Paper and thin

layer chromatography are the chromatographic techniques which readily provides qualitative information and through which it becomes possible to obtain quantitative data.

B. Paper chromatography

In paper chromatography a sheet of paper is used for the inert phase. One of the advantages of paper chromatography is that separations are carried out simply on sheets of filter paper, which acts as both support as well as medium for separation (Harborne, 1998). Another advantage is the considerable reproducibility of R_f (Retention factor) values determine on paper. In paper chromatography, filter paper used as solid phase, which is inert phase. A sample is placed near the bottom of the filter paper. Then this filter paper is placed in chromatographic chamber with solvent. The solvent move forwards by capillary action carrying soluble molecules along with it. Low porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity (Sherma, 1971).

C. Thin layer chromatography (TLC)

The first practical application of thin layer chromatography was given by Ashworth and Stahl (2013). Compared to paper chromatography, the special advantage of TLC is the versatility, speedy and sensitive. TLC is an adsorption chromatography (Hahn-Deinstrop, 2007) where samples are separated based on the interaction between a thin layers of adsorbent attached on the plate. The technique mostly employed for the separation of low molecular weight compounds. Different adsorbent used to separate various compounds.

D. Column chromatography (CC)

Column chromatography involves ion exchange, molecular sieves, and adsorption phenomenon. The flushing in conventional chromatography greatly dilutes the material, and the fractions usually require another step for concentration. A newer method called displacement chromatography elute with some compounds that has great affinity for the adsorbent. Fractions of elute materials can be more concentrated than the original solution applied to column.

E. Gas chromatography (GC)

Gas chromatography is a method for the separation of volatile compounds (Ingle *et al*., 2017). In this method, species distribute between gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate (Burchfield and Storrs, 1962). Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

F. High performance liquid chromatography (HPLC)

HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. (Ingle *et al*., 2017). The another name for HPLC is high–pressure liquid chromatography, separates compounds on the basis of their interactions with solid particles of tightly packed column and the solvent of the mobile phase. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent. High pressure up to 400 bars is required to elute the analyses through column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analyses to aid in their identification. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature, and it provides a good complement to gas chromatography for detection of compounds (Ingle *et al*., 2017).

G. High performance thin layer chromatography (HPTLC)

High performance thin layer chromatography (HPTLC) is a planar chromatography where separation of sample components is achieved on high performance layers with detection and data acquisition. These high performance layers are pre-coated plates coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns. The reduction in thickness of layer and particle size results in increasing the plate efficiency as well as nature of separation. HPTLC gives chromatogram i.e. separated samples after chromatography can be inspected by the eyes only in case of HPTLC (Srivastava, 2010).

2.6.4 Methods of identification of compounds

A. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two dimensional NMR techniques. Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabeled (Sherma, 1971) C-NMR is used to identify the types of carbon are present in the compound. H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

B. Mass spectrometry (MS)

Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. Through MS spectrum the molecular weight of sample can be determined. This method mostly employed for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously (De Vijlder et al., 2018).

2.7 Plants used in this study

Plants selected in this study were reported to have different families and classes of chemical compounds, some of were these compounds have many medicinal applications. Pharmacological screening of these plants followed by bioactivity-guided fractionation might lead to identification of promising bioactive compounds. The extracts, fractions and compounds were screened for biological activities and chemical analysis of some selected medicinal plants.

2.7.1 Anacardiaceae family

Anacardiaceae is one of the largest families of Sapindales and comprise 82 genera and ~800 species distributed mainly in tropical areas (Schulze-Kaysers *et al*., 2015). Members of the family are cultivated throughout the world for their edible fruits and seeds, medicinal compounds, valuable timber, and landscape appeal (Pell, 2004). Some of the products of Anacardiaceae, including mango (*Mangifera indica*), pistachio (*Pistacia vera*), cashew (*Anacardium occidentale*), and pink peppercorn (*Schinus terebinthifolia*), are enjoyed worldwide while other notables such as the pantropical Spondias fruits, the marula of Africa (*Sclerocarya birrea*), and the Neotropical fruits of Antrocaryon are restricted to local cultivation and consumption and are not generally transported far distances to larger markets (Wahid, 2012).

A. *Mangifera indica* L.

Mangifera indica L. (Mango) is a member of an Anacardiaceae family. It is one of the most popular fruits produced in tropical region of the world (Azim *et al*., 2014, Ayala-Silva *et al*., 2005). About more than 1000 varieties have been identified all over the world (Viruel et al., 2005, Jahurul *et al*., 2015). The annual world production is estimated to around 20 million tonnes. In Sudan, mango is considered as an essential horticultural crop, with about 57 varieties and around 0.4 million tonnes annual production (Nour *et al*., 2011).

A.1 Scientific classification of *Mangifera indica* (Parvez, 2016).

Kingdom	Plantae
Class	Magnoliopsida
Phylum	Mangoliophyta
Order	Sapindales
Family	Anacardiaceae
Genus	*Mangifera*
Species	*Mangifera indica*
Vernacular name	Mango

Figure (2.20): Seeds of *Mangifera indica*.

A.2 Botanical Description of *Mangifera indica*

Tree: The tree is medium to large (10-40 m in height), evergreen with symmetrical, rounded canopy ranging from low and dense to upright and open (Parvez, 2016). Bark: The bark is usually dark grey-brown to black, rather smooth, superficially cracked or inconspicuously fissured, peeling off in irregular, rather thick pieces. The tree forms a long unbranched long tap root (up to 6-8 m and more) plus a dense mass of superficial feeder roots. Effective root system of an 18- year old mango tree may observe a 1.2 m depth with lateral spread as far as 7.5m (Ali, 2016). Leaves: The leaves are simple alternately arranged, 15-45 cm in length. The petiole varies in length from 1 to 12 cm, always swollen at the base. Leaves are variable in shapes like oval-lanceolate, lanceolate, oblong, linear-oblong, ovate, obovate-lanceolate or roundish oblong (Parvez, 2016). The upper surface is shining and dark green while the lower is glabrous light green. Fruit: The fruit is more or less compressed, fleshy drupe, varies considerably in size, shape, colour, presence of fiber, flavour, taste and several other characters (Dar *et al*., 2016). Roots: The tree forms a long unbranched long tap root (up to 6-8 m and more) plus a dense mass of superficial roots. Effective root system of an 18- year old mango tree may reach a 1.2 m depth with lateral spread as far as 7.5m (Parvez, 2016).

A.3 Distribution of *Mangifera indica*

The mango is native to southern Asia, especially Burma and eastern India, and it spreads early on to Malaya, eastern Asia and eastern Africa (Fuller, 2011). Mangoes are grown throughout the tropics, from the Caribbean to Africa, South-East Asia, Australia, as well as India, where the history of the fruit goes back over 6,000 years and closely connected to the Hindu religion. As long ago as the 16th century, mangoes had been distributed via cultivation throughout the Indian subcontinent, and eventually to all tropical regions of the world (Dudley *et al*., 2010).

A.4 Distribution of *Mangifera indica* from Sudan

The mango distribution from Northern state, Khartoum state, Gezera state, River Nile state, Darfur state, Blue Nile state, Southern state, Nubba Mountains and South of Blue Nile state (Mohammed, 2014).

A.5 Ethno medicine uses of *Mangifera indica*

Various parts of mango are used for more than thousands of years as wide variety of ethno medicinal use (Khoo *et al*., 2016). Roots and Bark: Used as astringent, acrid, refrigerant, styptic, anti-syphilitic, vulnerary, anti-emetic, anti-inflammatory and constipating (Tarranum *et al*., 2015). They are useful in vitiated conditions of pitta, metrorrhagia, calonorrhagia, pneumorrhagia, leucorrhoea, syphilis, uteritis, wounds, ulcers and vomiting. The juice of fresh bark has a marked action on mucous membranes, in menorrhoea, leucorrhoca, bleeding piles and diarrhea (Tarranum *et al*., 2015). Paste of Mango roots applied on palms and soles cures fever. Paste of root helpful in healing of mouth wound (Sharma and Kumar, 2015). Leaves: Used as astringent, refrigerant styptic, vulnerary and constipating. They are also useful in vitiated conditions of cough, hiccup, hyperdipsia, burning sensation, hemorrhages, haemoptysis, haemorrhoids, wounds, ulcers, diarrhoea, dysentery, pharyngopathy, scorpion string and stomachopathy. The ash of burnt

leaves are useful in burns and scalds, and the smoke from burning leaves is inhaled for relief of throat diseases (Parvez, 2016). Flowers: Used as astringent, refrigerant, styptic, vulnerary, constipating and haematinic. The dried flowers are useful in vitiated conditions of pitta, haemorrhages, haemoptysis, wounds, ulcers, anorexia, dyspepsia, uroedema gleet, catarrh of bladder, diarrhoea, chronic dysentery and anemia (Parvez, 2016). Dried mango flowers serve as astringents in cases of diarrhea, chronic dysentery, and Powder help to reduce allergy (dermatitis) (Khan *et al*., 2017). Fruits: The unripe fruits are acidic, acrid, antiscorbutic, refrigerant, digestive and carminative (Divya, 2015). They are useful in dysentery ophthalmic, eruptions, urethrorrhoea and vaginopathy. The ripe fruits are refrigerant, sweet, emollient, laxative, cardiotonic, haemostatic, aphrodisiac, and tonic. They are also used in vitiated conditions vata and pitta, anorexia, dyspepsia, cardiopathy, haemoptysis, haemorrhages from uterus, lungs and intestine, emaciation, and anemia (Parvez, 2016). Also help to prevent colon cancer, calming inflammation, fruit Juice is act as a restorative tonic used in heat stroke (Khan *et al*., 2017). Stone: The seed kernel is rich source of protein (8.5%) and gallic acid, and is sweet, acrid, astringent, refrigerant, anthelmintic, constipating, haemostatic, vulnerary and uterine tonic. It is useful in vitiated conditions of pitta and cough, helminthiasis, chronic diarrhea, dysentery, haemorrhages, haemoptysis, haemorrhoids, ulcers, bruises, leucorrhoea, menorrhagia, diabetes, heat burn and vomiting (Parvez, 2016). Seeds: Seed kernel in hemorrhages and bleeding hemorrhoids, seed can also applied on burn, to treat Asthma (Khan *et al*., 2017). Stem and Bark: Aqueous extract traditionally used for the treatment of, syphilis, anemia, scabies, diabetes, cutaneous infections, menorrhagia, diarrhea. Bark infusion has been use as remedy for mouth infections in children (Scartezzini and Speroni, 2000).

A.6 Chemical constituents of *Mangifera indica*

The different chemical constituents of the plant, especially the polyphenolics, flavonoids, triterpenoids. Mangiferin a xanthone glycoside major bio-active constituent, isomangiferin, tannins & gallic acid derivatives (Nwoke *et al*., 2016). Bark: The bark of mango is reported to contain protocatechic acid, catechin, mangiferin, alanine, glycine, γ-aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (E)- en-20S,26-diol, C-24 epimers of cycloart-25 en 3β,24,27-triol and cycloartan-3β,24,27-triol (Kalita, 2021). Flower: The flower of mango contains alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propyl gallate, n-pentyl gallate, n-octyl gallate, 4-phenyl gallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid (Nwoke *et al*., 2016). Root: The root of mango contains the chromones, 3-hydroxy-2-(4'-methylbenzoyl)- chromone and 3-methoxy-2-(4'-methyl benzoyl)- chromone.An essential oil containing humulene, elemene, ocimene, linalool, nerol and many others have also been found in the leaves and flowers. The fruit pulp contains vitamins A and C, β- carotene and xanthophylls (Nwoke *et al*., 2016). Also, an unusual fatty acid, cis-9, cis-15-octadecadienoic acid has been isolated from the pulp lipids of mango. The most interesting constituents of *Mangifera indica* however are the polyphenolics, flavonoids, triterpenoids (Ali, 2016). Mangiferin (a xanthone glycoside and major

bio-active constituent), isomangiferin, tannins and gallic acid derivatives. Seeds: The seeds of mango contains the Polyphenols such quercetin, Kaempferol, gallicacid, tannin, xanthone (Shibahara *et al*., 1993). Leaves: Protocatechic acid, catechin, mangiferin, alanine, glycine, kinic acid, shikimic acid , tetracyclic triterpenoids cycloart-24-en-3β,26 diol, 3- ketodammar-24(E)-en-20S,26- diol (Shah *et al*., 2010). Fruits: The fruits of mango contains the Mangiferin, cis-9, cis-15-octadecadienoic acid, Xanthophyll esters, carotenes, and tocopherols (Khan and Khan, 1989).

A.7 Biological Activity of *Mangifera indica*

A number of *in-vitro* and *in-vivo* studies have been carried out to reveal various pharmacological potentials of *M. indica*. Different parts of *M. indica* trees have been demonstrated to exert antibacterial, antifungal, antiprotozoal, anthelmintic antioxidant and other effects (Ediriweera *et al*., 2017). Many of these pharmacological studies on different parts (as organic extracts or decoctions) of *M. indica* trees have been carried out to validate the ethnomedical uses of the plant in traditional medicine in the treatment of several diseases and conditions. Some experimentally proven pharmacological properties of different parts of *M. indica* trees have been described in detail in the following section.

A.7.1 Antibacterial activity of *Mangifera indica*

Manzur *et al*. (2020) evaluated antibacterial effectiveness of aqueous and ethanolic leaf extracts of *Mangifera indica* L. as a natural alternative for reduction of *Staphylococcus spp*. biofilms. The ethanolic extract was the most effective, showing minimum inhibitory concentrations and minimum bactericidal concentrations between 1.8–7.5 and 15.1–45.3 mg/ml, respectively. After tannin removal, the extracts were less effective, indicating these secondary metabolites as one of the main antibacterial compounds. The ethanolic extract at 45.3 mg/ml reduced mature biofilms in teat rubber (average: 3.91 log reduction) and stainless steel (average: 3.87 log reduction) after 5 min of contact. The extracts of *M. indica* leaves represent natural alternatives against *Staphylococcus spp*. strains and the ethanolic extract shows potential as a natural sanitizer.

Raju *et al*. (2019) collected 15 local cultivars of *Mangifera indica* L. with a motto to screen the best cultivar having high total phenolic content, flavonoid content, antitumor property and antimicrobial activity. Banganapalli cultivar of mango showed high total phenolic content and total flavonoid content i.e. 63.5±1.1 mg GAE/g and 16.7±0.5 mg quercetin/g followed by Royal special cultivar (TPC-58.7±0.6 mg GAE/g TFC - 16.2±0.6 mg quercetin/g). *Mangifera indica* L. cultivar Banganapalli which showed highest total phenolic content and total flavonoid content was screened for its antitumor and antimicrobial properties. Antitumor property was tested by using potato disc assay which recorded 40.12% tumor inhibition. Antimicrobial activity was assessed by agar diffusion method by taking 3 test microorganisms' viz. *Bacillus subtilis* subsp. subtilis DSM 10, *Staphylococcus aureus* MTCC 737 and *Escherichia coli* MTCC 46. The measured area of inhibition is around *Staphylococcus aureus* MTCC 737 in 8.5±0.3 mm followed by *E. coli* MTCC 46 (8.2±0.3 mm) and *Bacillus subtilis* sub subtilis (6.6±0.5 mm).

Olasehinde *et al*. (2018) investigated the phytochemical composition and antimicrobial activities of aqueous and ethanolic extracts of leaves of *Mangifera indica*. Standard methods were employed to screen the phytochemicals. *S. aureus* showed highest sensitivity to the aqueous extracts with MIC 31.25mg/mL. Least sensitivity was observed in *K. pneumoniae* and *Candida albicans* with MIC 125mg/mL each in the two extracts. *M. indica* exhibited significant antimicrobial activity comparable to gentamicin which is used as control in this study.

Taddaow *et al*. (2018) studied the antibacterial activity of methanolic and aqueous extracts from the leaves of three *M. indica* varieties. The results from the disc diffusion assay showed that leaf extracts from all tested *M. indica* varieties could inhibit the growth of *Staphylococcus epidermidis*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Propionibacterium acnes* and *Pseudomonas aeruginosa*. Among those, the methanolic extracts of *M. indica* leaves had the strongest inhibitory effects. Based on the broth dilution assay, the methanolic extract of MN had the greatest antibacterial effect. The minimal inhibitory concentration (MIC) values were 3.91, 7.81, 15.63, 62.5 and 125 mg/ml for *S. epidermidis*, *S. aureus*, MRSA, *P. acnes* and *Ps. aeruginosa* and the minimal bactericidal concentration (MBC) values were 7.81, 15.63, 31.25, 125 and 250 mg/ml, respectively.

Kamath and Ramakrishna (2017) studied the antibacterial activity of ethanolic extract of *Tectona grandis* (teak), *Mangifera indica* (mango) and *Anacardium Occidentale* (cashew) leaves. Antibacterial activity was observed in the concentration range of 25-100 mg/ml for all the extracts except *T. grandis* leaves. That are effective at 50-100 mg/ml concentration.

Verma *et al*. (2015) identified the antibacterial activity, minimum inhibitory concentration and phytochemical content of *Mangifera indica* flower extract against six bacterial strains. The methanolic extract showed a maximum zone of inhibition (23±1.00 mm) against *S. typhi* and minimum zone of inhibition (13.00±1.21mm) with ethanolic extract against *Staphylococcus aureus*. Further, the ethanolic extract observed in maximum (21±1.00 mm) against *S. typhi* and minimum (15±0.57mm) zone of inhibition against *Staphylococcus aureus*.

Ashok *et al*. (2014) studied the plant active components of *M. indica* bark were extracted using four different extraction solvents namely distilled water, ethanol, methanol and ethyl acetate. In the present study bark powder extract of *M. indica* was studied by using 100%, 50%, 25%, 12.5% and 6.25% dilution of four different solvents against the tested pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The ethyl acetate extract (100%) was found to be most effective against *Klebsiella pneumoniae* (28 mm) followed by *Bacillus subtilis* (25 mm), *Pseudomonas aeruginosa* (22mm), *Staphylococcus aureus* and *Escherichia coli* (20 mm each). Distilled water extract of *Mangifera indica* bark was not effective for the entire tested pathogens.

De and Pal (2014) evaluated the effects of aqueous young leaves extract against different Gram (-) microorganisms causing gastro-intestinal disorders. The growths of all the tested organisms are inhibited

and the growth inhibition is dose dependent. Hence it could be concluded that the aqueous young leaves extract of *Mangifera indica* could be utilized in the management of gastro-intestinal disorders and its effect could be potentiating as required.

Talba *et al*. (2014) determined the phytoconstituents of *Mangifera indica* kernel and its antibacterial activity against *Aeromonas caviae*. Methanol extract of mango kernel was used to identify secondary metabolites of mango kernel. Methanol extract used at different concentrations showed varying degrees of inhibition against *A. caviae* (ranging from 16 ± 2.41 mm to 24 ± 0.58 mm) except that the aqueous extract was found to be slightly active against the organism at lower concentrations with zones of inhibition ranging from 8 ± 1.22 mm to 11 ± 1.23 mm with measurable zones of inhibition at higher concentrations.

Poongothai and Rajan (2013) determined the antibacterial activity of *Mangifera indica* flower extracts. The results showed that the flower extracts of *M. indica* have antimicrobial activity against Uropathogenic *E. coli*. Methanol extracts showed the highest inhibition zone diameter of 22.6 ± 1.2 mm. The plant extracts were shown to have a MIC 180 ± 050 µg/ml for aqueous extract and 055 ± 025 µg/ml for methanolic extract. Phytochemical screening of the extracts revealed the presence of phenolic compounds, flavonoids and tannins in both the extracts tested, which are known to inhibit bacterial growth by different mechanisms from those of synthetic drugs.

Mushore and Matuvhunye (2013) determined the antibacterial activity of *Mangifera indica* stem bark extracts. The results showed that the stem-bark extracts of *M. indica* have antimicrobial activity against *S. aureus*. Methanol extracts showed the highest inhibition zone diameter of 25 mm, followed by ethyl acetate, water and hexane extracts with inhibition zone diameter of 22 mm, 14 mm and 10 mm, respectively. The antibacterial activities of different extracts were found to be concentration dependent, in agar and broth dilution methods. The plant extracts were shown to have a MIC range of 0.62 mg/ml to 4.17 mg/ml, in agar dilution method. Results from the broth dilution method had a MIC range of 0.16 mg/ml to 1.25 mg/ml. The control (Ampicillin) was however, more effective than plant extracts since only a concentration of 0.03 mg/ml in agar dilution and 0.001 mg/ml in broth dilution method were effective to inhibit the growth of *S. aureus*. The extracts were shown to be bacteriostatic at low concentrations.

Sahrawat *et al*. (2013) studied the antibacterial activities of leaf extract of *Mangifera indica* against bacteria some as *Proteus vulgaris*, *Pseudomonas fluorescens*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhi*. The methanol, ethanol and benzene extract of *M. indica* leaf showed effectiveness against bacterial strains but the benzene extract showed the maximum growth inhibition 85.1% against *Pseudomonas fluorescen*s bacteria at 100µl/ml extract concentration which are drug resistance for Tabramycin, Cephaloridine, Lincomycin, Norfloxacin and Oleandomycin. Minimum inhibitory concentration of benzene extract was determined against *Pseudomonas fluorescens* 50×10-1 µl/ml.

Prakash (2012) examined the potential of mango kernel as natural antibacterial against two bacterial strains: *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The maximum antibacterial activity at 37°C for 24h

shows minimum number of colonies in plates. This finding would probably become an alternative source of new and natural antibacterial agents. A mango kernel extract has a bacteriostatic and antibacterial activity, and thus can be used in food products or cosmetics as a bacteriostatic and antibacterial agent.

Vaghasiya *et al*. (2011) evaluated the antibacterial activity of methanol extract of *Mangifera indica* L. seeds against 41 clinically isolated and 20 standard bacterial strains. The extract showed potent antibacterial activity against all the clinically isolated bacterial strains and most of the standard bacterial strains which was comparable with that of standard antibiotics studied. It can be stated that the antibacterial activity may be due to the presence of tannin and higher amount of total phenol content.

Kaur *et al*. (2010) evaluated the antibacterial activity of the methanolic extract of mango (*Mangifera indica* L.) seed kernel. The crude methanolic extract of mango seed kernel at a concentration of 100 mg/mL is found to have potential antimicrobial activity against MRSA and *E. coli* compared to *V. vulnificus*. Study on the antibacterial activity also indicated that there was no significant difference in the antibacterial activity of the single and assorted mango seed kernel extracts.

A.7.2 Antifungal activity of *Mangifera indica*

Salati *et al*. (2018) investigated the biological synthesis of silver nanoparticles by mango plant extract and its anti-Candida effects. Based on the results, the synthesized nanoparticles were cubic and 65 nm in size. Among the clinical isolates, *C. albicans* with the frequency of 52.38% and *C. glabrata* with the frequency of 4.76% had the highest and lowest frequency among the 21 clinical isolates. *C. glabrata* had the most sensitivity with the quantitative serial dilution method (microdilution) with minimum inhibitory concentration (MIC) of fungal growth (0.016 mg/mL) and the minimum lethal concentration of fungi (0.032 mg/mL) against the nanoparticles of mango core ($P \leq 0.001$).

Fontenelle *et al*. (2017) evaluated the *anti-Candida* spp. activity against strains isolated. The means of inhibition zones were 11 ± 0.71, 13.5 ± 3.54, 10.5 ± 0.71 and 13.5 ± 0.71mm to Tommy Atkins, Rosa, Moscatel and Jasmim varieties, respectively. For Tommy Atkins, the MIC ranged from 0.62 to 1.25 mg/mL; for Rosa, ranged from 0.31 to 1.25 mg/mL; for Jasmim ranged from 0.31 to 0.62 mg/mL; while for the Moscatel variety the MIC value was 1.25 mg/mL for all *Candida* strains.

Dorta *et al*. (2016) investigated the antioxidant and antifungal (anti-yeast) properties of mango (*Mangifera indica*) peel and seed by-products. All mango extracts showed antifungal activity. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) values were lower for seed than for peel extracts. MICs and MFCs ranged from values <0.1 to 5 and 5 to >30 mg GAE/mL, respectively.

Deressa *et al*. (2015) studied the antifungal activity of five invasive alien plant leaf extracts against *Colletotrichum gloeosporioides in-vitro* and *in-vivo*. The study revealed that the inhibitory effect of the extracts depends on the type of plant species used, method of extraction and time of application of the extracts. *Prosopis juliflora* and *Lantana camara* extracts were the most effective plant extracts that significantly reduced radial growth and conidia formation, and reduced disease development on mango

fruits. Thus, *P. juliflora* and *L. camara* extracts can serve as an alternative means of post-harvest mango anthracnose disease management.

Jeyasakthy *et al*. (2013) studied antifungal activity of sequentially extracted different cold organic solvents extracts. Overall, *A. indica* showed higher degree of antifungal activity compared to other tested plants. Hexane extracts of *M. indica* and *L. camara* did not exhibit activity at all. However hexane extracts of *C. alata* and *A. indica* only revealed the antifungal activity even though they had lower activity than the other tested extracts. The inhibitory effect of ethyl acetate of stem bark of *C. alata* on *Pythium sp* was higher (32 mm) than the other extracts after 48 hours. Both ethyl extracts of root bark of *M. indica* and stem bark of *L. camara* revealed significantly highest inhibition on *Alternaria spp*. were 29 mm and 25 mm respectively after 48 hours incubation. Besides this, approximately similar inhibition zone (29 mm) was produced by ethyl acetate extract of root bark of *A. indica* and leaf of *A. indica* against *Pythium sp.* at same concentration. Moreover, this study revealed that the leaf extracts produced prominent inhibitory effect against fungi.

Jain and Nafis (2011) evaluated the *in-vitro* antifungal activity of crude aqueous and methanolic extracts of amchur (dried pulp of unripe *Mangifera indica*) against four *Candida spp*. which are reported to be present in diabetic foot ulcers. The crude extracts exhibited potential antifungal activity. The extracts were most effective against *Candida glabrata* (45-47 mm). The MIC values for crude extract ranged between 0.3 and 0.6 mg/ml. The phytochemical analysis of methanolic extract of amchur revealed the presence of tannins and terpenoids.

Kanwal *et al*. (2010) isolated Five flavonoids, namely (−)-epicatechin-3-O-β-glucopyranoside (1), 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-g]chromene-4(8H)-one (Göksungur et al.), 6-(p-hydroxybenzyl)taxifolin-7-O-β-D-glucoside (tricuspid) (Smith et al.), quercetin-3-O-α-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (4) and (–)-epicatechin(2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (5), from the leaves of mango (*Mangifera indica* L.). All concentrations of the five test flavonoids significantly suppressed fungal growth. However, the specificity of different test compounds was evident against different fungal species. In general, antifungal activity of the flavonoids was gradually increased by increasing their concentrations. The highest concentration (of 1000 ppm) of compounds 1–5 reduced the growth of different target fungal species by 63–97%, 56–96%, 76–99%, 76–98% and 82–96%, respectively.

Islam *et al*. (2010) investigated the analgesic, anti-inflammatory, antibacterial and antifungal properties of ethanol leave extract of *Mangifera indica*. In antibacterial experiment, leaves extract showed 7.0 mm to 11.5 mm in diameter of zone of inhibition against six Gram positive bacteria (such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* and *Lactobacillus vulgaricus*) and two Gram negative bacteria (such as *Shigella flexneri* and *Shigella sonei*). But *Salmonella typhi* and *Proteus sp* had negative activity on leaves extract. The leaves extract of *Mangifera indica* showed

antifungal activity against three fungal species (*Aspergillus ustus*, *Aspergillus niger* and *Aspergillus ochraceus*).

A.7.3 Antiprotozoal activity of *Mangifera indica*

Orabi *et al*. (2020) investigated the anti-*Trichomonas vaginalis* activity of mango's gallotannins, an aqueous ethanol extract of fresh kernels of *M. indica* was phytochemical. They showed structural-dependent inhibitory effects on the growth of *T. vaginalis* trophozoites in an *in vitro* investigation. Ethyl gallate and 1,2,4, 6-tetra-*O*-galloyl-β-D-glucose exhibited elevated anti-*T. vaginalis* activity (IC_{50} = 1.3, 2.4 μg/ml, respectively).

Odediran *et al*. (2014) subjected to three *in-vivo* antimalarial test models using chloroquine-sensitive *Plasmodium berghei berghei* to determine the most active under each of the test models. Using the original formulation, MAMA (1:1:1:1) which gave ED_{50} and ED_{90} of 101.54 ± 2.95 and 227.18 ± 2.95, respectively, as reference for comparison, MAMA-1 (1:2:2:2), with 79.58 ± 1.30 and 170.98 ± 1.30, gave significantly ($p < 0.05$) higher survival at 85 and 340 mg/kg when 80 % of the mice survived for 15.6 and 17.8 days, respectively, while MAMA-2 (2:1:2:2), with 83.57 ± 1.93 and 164.23 ± 1.93, gave comparable survival except at 170 mg/kg with 60 % survivors for 12 days. MAMA-1 and MAMA-2 were the best curative formulations with MAMA-1 giving additional prophylactic activity. MAMA-3 (2:2:2:1) with 98.70 ± 0.91 and 220.17 ± 0.91, gave comparable ($p > 0.05$) survival at 85 mg/kg with 60 % survival for 13.2 days and significantly higher survival at 42.5 mg/kg for 17 days with 40 % survival. Both MAMA and MAMA-3 were the best chemosuppressive formulations plus additional curative activities. MAMA-4 (1:1:2:2), the best prophylactic formulation with 94.87 ± 2.43 and 201.20 ± 2.43 gave significantly higher ($p < 0.05$) survival at all doses except at 21.25 mg/kg which gave 60 % survival up to 10 days.

Ramos *et al*. (2014) tested for leishmanicidal activity against promastigotes forms of *L. amazonensis*, EOMiR and EOMiE showed IC_{50} (72 h) of 39.1 and 23.0 μg/mL, respectively. In macrophages, EOMiR and EOMiE showed CC_{50} of 142.84 and 158.65 μg/mL, respectively. However, both were more specific to the parasite than macrophages, with values of selectivity index of 6.91 for EOMiE and 3.66 for EOMiR. The essential oils were evaluated for their cytotoxicity against the human tumor cells HEp2, HT-29, NCI-H292, and HL-60. The EOMiR and EOMiE were most effective against the HL-60, with IC_{50} values of 12.3 and 3.6 μg/mL, respectively.

Malann *et al*. (2013) evaluated anti-malarial properties of crude leaf extract of *Mangifera indica* and C*asuarina equistifolia* using standard procedures in laboratory mice. The results showed that water extract of *M. indica* displayed a very good activity against *Plasmodium berghei* malaria parasites in a dose dependent fashion with a statistically significant difference ($p < 0.05$) while *C. equistifolia* extract showed no significant difference ($p > 0.05$) at all the dose levels chosen in the suppressive test. Furthermore, in the curative, all the test substances were able to reduce the parasitaemia level with a clear significant difference between the treated groups and the control ($p < 0.05$) although parasites were not totally cleared after

termination of treatment. The repository effect of the test substances produced a significant difference ($p< 0.05$) suppression of parasitaemia, but *C. equistifolia* failed to prove any repository effect as the parasitaemia level increased to 12.40± 1.91 than the control 11.67±1.56. The test extracts did not prevent body weight loss associated with malaria infection as only the 5mg/kg chloroquine phosphate (CQ) group prevented weight loss. Prevention of toxic effect resulting from treatment was determined based on the LD_{50} of the extracts to be 891.25mg/kg *M. indica* and 2884.03mg/kg for *C. equistifolia* inoculated intraperionneally in mice.

Ayoola *et al*. (2008) determined the antioxidant activities of four medicinal plants traditionally used in the treatment of malaria in southwestern Nigeria. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars. *M. indica* did not contain cardiac glycosides and alkaloids while, *P. guajava* also showed the absence of alkaloids and anthraquinones. Anthraquinones was similarly absent from *V. amygdalina*. Concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as 0.04 mg/ml, 0.313 mg/ml, 0.58 mg/ml, 2.30 mg/ml and 0.054 mg/ml for *P. guajava, M. Indica, C. papaya, V. amygdalina* and Vitamin C, respectively.

A.7.4 Anthelminthic activity of *Mangifera indica*

Muhammadi *et al*. (2020) screened the aqueous, methanol and DMSO extracts from leaves of *Mangifera indica* and *Morus alba* for bioactive compounds. All extracts were also caused paralysis followed by subsequent death (13.5/26.87 to 39/60.06mint) of *P. posthuma*, of these methanolic extract from *M. indica* was least than that by albendazole (30.45/52mint) and piperazine citrate (14.35/36mint).

Giovanelli *et al*. (2018) investigated the *in-vitro* anthelmintic activity on sheep gastrointestinal strongyles (GIS) of four plant-derived pure compounds, mangiferin (at 0.25%, 0.125% and 0.0625%), rutin (at 1%, 0.75%, 0.5%), quercetin (at 1%), and β-sitosterol (at 1%, 0.75%, 0.5%). For comparison, untreated and treated (0.1% thiabendazole, 0.1% TBZ) controls were used. Six repetitions were made throughout the experiment. Data were statistically elaborated using the χ^2 test. The concentration able to inhibit the development of the 50% of L1s to L3s and causing the mortality of the 50% of L3s (EC_{50}) was also calculated. L3s recovered from untreated Petri dishes were identified at the genus level. In EHT, all tested compounds at all concentrations significantly ($p < 0.01$) inhibited the hatch of the eggs when compared to the untreated controls, but none of them was as effective as 0.1% TBZ. In LDT, rutin (at 1%, 0.75% and 0.5%), mangiferin (at 0.25% and 0.125%), β-sitosterol (at 1%) and 0.1% TBZ completely prevented the larval development from L1 to L3 in respect to the untreated controls ($p < 0.01$). In LMT, all tested compounds significantly ($p < 0.01$) increased the death of L3s compared to the untreated controls, except for β-sitosterol at 0.5%. However, only rutin at all concentrations and 0.25% and 0.125% mangiferin were as effective as 0.1% TBZ. *Haemonchus*, *Trichostrongylus*, *Chabertia* and *Teladorsagia/Ostertagia* GIS genera, were identified.

Dasrao *et al*. (2014) evaluated anthelmintic activity of methanolic extract *Mangifera indica* (leaves) against Indian earthworm *Phertima posthma*. Methanolic extract of *Mangifera indica* leaves were show concentration dependent anthelmintic activity. Albendazole at concentration 20 mg/ml was used as standard reference drug.

El-Sherbini and Osman (2013) evaluated, extracts of immature fruits of the mango *Mangifera indica* L. for inhibition of larval development. Aqueous extracts of immature fruits at 100 mg ml 1 showed 100 % inhibition of larval development. *In vitro* results indicate that this fruit could assist Strongyloides stercoralis control.

Latha *et al*. (2010) evaluated the crude ethyl acetate and ethanol extracts of *Mangifera indica* L. for anthelmintic activity using adult earthworms, in which *Mangifera indica* root extracts exhibited a dose dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. With higher doses (30 mg/ml of ethyl acetate extract), the effects were comparable with that of 3% piperazine citrate.

A.7.5 Antioxidant activity of *Mangifera indica*

Coelho *et al*. (2019) evaluated bioactive compounds by reversed-phase high-performance liquid chromatography coupled to diode array detection and fluorescence-detection (RP-HPLC/DAD/FD) and *in-vitro* antioxidant activity (AOX), for by-product potential reuse. Alcoholic maceration in wine ethanol (65% *v*/*v*) produced liqueurs with higher phytochemical and AOX content. Maceration with pectinase resulted in liqueurs with higher quercetin-3-*O*-glucopyranoside content. In relation to mango varieties, Haden liqueurs presented higher bioactive content than Tommy Atkins liqueurs. The liqueurs presented high antioxidant activity. The main bioactive compounds found were flavanols (epicatechin-gallate, epigallocatechin-gallate), flavonols (quercetin-3-*O*-glucopyranoside and rutin), and phenolic acids (gallic acid, *o*-coumaric acid, and syringic acid).

Etuh *et al*. (2019) evaluated *in-vivo* antioxidant activity of *Mangifera indica* cold aqueous leaf extract. The % mortality of flies after 5 days showed 32.5%, 0%, 15.5% and 37% in the control (10 g diet with 200 µl of distilled water), 2.5 mg/10 g diet, 5 mg/10 g diet and 10 mg/ 10 g diet respectively. There was elevation in total thiol content and high GST and CAT activity in 2.5 mg/10 g diet and 5 mg/10 g diet treated flies.

Vazquez-Olivo *et al*. (2019) evaluated the potential use of this plant material for human consumption. In this study, the phenolic content from the MBE from four varieties (Kent, Keitt, Ataulfo and Tommy Atkins) was analyzed by high-performance liquid chromatography coupled to photodiode array detector (HPLC-DAD) and liquid chromatography coupled with time-of-flight mass spectrometry (LC/MS-TOF). Furthermore, the Ataulfo variety showed the highest cellular antioxidant activity (67%) at the concentration of 100 µg mL^{-1}. The intestinal permeability of mangiferin present in the bark extracts was 3- to 4.8-fold higher than those of mangiferin as standard, whereas the intestinal permeability of gallic acid varied among the tested extracts.

Abbasi *et al*. (2017) evaluated cellular antioxidant activity (CAA) and inhibition of hepato-cellular carcinoma (HepG2) proliferation for the first time in the pulp and peel of mango cultivars. Comparatively, peel had high flavonoids and tocopherols content and showed significant antioxidant activity. Among all

the studied cultivars, the Xiao Tainong peel was predominant with highest fistein, mangiferin and alpha-tocopherol content and significant cellular antioxidant activity value 2986 ± 380 μmol QE/100 g FW. The HepG2 cells antiproliferation was maximum in the peel of Da Tainong and pulp of Aozhou with lowest EC_{50} values, 2.35 ± 0.65 and 185.4 ± 10.9 (pulp) mg mL^{-1}, in a dose-dependent manner. Negative associations of flavonoids and tocopherol compounds with CAA and antiproliferative activity in mango confirmed synergistic, additive or antagonistic actions of phytochemicals. The current study suggests that mango peel could be used as a value added ingredient or functional food and may contribute considerably to promote consumer health.

Santhirasegaram *et al*. (2015) subjected freshly squeezed Chokanan mango juice to thermal treatment (at 90 °C for 30 and 60 s), sonication (for 15, 30 and 60 min at 25 °C, 40-kHz frequency) and UV-C treatment (for 15, 30 and 60 min at 25 °C). The results showed better retention of individual phenolic compounds in non-thermal-treated juice, when compared to the control and thermally treated juice. A significant enhancement in antioxidant activities were observed after non-thermal treatment. The sensory evaluation verified that non-thermal-treated juice was preferred more than thermally treated juice.

Meneses *et al*. (2015) tested antioxidant extract by solid–liquid extraction using aqueous acetone (80% v/v) and subsequent adsorption/desorption in C18 cartridge. Recovery of antioxidants was performed by SAE using SC-CO_2 as anti-solvent. The product obtained was a dry powder, spherical nanoparticles with a particle diameter of 143 ± 40 nm with an antioxidant activity of 851.9 μMol TE/g and a half inhibition concentration of DPPH radical of 90 μg/mL. The analyses performed indicated that SAE processing of natural extracts is effective for improving the activity of natural antioxidant compounds by producing nanoparticles.

Mohan *et al*. (2013) evaluated the anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica* in *in vitro* conditions. In *in-vitro* DPPH radical scavenging activity, the MEMI, EMEMI and BMEMI have offered significant antioxidant activity with IC_{50} values of 13.37, 3.55 and 14.19 μg/mL respectively. Gallic acid, a reference standard showed significant antioxidant activity with IC_{50} value of 1.88 and found to be more potent compared to all the extracts and fractions. In *in vitro* LOX inhibition assay, the MEMI, EMEMI and BMEMI have showed significant inhibition of LOX enzyme activity with IC_{50} values of 96.71, 63.21 and 107.44 μg/mL respectively. While, reference drug Indomethacin also offered significant inhibition against LOX enzyme activity with IC_{50} of 57.75.

Arogba and Omede (2012) evaluated antioxidant activity of processed mango (*Mangifera indica*) kernel (PMK), processed Bush Mango (*Irvingia gabonensis*) kernel (PBMK) and the mixture of both (MKK) at 50: 50 using 2, 2-diphenyl -1-picryl hydrazyl (DPPH). The median inhibitory concentrations (IC_{50}) of the three samples showed better correlation with that of reference Quercetin than the reference Vitamin C used. The three samples showed relatively higher radical scavenging effect than the reference samples. The result was significant at $p < (0.05)$, indicative of high level flavonoid and phenolic acid content.

Fernández-Ponce *et al*. (2012) applied supercritical fluid extraction (SFE) and subcritical water extraction (SWE) from mango leaves in order to obtain extracts with high phenolic content and potent antioxidant activity. The antioxidant activity was evaluated by DPPH assay, and the quantification of the main polyphenols of mango leaves by HPLC analysis. SWE showed global yields up to 35% for Kent variety, and extracts with antioxidant activities superior to (+)-α-tocopherol related with their high content on the polyphenols mangiferin and quercetin.

Sultana *et al*. (2012) determined antioxidant activity of leaves, peels, stem bark, and kernel of mango varieties langra and chonsa. Total phenolic (TPC) and total flavonoid contents (TFCs) in segments of langra ranged from 63.89 to 116.80 mg GAE/g DW and 45.56 to 90.89 mg CE/g DW, respectively, and that of chonsa were 69.24 to 122.60 mg GAE/g DW and 48.43 to 92.55 mg CE/g DW, respectively. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and linoleic inhibition capacity in segments of langra ranged from 53.30% to 61.10% and 40.0% to 47.20%, respectively, whereas for chonsa; 56.40% to 66.0% and 48.1% to 49.0%, respectively. The reducing potentials of different segments of langra and chonsa at concentration of 10 mg/mL were 0.512 to 0.850 and 0.595 to 0.665 mV, respectively. Comparison between both varieties showed chonsa exhibited better antioxidant activity. Data were analyzed by analysis of variance (ANOVA) using completely randomised design (CRD) under factorial.

Palafox-Carlos *et al*. (2012) investigated how the ripening stage affects their total phenolic content and antioxidant activity. The quality parameters were significantly different ($P \leq 0.05$) in fruits of different RS, except for firmness and pulp color that were similar in fruits from RS3 and RS4. Mango fruits from RS2 and RS3 accumulated the highest phenol content (174 mg EAG/100 g FW) and antioxidant capacity measured by DPPH (93% inhibition). In general, the antioxidant capacity in fruit from the four stages measured by DPPH and FRAP was similar (8.2 μMET/g).

Varakumar *et al*. (2011) identified Carotenoids and quantified in puree and wine. Results showed that the percentage decrease in xanthophylls levels in wine were in the range of 69.3–89.7%, and >80% degradation was noted in Banginapalli, Neelam, Sindhura and Totapuri and 15.3–26.5% for β-carotene. However, significant degradation of β-carotene was observed in only Totapuri wine, indicating that lutein was more sensitive to degradation than β-carotene during fermentation. Antioxidant activity of mango wine was evaluated using *in vitro* models. The highest radical-scavenging activity was shown by Alphonso (91), Sindhura (90) and Banginapalli (88%), respectively, whereas Alphonso (71), Banginapalli and Sindhura (68.5%) had shown higher inhibitory effect on low-density lipoprotein oxidation. The Hunter color values of the mango wines were also evaluated and the results are presented.

Kim *et al*. (2010) investigated the antioxidant and antiproliferative properties of flesh and peel of mango (*Mangifera indica* L.). The mango peel extract exhibited stronger free radical scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and alkyl radicals than mango flesh extract, regardless of ripeness. Similarly, peel extract exhibited significant antiproliferative effect against all tested cancer cell lines,

compared to that of flesh extract, in a dose-dependent manner. The result also showed that the antiproliferative activity of mango flesh and peel extracts correlated with their phenolic and flavonoid contents. Thus, mango peel, a major by-product obtained during the processing of mango product, exhibited good antioxidant activity and may serve as a potential source of phenolics with anticancer activity.

A.7.6 Toxicity of *Mangifera indica*

Reddeman *et al*. (2019) performed a battery of OECD- and GLP-compliant toxicological studies on mango leaf extract (*Mangifera indica*) containing 60% mangiferin. No evidence of genotoxicity was found in a bacterial reverse mutation test (Ames). While evidence of clastogenic activity was noted in an *in-vitro* chromosomal aberration test, an *in-vivo* mammalian micronucleus test showed no findings up to the limit dose (2000 mg/kg bw). A 90-day repeated dose oral toxicity study was conducted in rats using doses of 0 (vehicle control), 500, 1000, and 2000 mg/kg bw/day. Based on the lack of mortality or toxic effects in the 90-day study, the NOAEL for MLE in Han:Wist male and female rats was determined to be 2000 mg/kg bw/day, the highest dose tested.

Prado *et al*. (2015) investigated pharmacological properties of mangiferin have been reported, but few studies mangiferin toxicity. Single doses of mangiferin were administered by oral or i.p. route or were applied dermally to Sprague-Dawley rats and Balb/C mice. Clinical symptoms of animals were observed during 14 days after treatment. Animals also received single oral doses daily for 28 consecutive days. Blood biochemistry, hematology and pathology findings were reported. : In the acute study, no toxic effects were observed after dermal exposure to mangiferin 2000 mg/kg but transient dyspnea, flank position and piloerection were observed after oral administration to this xanthone. I. p. administration induced similar toxicity signs, but at the highest dose (2000 mg/kg) all mice, one female rat and one male rat died. Rats orally treated with mangiferin (250-1000 mg/kg) for 28 days did not show any abnormal clinical signs or hematology alterations, when compared to control group animals. Histopathological alterations like vacuolar degeneration, necrosis and increment of apoptosis of the acinar cells were observed in the exocrine pancreas of rats at 1000 mg/kg. This suggesting that exocrine pancreas was the target organ for mangiferin's toxicity.

2.7.2 Lamiaceae family

The family Lamiaceae or Labiatae contains many valuable medicinal plants. In the family are 236 genera and between 6900 and 7200 species. To the most abundant genera belong Salvia (900species), Scutellaria (360), Stachys (300), Plectranthus (300), Hypis (280), Teucrium (250), Vitex (250), Thymus (220), and Nepeta (200). Lamiaceae plants rich in essential oils have great worth in natural medicine, pharmacology, cosmetology, and aromatherapy (Karpiński, 2020). It contains important aromatic plants used in traditional and modern medicine and in the food and pharmaceutical industries. Rosemary, thyme, oregano, and sage are the most popular plants in Spanish traditional remedies and are often used for the treatment of gastritis, infections, dermatitis, bronchitis, and inflammation.

Particularly, rosemary and sage have been extensively studied for their anti-oxidative and antimicrobial activity (Degirmen, 2010, González-Minero *et al*., 2020).

A. *Rosmarinus officinalis* L.

Rosmarinus officinalis L., commonly known as rosemary, belongs to the Lamiaceae family. It is an aromatic plant with needle-like leaves that is cultivated worldwide (Kompelly *et al*., 2019). Rosemary has therapeutic properties and has been used in folk medicine as an oral preparation to relieve renal colic, dysmenorrhea, and muscle spasms. Rosemary has antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antithrombotic, antinociceptive, antidepressant, antiulcerogenic, and antioxidant activities (Uritu *et al*., 2018).

A.1 Scientific classification of *Rosmarinus officinalis*

Kingdom	Plantae
Class	Magnoliopsida
Sub class	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Rosmarinus*
Species	*Rosmarinus officinalis*
Vernacular name	Rosemary

(Begum *et al.*, 2013b).

Figure (2:21): plant of *Rosmarinus officinalis* (Hussain *et al*., 2020).

A.2 Botanical Description of *Rosmarinus officinalis*

Rosemary is a perennial shrub and usually grows to about 1 metre (3.3 feet) in height, though some plants can reach up to 2 metres (6.6 feet) tall. The linear leaves are about 1 cm (0.4 inch) long and somewhat resemble small curved pine needles. They are dark green and shiny above, with a white underside and curled leaf margins. The small bluish flowers are borne in axillary clusters and are attractive to bees. Rosemary is fairly resistant to most pests and plant diseases, though it is susceptible to certain fungal infections, such as powdery mildew, in humid climates. It is also a common host to spittlebugs. The plants are easily grown from cuttings (Foote, 2008). Stem Rosemary is an evergreen, shrubby herb that grows to a height of 1 to 2 m, with a unique aromatic odour and a camphoraceous undertone. The erect stems are divided into numerous long, slender branches that have ash-coloured and scaly bark. Leaves The branches bear opposite, leathery thick leaves which are lustrous, linear, dark green above and downy white below. Flower: The flowers are small and pale blue to deep blue. Much of the volatile essential oils reside in their calyces.

A.3 Distribution of *Rosmarinus officinalis*

R. officinalis is native to the Mediterranean region, from where it has been introduced into all continents. In India, it is reportedly often cultivated in gardens, while it is much rarer in Pakistan. The species is an introduced species in the West Indies and is reported to be invasive to Cuba (Acevedo-Rodríguez *et al*., 2012, Oviedo *et al*., 2012). The main producers are Italy, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa (Svoboda and Deans, 1992).

A.4 Ethno medicine uses of *Rosmarinus officinalis*

The traditional medicine of *R. officinalis*, the leaves of *R. officinalis* L. are used based on their antibacterial activities (Bozin *et al*., 2007), carminative (Begum *et al*., 2013b, Evans, 2009a, Heinrich *et al*., 2017) and as analgesic in muscles and joints (Wichtl, 2004, Begum *et al*., 2013a). Also, rosemary's essential oils and extracts obtained from flowers and leaves are used to treat minor wounds, rashes, headache, dyspepsia, circulation problems, but also as an expectorant, diuretic and antispasmodic in renal colic (Begum *et al*., 2013a, Ulbricht *et al*., 2010).

A.5 Chemical constituents of *Rosmarinus officinalis*

R. officinalis contains secondary metabolites with therapeutic potential, such as carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids (De Macedo *et al*., 2020). Polyphenols are antioxidant chemical compounds primarily responsible for the fruit coloring, which are classified as phenolic acids, flavonoids and nonflavonoids (Doughari, 2012). In addition to their antioxidant properties, they play a very important role in the plant defenses against herbivores, pathogens and predators; therefore, they have an application in the control of infectious agents in humans (Doughari, 2012). In *R. officinalis*, the most common polyphenols are apigenin, diosmin, luteolin, genkwanina and phenolic acids (>3%), especially rosmarinic acid, chlorogenic acid and caffeic acid (Al-Sereiti *et al*., 1999, Samuelsson and Bohlin, 2017). Other major compounds common in rosemary are terpenes, usually present in essential oils and resins, which include over 10,000 compounds divided into mono-, di-, tri- and sesquiterpenes, depending on the number of carbon atoms and isoprene groups (Choi and Moore) (Lovkova *et al*., 2001). It is possible to find in rosemary terpenes such as epirosmanol, carnosol, carnosic acid (tricyclic diterpenes), ursolic acid and oleanolic acid (triterpenes) (Ulbricht *et al*., 2010, Samuelsson and Bohlin, 2017).

A.6 Biological Activity of *Rosmarinus officinalis*

A number of *in-vitro* studies has been carried out to reveal various pharmacological potentials of *R. officinalis*.

A.6.1 Antibacterial activity of *Rosmarinus officinalis*

Soulaimani *et al*. (2021) evaluated antibacterial effects of essential oil (EOs) obtained from *Lavandula maroccana* (Murb.), *Thymus pallidus* Batt., and *Rosmarinus officinalis* L. against three multi-drug resistant Gram-negative bacteria. EOs tested individually demonstrated moderate antibacterial activity with MIC and MBC values from 1.3 mg/mL to72 mg/mL, whereas the FICI of their binary combinations revealed

interesting interactions. The combination of EOs obtained from *T. pallidus* and *L. maroccana*s showed the highest number of effective combinations, with synergistic effects against *P. aeruginosa* and *K. pneumoniae,* and an additive effect against *E. coli*. The mixture of *L. maroccana* and *R. officinalis* EOs showed synergistic effects against *E. coli* and *P. aeruginosa* with an additive effect against *K. pneumoniae*. The combination of *R. officinalis* and *T. pallidus* EOs showed a synergistic effect against *K. pneumoniae* only.

Al Zuhairi *et al*. (2020) evaluated the essential oil components by Gas Chromatography-Mass Spectrometry. The essential oil showed antibacterial activity against all the tested strains, with inhibition zones and minimum inhibitory concentrations (MIC) in the range of 7.00 ± 0.00–9.6 ± 0.32 mm and 0.06 ± 0.00 to 0.16 ± 0.07 mg/mL, respectively. The minimum bactericidal concentration (MBC) values were equal to MIC values. The greatest microbial inhibitory effect was against *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Proteus vulgaris*, respectively.

Silva *et al*. (2019) evaluated the efficacy of different concentrations of essential oils combined with calcium hydroxide against *Enterococcus faecalis*. MICs in the range of 0 to 100 mg/ml were only observed in the ROH group. The RO, ROH, AB, ZO, and ZOH presented absolute data for MBC. Bacterial growth was detected in the DWH group at all concentrations tested. The combination of the essential oils tested here with calcium hydroxide appears promising as an intracanal medication in endodontic treatment because of its effectiveness against *Enterococcus faecalis*. Essential oils are important in endodontic therapy since calcium hydroxide, the gold standard intracanal medication, is not effective against *E. faecalis*.

Stojiljkovic *et al*. (2018) investigated antibacterial effects of rosemary essential oils on some Gram-positive and Gram-negative bacteria: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa. Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium.* The antimicrobial and other biological activities of rosemary essential oil are directly correlated with the presence of bioactive volatile components. And it has been used as alternative antimicrobial food preservatives.

Satyal *et al*. (2017) obtained the essential oils of *R. officinalis* from plants growing in Victoria (Australia), Alabama (USA), Western Cape (South Africa), Kenya, Nepal, and Yemen. Chemical compositions of the rosemary oils were analyzed by gas chromatography-mass spectrometry as well as chiral gas chromatography. The oils were dominated by (+)-α-pinene (13.5%–37.7%), 1,8-cineole (16.1%–29.3%), (+)-verbenone (0.8%–16.9%), (−)-borneol (2.1%–6.9%), (−)-camphor (0.7%–7.0%), and racemic limonene (1.6%–4.4%). Hierarchical cluster analysis, based on the compositions of these essential oils in addition to 72 compositions reported in the literature, revealed at least five different chemotypes of rosemary oil. Antifungal, cytotoxicity, xanthine oxidase inhibitory, and tyrosinase inhibitory activity screenings were carried out, but showed only marginal activities.

Mekonnen *et al*. (2016) evaluated the *in-vitro* antimicrobial activities of four plant essential oils (*T. schimperi, E. globulus, R. officinalis*, and *M. Chamomilla*). Results of this study revealed that essential oils of *T. schimperi, E. globulus,* and *R. officinalis* were active against bacteria and some fungi. The antimicrobial effect of *M. chamomilla* was found to be weaker and did not show any antimicrobial activity. The minimum inhibitory concentration values of *T. schimperi* were 15.75 mg/mL for most of the bacteria and fungi used in this study. The minimum inhibitory concentration values of the other essential oils were in the range of 15.75–36.33 mg/mL against tested bacteria. This study highlighted the antimicrobial activity of the essential oil of *E. globulus*, *M. chamomilla, T. Schimperi*, and *R. officinalis*.

Pesavento *et al*. (2015) investigated antimicrobial activity of five essential oils up to 72 h against foodborne pathogens (*Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis, Campylobacter jejuni*). The most active EOs were *Thymus vulgaris* and *Origanum vulgare,* followed by *Cinnamomum zeylanicum, Rosmarinus officinalis,* and *Salvia officinalis.* The antimicrobial activity of *O. vulgare, Rosmarinus officinalis* and *T. vulgaris* was investigated against five enterotoxin producers of *S. aureus* and five *L. monocytogenes* strains, for different amounts of time (up to 14 days), at 4 °C, in meatballs. Concentrations of 2% and 1% restricted the growth of both the pathogens but, as a result of panel tests, altered the meat flavor. The cooked meatballs containing 0.5% of EO were acceptable in terms of taste, and the oils were able to suppress concentrations of $<10^2$ CFU/g of the pathogens, revealing the potential use of *R. officinalis, T. vulgaris* and *O. vulgare* as food preservatives at this concentration.

Ceylan *et al*. (2014) investigated the antibiofilm activity of the essential oil from *Rosmarinus officinalis* against biofilm formation of *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus cereus* and *Bacillus subtilis*. The antibiofilm effect of MBC, MIC, MIC/2, MIC/4, MIC/8 and MIC/16 concentrations of *Rosmarinus officinalis* essential oil was assessed by the microplate biofilm assay. The essential oil exhibited significant antibacterial activity against all tested bacteria. In contrast to the antibacterial activity, MBC and subinhibitory concentrations of essential oil showed limited antibiofilm activity. MBC concentrations of essential oil attenuated the biofilm formation at 60.76% and 74.7% for *Staphylococcus aureus* MU 47 and *Pseudomonas aeruginosa* MU 187, respectively.

Jordán *et al*. (2013) determined in individual *Rosmarinus officinalis* L, the effect of the order of abundance among the components that define the rosemary essential oil chemotype (eucalyptol, camphor, α-pinene), on the antimicrobial activity. Determination of the diameter of inhibition in *Salmonella typhimurium* pointed to a positive contribution effect of eucalyptol and α-pinene. A high proportion of α-pinene increases the effectiveness of the oil against *Staphylococcus aureus*, while the presence of eucalyptol, as the most abundant compound, considerably decreases the efficiency of rosemary oil. In contrast, the efficacy of these oils against *Listeria monocytogenes* and *Escherichia coli* was not affected by this condition. As regards the minimum inhibitory (MIC) and bactericide (MBC) concentrations, the strong

activities exhibited by these essentials oils (<0.5 µL/mL) did not allow the chemotypes and antibacterial activities to be differentiated.

Wang *et al*. (2012) evaluated of *Rosmarinus officinalis* L. essential oil and three of its main components 1,8-cineole (27.23%), α-pinene (19.43%) and β-pinene (6.71%) for their *in vitro* antibacterial activities and toxicology properties. *R. officinalis* L. essential oil possessed similar antibacterial activities to α-pinene, and a little bit better than β-pinene, while 1,8-cineole possessed the lowest antibacterial activities. .

Bendeddouche *et al*. (2011) evaluated the antimicrobial activity against three pathogenic bacteria: Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*). The essential oil exhibited strong antibacterial activity against *E. coli* and *P. aeruginosa*, and was also active against *Staphylococcus aureus.*

Jarrar *et al*. (2010) determined the antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) and to investigate the synergistic effects of this extract combined with ceforuxime against methicillin resistant *Staphylococcus aureus* (MRSA). The minimum inhibitory concentrations (MICs) of the ethanol extract of rosemary were in the range of 0.39-3.13 mg/mL. The minimum bactericidal concentrations (MBCs) were usually equal to or double that MICs. The antimicrobial activity of combinations of the ethanol extract of rosemary and cefuroxime indicated their synergistic effects against all MRSAs.

A.6.2 Antifungal activity of *Rosmarinus officinalis*

Achimón *et al*. (2021) evaluated the chemical composition of *Curcuma longa, Pimenta dioica, Rosmarinus officinalis,* and *Syzygium aromaticum* essential oils and their antifungal and anti-conidiogenic activity against *Fusarium verticillioides*. *S. aromaticum* EO exhibited the highest antifungal effect, followed by *P. dioica* and to a lesser extent *C. longa*. *Rosmarinus officinalis* poorly inhibited fungal growth; however, it was the only EO that inhibited conidial production, with its major components being 1,8-cineole (53.48%), α-pinene (15.65%), and (−)-camphor (9.57%). The results showed that some compounds are capable of decreasing mycelial growth without affecting sporulation, and vice versa. However, not all the compounds of an EO are responsible for its bioactivity.

Da Silva Bomfim *et al*. (2020) evaluated the antifungal and antiaflatoxigenic activity of *Rosmarinus officinalis* L. essential oil (REO) against *Aspergillus flavus* Link. REO by hydrodistillation and its major components were identified as 1,8-cineole (eucalyptol, 52.2%), camphor (15.2%) and α-pinene (12.4%) by GC/MS and NMR. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were both 500 µg/mL. REO reduced the mycelial growth of *A. flavus* at a concentration of 250 µg/mL (15.3%). The results obtained from scanning electron microscopy (SEM) demonstrated a reduction in the size of conidiophores and in the thickness of hyphae in *A. flavus* caused by treatment with REO (250 µg/mL). The production of ergosterol and the biomass of mycelium were both reduced as the REO treatment concentration increased. The production of aflatoxins B_1 and B_2 was inhibited after treatment with 250 µg/mL REO, a concentration below the MIC/MFC, indicating that the antiaflatoxigenic

effect of REO is independent of its antifungal effect and is likely due to its direct action upon toxin biosynthesis.

Kaab *et al*. (2019) aimed to assess the antifungal and herbicidal activities of *Rosmarinus officinalis* essential oil (EO) to find an alternative to synthetic pesticides. It was confirmed that this EO significantly inhibits spore germination of *Fusarium oxysporum*, *Fusarium culmorum* and *Penicillium italicum* at 6 mM, the percentage of inhibition reached 100% on *Fusarium oxysporum*. Indeed, EO slows down seedling growth of *Trifolium incarnatum*, *Silybum marianum*, and *Phalaris minor*. In fact, EO at 5 mM completely inhibits seed germination.

Diánez *et al*. (2018) screened the antifungal properties of 12 Eos, that is, *Syzygium aromaticum, Pelargonium graveolens, Lavandula angustifolia, Cupresus sempervirens, Mentha piperita, Santolina chamaecyparissus, Citrus sinensis, Pogostemon patchouli, Thymus mastichina, Thymus vulgaris, Eucalyptus globulus* and *Rosmarinus officinalis*. The mycelial growth inhibition and ED_{50} were calculated. The chemical analysis of the EOs was analysed using gas chromatography–mass spectroscopy. A total of 58 compounds were identified in the 12 EOs. All essential oils analysed showed antifungal activity against the test pathogens in the range of 5·32–100%. The inhibitory effect of oils showed dose-dependent activity on the tested fungus. Based on the ED_{50} values, clove, rose geranium, peppermint and patchouli were the most effective.

Ksouri *et al*. (2017) studied the antifungal activity of the essential oils from *Origanum floribundum* Munby., *Rosmarinus officinalis* L.and *Thymus ciliatus* Desf. against a *Candida albicans* reference strain and ten *C. albicans* isolated strains from bovine clinical mastitis. The three essential oils revealed highly effective anticandidal activity, with an MIC of 80% values ranged from 15.02 to 31.08 μg/mL.

Sepehri *et al*. (2016) investigated the antifungal effects of *Echinophora platyloba* and *Rosmarinus officinalis* extracts on *C. albicans* species. The minimum inhibitory concentration was determined by the microtitration technique. The results showed that the leaf extracts of *E. platyloba* and *R. officinalis* had strong antimicrobial activities. Also, based on the findings, *R. officinalis* leaf extracts exhibited higher antimicrobial activity. The ethanolic leaf extracts of *E. platyloba* and *R. officinalis* showed good antimicrobial activity against *C. albicans* strains. However, the aqueous extracts did not show any major activities against the tested *C. albicans* strains. On the other hand, the ethanolic extracts exhibited major antimicrobial properties against *C. albicans* strains. The highest minimum inhibitory concentration was reported in *E. platyloba* leaf extracts.

Felsociova *et al*. (2015) screened 15 essential oils of selected plant species, *Lavandula angustifolia*, *Carum carvi*, *Pinus mungo* var. pulmilio, *Mentha piperita*, *Chamomilla recutita* L., *Pinus sylvestris*, *Satureia hortensis* L., *Origanum vulgare* L., *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abietis albia* etheroleum, *Chamomilla recutita* L. Rausch, *Thymus vulgaris* L., *Origanum vulgare* L. for

antifungal activity against five Penicillium species: *Penicillium brevicompactum, Penicillium citrinum, Penicillium crustosum, Penicillium expansum* and *Penicillium griseofulvum*. There were five essential oils of the 15 mentioned above which showed a hopeful antifungal activity: *Pimpinella anisum, Chamomilla recutita* L., *Thymus vulgaris, Origanum vulgare* L. The most hopeful antifungal activity and killing effect against all tested penicillia was found to be *Origanum vulgare* L. and *Pimpinella anisum*. The lowest level of antifungal activity was demonstrated by the oils *Pinus mungo* var. pulmilio, *Salvia officinalis* L., *Abietis albia* etheroleum, *Chamomilla recutita* L. Rausch, *Rosmarinus officinalis*.

Stupar *et al*. (2014) investigated the antifungal activity of *Origanum vulgare*, *Rosmarinus officinalis* and *Lavandula angustifolia* (Lamiaceae) essential oils and biocide benzalkonium chloride against fungi isolated from stone (*Bipolaris spicifera* and *Epicoccum nigrum*) and wooden substrata (*Aspergillus niger*, *Aspergillus ochraceus*, Penicillium sp. and *Trichoderma viride*) of cultural heritage objects. Carvacrol (64.06%) was the main component of O. vulgare essential oil, while linalool (37.61%) and linalool acetate (34.86%) dominated in *L. angustifolia* essential oil. The main component of *R. officinalis* essential oil was 1.8-cineole (44.28%). To determine fungistatic and fungicidal concentrations (MIC and MFC) micro-, macrodilution and microatmosphere methods were used. Mycelial growth and spore germination of fungal isolates were inhibited with different concentrations of antifungal agents. The oil of *O. vulgare* and *biocide benzalkonium* chloride displayed the strongest antifungal activities followed by *R. officinalis* and *L. angustifolia* essential oils. MIC and MFC values obtained in microatmosphere and microdilution method for *O. vulgare* essential oil ranged from 0.1 to 2.0 μL mL^{-1}, while for *R. officinalis* and *L. angustifolia* ranged from 10.0 to 100.0 μL mL^{-1}. The most susceptible fungus to essential oil treatments was *E. nigrum*. MIC and MFC values for benzalkonium chloride ranged from 0.1 to 4.0 μL mL^{-1}. Tested isolates, *A. niger* and *A. ochraceus*, were the most susceptible for biocide treatment.

Matsuzaki *et al*. (2013) evaluated the antifungal activities against *C. albicans* of essential oils from seven aromatic plants from three manufacturers, and of three chemotype essential oils from rosemary (*Rosmarinus officinalis*). As a result, we found that the antifungal activity was increased several times by the addition of Tween 80. All the tested essential oils showed stable antifungal activity, however, the variation was observed among the manufacturers of rosemary and eucalyptus. Rosemary has three chemotypes; Cineol, Camphor and Verbenon. They derived from a same plant species, but contain different chemical components. The Cineol, dose-dependently decreased the number of *C. albicans* in the time-kill assay.

Mugnaini *et al*. (2012) evaluated the anti-*M. canis* activity of some EOs chemically characterized both *in vitro* and *in vivo*. Eleven feline isolates of *M. canis* were tested by microdilution against EOs extracted from *Thymus serpillum, Origanum vulgare, Rosmarinus officinalis, Illicium verum* and *Citrus limon*. A mixture composed by 5% *O. vulgare*, 5% *R. officinalis* and 2% *T. serpillum*, in sweet almond oil was

administered to seven infected, symptomatic cats. *T. serpillum* and *O. vulgare* showed the lowest MICs, followed by *I. verum, R. officinalis* and *C. limon*. The assay performed on mixture showed that antimycotic activity of each component was enhanced. Four out of seven treated cats recovered both clinically and culturally. *T. serpillum* and *O. vulgare* EOs showed a strong antifungal activity. Preliminary data suggest a possible application in managing feline microsporiasis

Makhloufi *et al*. (2011) tested the antifungal activity of this oil against seven fungal strains isolated from dates and wheat. The inhibitory effect of this oil on fungal growth suggests prospects for its application in the food industry. The microbiological analyses of dates showed the existence of a fungal flora dominant by Penicillium and Aspergillus. Besides, the influence of the addition of *R. officinalis* L. and/ or its essential oil on the physicochemical and microbiological quality of dates showed a decrease in humidity, the pH, a light increase of the acidity and a reduction of the microbial flora.

Centeno *et al*. (2010) tested the antifungal activity of ethanolic extracts of *Rosmarinus officinalis* and *Thymus vulgaris* against strains of *Aspergillus flavus* and *A. ochraceus*, since these two species are common contaminants of cereals and grains and are able to produce and accumulate mycotoxins. The results obtained suggest that the assayed extracts affect the proper development of *A. flavus* and *A. ochraceus*; leading to a lower MIC (1200 ppm) and MFC (2400 ppm) for *T. vulgaris* extract against *A. ochraceus* than against *A. flavus*.

A.6.3 Antiprotozoal activity of *Rosmarinus officinalis*

Anacarso *et al*. (2019) studied the anti-amoebic activity of essential oils, derived from rosemary (*Rosmarinus officinalis* L.) and cloves (*Syzygium aromaticum* L. Merr. & Perry), against *Acanthamoeba polyphaga* strain. This activity was evident after few hours, with encouraging results obtained in particular with cloves EO, able to act at the lower concentrations and after 1 h, probably for its high eugenol content (65.30%).

Dahab (2015) assessed two herbs (*Syzygium aromaticum* and *Rosmarinus officinalis*) for their antimicrobial activity against *Giardia lamblia* causing giardiasis in humans and *Streptococcus agalactiae*, a major fish pathogen causing streptococcosis. Methanol extract from *R. officinalis* was strongly active against *G. lamblia* (IC_{50} = 0.689 mg/mL) and *Syzygium aromaticum* was good activity against *G. lamblia* (IC_{50} = 0.755 mg/mL). On the other hand, ethanol extract from *Syzygium aromaticum* was strongly active against *G. lamblia* (IC_{50} = 1.227 mg/mL) and *R. officinalis* was good active against *G. lamblia* (IC_{50} = 2.874 mg/mL). None of the aqueous extracts of the two plants were active against tested protozoan. Methanol extracts of *Syzygium aromaticum* and *Rosmarinus officinalis* produced the largest (22.2±1.2 mm) and (25.4±0.9 mm) inhibition zones, respectively. Determination of minimal inhibitory concentration (MIC) of herb extracts against *S. agalactiae* showed that the methanol extract of *Rosmarinus officinalis* had the lowest MIC value (6.25 mg/L). methanol extract of *Syzygium aromaticum* was a moderate MIC (12.5 mg/L).

Azeredo *et al*. (2014) analyzed the biological activity of different EOs on *Trypanosoma cruzi*, as well as their cytotoxicity on Vero cells. Evaluated on *T. cruzi* epimastigote forms: *Cinnamomum verum, Citrus limon, Cymbopogon nardus, Corymbia citriodora, Eucalyptus globulus, Eugenia uniflora, Myrocarpus frondosus,* and *Rosmarinus officinalis. Cinnamomum verum* EO was the most effective against *T. cruzi* epimastigotes (IC_{50}/24 h = 24.13 μg/ml), followed by *Myrocarpus frondosus* (IC_{50}/24 h = 60.87 μg/ml) and *Eugenia uniflora* (IC_{50}/24 h = 70 μg/ml). The EOs of *C. citriodora, E. globulus,* and *R. officinalis* showed no activity at concentrations up to 300 μg/ml. *T. cruzi* metacyclic trypomastigotes and intracellular amastigotes with *C. verum* EO resulted in IC_{50}/24 h values of 5.05 μg/ml and 20 μg/ml, respectively. Therefore, trypomastigotes are more susceptible than epimastigotes, with selectivity index (SI) about 4.7-fold higher (9.78 and 2.05, respectively).

Elbadr et al. (2013b) selected for new natural anti-protozoal chemotherapy, two plants of the family Lamiaceae, *Rosmarinus officinalis* and *Ocimum basilicum*. Petroleum ether extract from *R. officinalis* was strongly active against *G. lamblia* (IC_{50} = 4.382 mg/ml^{-1}) and good activity of *O. basilicum* (IC_{50} = 14.9 mg/ml^{-1}). Ethyl acetate extract from *R. officinalis* was strongly active against *G. lamblia* (IC_{50} = 2.02 mg/ml^{-1}) and moderate activity of *O. basilicum* (IC_{50} = 25.4 mg/ml^{-1}). Methanol extract from *R. officinalis* was strongly active against *G. lamblia* (IC_{50} = 2.383 mg/ml^{-1}) and moderate activity of *O. basilicum* (IC_{50} = 33.8 mg/ml^{-1}). None of the aqueous extracts of the two plants were active against tested protozoan.

Ahmed *et al*. (2011) tested 10 essential oils extracted from 10 plants issued from Sned region (Tunisia) both their leishmanicidal effects against *Leishmania major* and *L. infantum,* and their cytotoxicity against murine macrophage cell line RAW 264.7 (ATCC, TIB-71). The results showed were significantly active against both *L. major* and *L. infantum*, whereas *Ruta chalepensis* EO (rich on 2-undecanone at 84.28%) is only active against *L. infantum*. Both oil extracts showed low cytotoxicity towards murine macrophages. The characteristic ratios (IC_{80} Raw264.7 cells/IC_{50} *L. infantum* and IC_{80} Raw264.7 cells/IC_{50} *L. major*) were, respectively, 2.7 and 1.57 for *T. hirtus* sp. *algeriensis*, and 1.34 and 0.19 for *R. chalepensis*.

A.6.4 Anthelminthic activity of *Rosmarinus officinalis*

Pinto *et al*. (2019) evaluated the *in-vitro* ovicidal and larvicidal activity of essential oil of *Rosmarinus officinalis*. The assays of *R. officinalis* oil showed a significant ($p<0.05$) 97.4% to 100% inhibition of egg hatching and a significant ($p<0.05$) 20% to 74% inhibition of larval migration.

Zoral *et al*. (2017) examined the anthelmintic activity of rosemary extract against the monogenean (*Dactylogyrus minutus*) *in-vitro* and *in-vivo*. Parasites were dead at 61.8 ± 5.6 and 7.8 ± 1.4 min when exposed to 100 and 200 g aqueous rosemary extract solution/L of water respectively. It took 166.7 ± 48.2 and 5.4 ± 1.01 min to kill the parasites when exposed to 1 and 32 g ethanol rosemary extract solution/L of water respectively. Moreover, pure component of rosemary extract obtained commercially used in *in-vitro* experiments showed that 1,8-Cineole was the most toxic component of the main components tested. Parasite intensity and prevalence in fish exposed to 50 and 100 g aqueous rosemary solution/L water for

30 min were significantly lower than they were in controls ($p < 0.05$). In oral treatment experiments, diets of *Cyprinus carpio* were supplemented with eight different concentrations of aqueous rosemary extract. The intensity of parasites was significantly less in fish fed for 30 days with feed containing 60, 80 and 100 ml aqueous extract/100 g feed than in control ($p < 0.05$).

A.6.5 Antioxidant activity of *Rosmarinus officinalis*

Fatemeh *et al*. (2021) investigated the effect of *Rosmarinus officinalis* L. (rosemary) extract on acetyl cholinesterase (AChE) activity and oxidative stress biomarkers in healthy volunteers. Administration of 1000 mg of rosemary for 30 days significantly decreased AChE activity compared to AChE activity before rosemary treatment (p value < 0.001) and also in the placebo group (p value < 0.01). Notably, administration of rosemary considerably improved TAC (p value < 0.01) and protein carbonylation (*p* value < 0.05) compared to those values before rosemary treatment and in the placebo group. After administration of rosemary, no significant changes were observed in lipid peroxidation (p value > 0.05) compared to that value before rosemary treatment and in the placebo group.

Bouzekri (2020) studied the valorization of the medicinal plants of Morocco, the *Rosmarinus officinalis* a very potential plant, belong to the Lamiaceae family to evaluate its antimicrobial and antioxidant activities. The essential oil of *Rosmarinus officinalis* can be used for improving the quality and shelf life of food products. Naiel *et al*. (2019) designed to inspect the ameliorative effect of Rosemary (*Rosmarinus officinalis*)-supplemented diets on aflatoxin B1 (AFB1) toxicity in terms of the performance, residual AFB1 concentration in tissue, whole-body composition, hepatosomatic index, mortality rate, serum biochemical parameters, antioxidative enzymes, immunity parameters and liver histopathology of *Oreochromis niloticus*. The antioxidant activity and innate immunity status were significantly improved by high level of Rosemary.

Baj *et al*. (2018) demonstrated the usefulness of the simplex-lattice mixture design method for the design of essential oil mixtures with potent antioxidant properties, as well as generate a discussion of potential interactions existing between essential oils. The highest antioxidant activity was obtained for a 75:8:17 percentage composition mixture respectively. However, the proposed composition of the individual ingredients in the design mixture showed a higher percentage of inhibition (90%) compared to the activity of the single component (ol. marjoram 88%) which was the most active.

Bajalan *et al*. (2017) obtained the leaf essential oil from seven populations of rosemary growing in Western Iran by hydrodistillation and analyzed by GC-FID and GC–MS. Overall, the essential oils from the seven populations, at 3.2 mg/ml, exhibited high antioxidant activity (more than 50% radical inhibition).

Wollinger *et al*. (2016) investigated the residual antioxidants after hydro-distillation, especially rosmarinic acid and carnosic acid. The total antioxidant activity of the extracts and of the pure compounds was determined by DPPH assays. It is shown that after 2.5 h of hydro-distillation the amount of rosmarinic acid and the antioxidant activity in the water residue reaches a maximum value.

Ladan Moghadam (2015) investigated the antioxidant potential of *Rosmarinus officinalis* on the basis of the chemical compositions of oils obtained by hydrodistillation. The main constituents of the essential oils in aerial parts were α-pinene (43.12 %), camphene (10.5 %), 1,8-cineole (10.02 %), camphor (8.07 %), linalool (8.09 %) and limonene (6.12 %). Other components were amounts less than 2 %. The samples were screened for their antioxidant activities using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical assay methods. 1,8-Cineole (11.01±0.41 µg/ml) showed appreciable antioxidant activity in DPPH test.

Rašković *et al*. (2014) evaluated of our study the protective effect of rosemary essential oil on carbon tetrachloride-induced liver injury in rats and to explore whether its mechanism of action is associated with modulation of hepatic oxidative status. Pre-treatment with studied essential oil during 7 days significantly reversed the activities of antioxidant enzymes catalase, peroxidase, glutathione peroxidase and glutathione reductase in liver homogenates, especially in the dose of 10 mg/kg.

Kasparavičienė *et al*. (2013) produced ethanolic extracts of rosemary leaf by maceration and percolation and different ethanol concentrations. The most potent solvent concentration was 50% for the evaluation of total polyphenols 47.39 ± 0.21 mg/mL RAE. All tested rosemary extracts showed high DPPH radical inactivation which ranged from 75.96 ± 1.12% to 85.81 ± 0.14%, but no statistically significant difference ($P > 0.0624$) was observed. The dry residue was also evaluated and it was higher in the liquid extracts than in tinctures. Stirring of rosemary leaves increased the total phenolics (from 42 till 53 mg/ml RAE) in the 50% ethanolic rosemary tincture.

Khalil *et al*. (2012) examined the action of *R. officinalis* against experimental diabetes as well as the antioxidant potential of the leaf extract. This was evident from a significant decrease in blood sugar level and oxidative stress makers including serum TBARS and nitric oxide (NO). Serum enzymatic (glutathione transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and non enzymatic antioxidants (vitamin C and reduced glutathione) were found to be increased by the administration of *R. officinalis*.

Beretta *et al*. (2011) isolated the antioxidant constituents of essential oils of *Rosmarinus officinalis* L. (α-pinene chemotype) at the flowering (A), post-flowering (B), and vegetative stages (C). Radical scavenging capacity (DPPH test, IC_{50} 36.78 ± 0.38, 79.69 ± 1.54, 111.94 ± 2.56 µL) and anti-lipoperoxidant activity (TBARS, IC_{50} 0.42 ± 0.04, 1.20 ± 0.06 µL, 4.07 ± 0.05 µL) differed widely in the three stages. Antioxidant activity, identified after silica gel fractionation chromatography, was closely related ($R^2 = 0.9959$) to each EO's content of hydroxilated derivatives, (containing alcohols, phenols and 1,8 cineole): 15.26 ± 0.12%, 7.22 ± 0.06%, and 5.09 ± 0.10% in EOs from A, B, and C. Modeling the C, H, O terpenes in a simulated phospholipid bilayer indicated that anti-lipoperoxidant activity depended on the stability and rapidity of their interactions with the membrane bilayer components, and their positioning over its surface. investigated the antiproliferative, antioxidant and antibacterial activities of *Rosmarinus officinalis* essential oil, native to Pakistan. The essential oil content from the leaves of *R. officinalis* was 0.93 g $100g^{-1}$. The antioxidant

activity was evaluated by the reduction of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and measuring percent inhibition of peroxidation in linoleic acid system.

A.6.6 Toxicity of *Rosmarinus officinalis*

Almakhatreh *et al*. (2019) investigated the possible protective effect of rosemary extract against Etoposide -induced changes in liver and kidney functions, and DNA damage in rats. This increased in ALT, AST, ALP, creatinine, urea, potassium ions, chloride ions, and DNA damage was reduced after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4. Also, this decreased in albumen, total proteins, sodium ions, and calcium ions was increased after administration of rosemary when co-treated with etoposide (G4), or post-treated after etoposide (G5) for four weeks with lowest damage in G4.

Kiran and Prakash (2015) studied explores the efficacy of *Rosmarinus officinalis* essential oil (EO) as a plant based insecticide and its effect on acetylcholinesterase (AChE) and antioxidant enzyme system in *Sitophilus oryzae* and *Oryzaephilus surinamensis*. As a fumigant, at 0.15 µl/ml EO exhibited 100% toxicity and antifeedant activity against the test insect pests without affecting seed germination.

Alnamer *et al*. (2011) evaluated the central nervous system psychotropic effects of the essential oil from the leaves of *R. officinalis* and the flowers of *L. officinalis*. The intraperitonial administration in mice of essential oil from *L. officinalis* at 300 and 600 mg/kg i.p. induces strong sedative effects compared to reference substance diazepam in mice, and an hypnotic effects at doses 1000 and 1500 mg/kg. However, the essential oil extracted from *R. officinalis* at the doses 50 and 100 mg/kg, produced no sedative activity significant on the central nervous system.

2.7.3 Menispermaceae family

The family Menispermaceae consists of 75 genera, with 520 species (De Wet and Van Wyk, 2008). It is a large glabrous deciduous climbing shrub. The species have been widely distributed throughout the tropical and subtropical parts of Asia, Africa, and Australia (Chi *et al*., 2016). It is mainly composed of tropical lianas (Jacques and Bertolino, 2008). Because of its richness in bisbenzylisoquinoline alkaloids, this family is used worldwide in traditional medicine to treat a variety of ailments (De Wet *et al*., 2005, Barbosa-Filho *et al*., 2000, De Wet *et al*., 2004). This family contains a number of plants with scientifically recognized important pharmacological activities (Jahan *et al*., 2010). These six species were *Cocculus hirsutus*, *Stephania glabra*, *Stephania japonica*, *Tinospora cordifolia*, *Tinospora crispa*, and *Tinospora sinensis*. Of the six species, *Stephania japonica* and *Tinospora cordifolia* were most frequently used for treatment of ailments like diabetes, edema, pain, bone fracture, debility, gastrointestinal disorders, respiratory tract disorders, helminthiasis, malaria, hepatic disorders, tuberculosis, measles, urinary tract disorders, and hypertension (Jahan *et al*., 2010). The folk medicinal use of several of these plants has been validated through scientific studies.

A. *Tinospora bakis* (A.Rich.) Miers

Tinospora bakis (A.Rich) Miers in Hook. Niger. Fl.: 215 (1849) belongs to the family Menisperaceae. It is known in Sudan as Irg al-hagar, andfound at low plains and distributed in West and Central Africa (El Ghazali *et al*., 2003). It is an ethno pharmacologically important Tinospora species and widely used in various regions of Africa. In view of its various medicinal uses several pharmacological screenings have been carried out (Wangchuk *et al*., 2017). *T.bakis* is a plant of the folk medicine used by healers in Burkina Faso for the treatment of malaria (Ouattara *et al*., 2006b).

A.1 Scientific classification of *Tinospora bakis*

Kingdom	Plantae
Class	Magnoliopsida
Sub class	Magnoliidae
Order	Ranunculales
Family	Menisperaceae
Genus	*Tinospora*
Species	*bakis*
Vernacular name	Irg al-hagar

Figure (2:22): Plant of *Tinospora bakis*.

A.2 Distribution of *Tinospora bakis*

Tinospora bakis is found at low plains and distributed in West and Central Africa. In Sudan Komul, Ingassana, Blue Nile state (El Ghazali *et al*., 2003).

A.3 Botanical Description of *Tinospora bakis*

Slender glabrous lianes. Branchlets with lenticels. Leaves alternate, simple and entire; petiole 0.5–4(–8) cm long, sparsely hairy or glabrous; blade broadly ovate-triangular, 3–5 cm × 3–5 cm, base cordate, apex acuminate, pale green, palmately veined with 5–7 main veins, short-hairy or glabrous below. Bark flaking off in orange scales; branches glabrous, with numerous warty lenticels. Flowers unisexual, small, yellowish green; pedicel 2–3 mm long; sepals 6 in 2 whorls, translucent or membranous, outer sepals ovate-triangular, 1–1.5 mm × c. 0.5 mm, inner sepals 2.5–4 mm × 2–3 mm; petals 6, 2–3 mm × 1–2 mm, fleshy; male flowers with 6 free stamens 2.5–3 mm long; female flowers with 6 staminodes, ovary superior, consisting of 3 free carpels 1–2 mm long. Fruit consisting of up to 3 drupes, 6–9 mm × 4–5 mm, each 1-seeded. *Tinospora bakis* flowers towards the start of the rainy season (El Ghazali *et al*., 2003).

A.4 Ethno medicine uses of *Tinospora bakis*

Tinospora bakis is used traditionally in Sudan as antidiabetic (Alamin *et al*., 2015), antitheilerial (Farah *et al*., 2013). This plant is also used for the treatment of headache (El Ghazali *et al*., 2003). The root of plant has a high character in West Africa as a diuretic and febrifuge (Domis and Oyen, 2008), also the root is used against jaundice, hematuria, bilious fever and yellow fever, anti-malaria (Ouattara *et al*., 2006b) and schistosomiasis. Antidiabetic activity of aqueous extracts of *T. bakis* on streptozotocin-induced diabetes rats (Alamin *et al*., 2015).

A.5 Chemical constituents of *Tinospora bakis*

The root contains alkaloids, including protoberberine type alkaloid palmatine, and 2–4% columbin, a diterpenoid furanolactone (Farah *et al*., 2018, Domis and Oyen, 2008). The aqueous extract of the root containing the alkaloidal fraction showed moderate activity against a chloroquine-resistant strain of *Plasmodium falciparum in-vitro* (Ouattara *et al*., 2006b). Columbin, in small doses, was found to increase the secretion of the bile and the glands of stomach and intestines; at higher doses it produces greasy degeneration of the liver. Palmatine shows a stronger antipyretic effect than a total alkaloid extract in rabbits. It paralyses the respiratory centre more than morphine.

A.6 Biological Activity of *Tinospora bakis*

A number of *in-vitro* study has been carried out to reveal various pharmacological potentials of *Tinospora bakis*.

A.6.1 Antiprotozoal activity of *Tinospora bakis*

Abdelrahman and Khalil (2012) evaluated of *Tinospora bakis* *in-vivo* against *Trypanosoma evansi* infection in rats. The result was compared to Cymelarsan which was given at the recommended dose of 2.5 mg kg^{-1}, b.wt. subcutaneously. Daily programs for the parasitaemia for all methanolic and chloroformic extracts in infected or uninfected rats were followed for 30 days after treatment. Blood was collected every week for analysis. *Tinospora bakis* extract was found to be effective in cleaning the parasite for a considerable time.

Ouattara *et al*. (2006b) screened aqueous, methanol, hydromethanol extracts from the roots *T. bark* of *S. madagascariensis*, methanol and hydromethanol extracts from the leaves of *C. glutinosum* and aqueous and alkaloidal extracts from the roots of *T. bakis* were also made and their antimalarial activity against *Plasmodium falciparum* chloroquine-resistant strain W2 *in-vitro*. The screening showed that the methanol and hydromethanol extracts of *Swartzia madagascariensis*, hydromethanol extracts of *C. glutinosum* and alkaloidal extracts of *T. bakis* were active (5µg/ml < IC_{50} < 50 µg/ml).

A.6.2 Anthelminthic activity of *Tinospora bakis*

Farah *et al*. (2013) screened the aqueous extract of *Tinospora bakis* roots for the discovery of its *in-vitro* activity against *Theileria lestoquardi*. *In-vitro* screening of the extract against *T. lestoquardi* which is the causative agent of malignant theileriosis in sheep showed activity of 17.66 and 30.00% at concentrations of 5000 and 10000 ppm, respectively. Concentrations of 250 and 500 ppm had no activity. Lethal concentration 50% (LC_{50}) was 184268.54 ppm. The plant extract significantly ($P<0.05$) decreased the number of macroschizonts per cell, number of dividing cells (binucleated and multinucleated) and mean number of viable cells at concentrations of 5000 and 10000 ppm. However, the number of cells with extra cellular macroschizonts did not significantly ($P>0.05$) increase.

A.6.3 Toxicity of *Tinospora bakis*

Farah *et al*. (2018) evaluated the toxic effect of *Tinospora bakis* roots on body weight, hematology, biochemistry and histopathology on rats. The extract had no adverse effects on hematology, biochemistry and histology of rats at doses of 50 and 500 mg/ kg, but caused significant alteration at dose 2000 mg/kg. White blood cells (WBCs) were significantly ($P<0.05$) increased; Red blood cells (RBCs), Hemoglobin (Hb) and Packed cell volume (PCV) were significantly ($P<0.05$) decreased. Total protein and albumin were significantly ($P< 0.05$) decreased whereas Urea, creatinine, Alanin Transaminase (ALT), Asparate Transaminase (AST) and Alkaline phosphatase were significantly elevated.

Farah *et al*. (2013) screened the aqueous extract of *Tinospora bakis* roots for the discovery of its *in-vitro* activity against *Theileria lestoquardi*. The extract also brought about a slight cytotoxicity at concentration of 10000 ppm.

Abdelrahman and Khalil (2012) evaluated of *Tinospora bakis in-vivo* against *Trypanosoma evansi* infection in rats. The plant was found to be toxic only with the high dose (500 mg kg^{-1} b.wt.) of the chloroformic extract.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Plant materials

The seeds of *Mangifera indica* and the *Rosmarinus officinalis* pods were collected from the market at Khartoum town, and *Tinospora bakis* whole plant was collected from the Blue Nile state during January and May 2019. The plants were taxonomically identified and authenticated by the taxonomists Mr. Yahya Sulieman Mohamed and Mr. Mubarak Khalid at the herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The sample materials were air-dried, under the shadow with good ventilation and then ground finely until their uses for extracts preparation.

3.1.2 Chemicals and Reagents

Analytical grades of the following chemicals and reagent were used for different analyses during the study along with their company identification:

Type of Assay	Chemical	Company
Extractions	Ethanol	Techno pharmchem, India
	Distilled water	NS
Fractionation	N-hexane	SDH Laboratory Reagent, India
	Ethyl acetate	British Drug House, England
	N-butanol	British Drug House, England
	Distilled water	NS
Antimicrobial	Crystal violet	The British Drug House, England.
Activity	Glucose phosphate peptone water	The British Drug House, England.
	Hydrogen peroxide (H_2O_2)	British Drug House, England.
	Immersion oil	British Drug House, England.
	Iodine	Hopkins and William England
	Lactose	Hopkins and William England
	Methanol	Loba Chemie PVT Ltd.
	Methyl red	British Drug House, England.
	Phenol red	British Drug House, England.
	Safranin red	British Drug House, England.
	Sucrose	Oxoid limited, England Sulphuric acid British
	Urea powder	Abott Ltd.
	DMSO	Merck Chemicals, Darmstadt, Germany
	Resazurin	Sigma-Aldrich, Germany
Anti-protozoal	RPMI 1640	Gibco-Brl, Life Technology
Activity	Fetal bovine serum	Bio west, France
	Trepan blue	Gibco, Grand Island, N.Y and USA
	Sodium chloride	British Drug House, England
	Sodium Bicarbonate	British Drug House, England
	Phosphate buffer saline (PBS)	British Drug House, England
	L- Glutamine	British Drug House, England

Type of Assay	Chemical	Company
	DMSO	Merck Chemicals, Darmstadt, Germany
	Distilled water	NS
	Liquid nitrogen	Radiation Safety Institute
Anti-schistosoma activity	Distilled water	NS
Anti-oxidant Activity	DMSO	Merck Chemicals, Darmstadt, Germany
	DPPH	Sigma-Aldrich, USA
	Propyl gallate	Sigma-Aldrich, USA
	Ethanol	Techno pharmchem, India
Cytotoxicity	RPMI 1640	Gibco-Brl, Life Technology
	Sodium chloride	British Drug House, England
	Sodium Bicarbonate	British Drug House, England
	Phosphate buffer saline (PBS)	British Drug House, England
	L- Glutamine	British Drug House, England
	DMSO	Merck Chemicals, Darmstadt, Germany
	Fetal bovine serum (FBS)	Bio west, France
	3-(4,5-Dimethylthiazole-2-yl)-2,5diphenyl tetrazolium bromide (MTT)	Sigma, USA
	Trypsin	Bio west, France
	Liquid nitrogen	Radiation Safety Institute
	trypan blue	Gibco, Grand Island, N.Y and USA Dimethyl
	Distilled water	NS
Phytochemical Screening	Ferric chloride ($FeCl_3$)	Loba chemie, India
	Potassium hydroxide (KOH)	SDH Laboratory Reagent, India
	Hydrochloric acid (HCl)	Loba chemie, India
	Lead acetate (PbAc)	Techno pharmchem, India
	Sulphuric acid (H_2SO_4)	Techno pharmchem, India
	Distilled water	NS
Total Phenols	Folin- Ciocalteau	Techno pharmchem, India
	Sodium carbonate	Scharlau Chemie S.A. Spain
	Gallic acid	Sigma-Aldrich, USA
Total Flavonoids	Sodium nitrite ($NaNO_2$)	Techno pharmchem, India
	Aluminum chloride ($AlCl_3$)	Techno pharmchem, India
	sodium hydroxide	Techno pharmchem, India
Total Tannins	Ferric chloride ($FeCl_3$)	British Drug House, England
	Gelatin test	British Drug House, England
	$K_3Fe(CN)_6$	British Drug House, England
	distilled water	NS
TLC	silica gel	Merck, India
	n-butanol	SDH Laboratory Reagent, India
	acetic acid	Techno pharmchem, India
	Acetic acid	British Drug House, England
	Chloroform	British Drug House, England
	Ethyl acetate	British Drug House, England

Type of Assay	Chemical	Company
	Formic acid	British Drug House, England
	Ethanol	Loba Chemie PVT Ltd
	Methanol	Loba Chemie PVT Ltd
	n-Butanol	British Drug House, England
	Para-dimethyl-amino benzaldehyde	Oxoid Ltd England
	Petroleum ether	British Drug House, England
	Phenol red	British Drug House, England
	Sodium chloride	British Drug House, England
	Sulphuric acid	British Drug House, England
	Tetra methyl-p phenylene diamine-dihydrochloride.	British Drug House, England
	Toluene	(Scharlau ACO154 Euorpean (U) union).
	Acetic acid	Fluka, Switzerland
	Pre-coated Plates DC	Riedel-deHaen, Germany
	Silica gel DG	Riedel-deHaen, Germany
	Silica gel GF254	Riedel-deHaen, Germany
	Vanillin	BDH Chemicals, England
GC-MS	Helium	Liquid air company, Sudan
HPLC	Methanol (HPLC grade)	Scharlu, Spain
	Dichloromethane (HPLC grade)	Scharlu, Spain
NMR	Methanol (CD_3OD)	British Drug House, England
Spectrophotometer	Dichloromethane ($CDCl_3$)	British Drug House, England

Key: NS: Not Specified**.**

3.1.3 Chemotherapeutic agents (Antibiotics drugs)

The chemotherapeutic used for different analyses during the study along with their company identification.

Bio-assay Used	Antibiotics/Standard	Company
Antibacterial	Ampicillin	Amipharma laboratories Ltd. Sudan.
	Gentamicin	Roussel, England**.**
Antifungal	Clotrimazole	General Medicine Co. Sudan.
	Nystatin	Sigma Chemical Company USA.
Antiprotozoal	Metronidazole (Flagyl)	Company; Wujiang Litown pharmaceutical
Anti-oxidant	Propyl gallate	Sigma-Aldrich, USA
Cytotoxicity	Triton X-100	Sigma-Aldrich, USA

3.1.4 Culture media

The culture media used for different analyses during the study along with their company identification.

Bio-assay	Culture Media	Company
Antimicrobial	Muller and Hinton agar	Hi media Laboratories Pvt. Ltd., India
	Muller and Hinton broth	Hi media Laboratories Pvt. Ltd., India
	Nutrient agar	Hi media Laboratories Pvt. Ltd., India
	Nutrient broth	Hi media Laboratories Pvt. Ltd., India
	Blood agar base	Hi media Laboratories Pvt. Ltd., India
	Koser citrate agar	Hi media Laboratories Pvt. Ltd., India
	MacConkey's agar	Hi media Laboratories Pvt. Ltd., India

Bio-assay	Culture Media	Company
	Mannitol salt agar	Hi media Laboratories Pvt. Ltd., India
	Peptone	Hi media Laboratories Pvt. Ltd., India
Antifungal	Sabouraud Dextrose agar	Hi media Laboratories Pvt. Ltd., India
Antiprotozoal	RPMI 1640	Gibco-Brl, Life Technology
Cytotoxicity	RPMI 1640	Gibco-Brl, Life Technology

3.1.5 Equipment and Instruments

The equipment and instruments used for different analyses during the study along with their company identification.

Equipment and Instruments	Company
Autoclave	Griffin and George Ltd, England
Automatic Pipette	Greiner bio-one
Balance type H 6T E.	Mettler, England
Beakers	Isolab – Germany
Capillary tubes	Marienfeld – Germany
Centrifuge	Braun, Centrifuge PLC series
Chromatographic jar	Superfit – India
Conical flasks	Isolab – Germany
Disposable 96-wells plate	Costar, Germany
Eppendorf Tube 1.5	Nalge Nunc, Inter
Falcon Tube 15 ML	Nalge Nunc, Inter
Filter papers	EMD Chemicals Inc. Germany
Filter units (0.45 um)	Nalge Nunc, Inter
Flat bottomed culture tissue	Costar, Germany
Freeze-dryer	Trivac, USA
Funnels	Isolab- Germany
GC-MS	Shimadzu, Japan
Glass column	Duran, Germany
Glass wares	Griffin and George Ltd, England
Hair dryer	Philips-China
Hot air oven	Gallenkamp, England
Incubators	Baird and Tatlock Ltd, England
Measuring cylinders	Witg – Germany
Micro Pipettes and tips	NICHIRYO, Japan
Microscope	Will Wetzlar, Germany
Nuclear Magnetic Rezones (NMR)	Bruker Germany
Oven	Widnes – England
Pasteur pipette	Nalge Nunc, Inter
Petri dishes	Costar, Germany
PH meter	JENWAY, UK
Reflux Condenser	Grant Instruments Ltd
Rotary evaporator	Stuant, Germany
Separatory funnel	Isolab – Germany
Shaker	Sturart scientific, flask Shaker, UK
Sonicator	ELMA, Germany
Soxhlet	Grant Instruments Ltd. England
Spectrum spectrophotometer	Thermo Scientific, Finland

Equipment and Instruments	Company
Spreader	Desaga, Germany
Sterile filter cup	Nalge Nunc, Inter
Syringe	Socorex
Test tube	Nalge Nunc, Inter
UV lamp	UVP – England
UV/Vis. Spectrophotometer	Jenway, England
Vortex	As One, China
Water bath	Grant Instruments Ltd

3.1.6 Microorganisms used to test the biological activities of the plants

Strains Used	Species of microorganisms	Cultivation & conditions	Source
Bacteria	*Bacillus subtilis*		NCTC 8236 (Gram + ve bacteria)
	Staphylococcus aureus	MHA & 37ºC	ATCC 25923 (Gram +ve bacteria)
	Escherichia coli		ATCC 25922 (Gram -ve bacteria)
	Pseudomonas aeruginosa		ATCC 27853 (Gram -ve bacteria)
Fungí	*Candida albicans*	DSA & 25ºC	ATCC 7596 (Yeast fungi)
Parasites	*Entamoeba histolytica*	RPMI & 37ºC	Isolated parasites
	Giardia lamblia		
	Biomphalaria pfeifferi	DW & RT	Snail
Cytotoxicity	Brine shrimp	SW & RT	Larva
	BHK	RPMI & 37ºC	Normal cell line

Key: *Obtained from Department of Microbiology and Parasitology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), **BHK=** Baby hamster kidney, **MHA=** Mueller Hinton Agar, **DSA**= Sabouraud Dextrose Agar, **RPMI**= Roswell Park Memorial Institute, **DW**= Distilled Water, **SW**= Sea water, **RT**= Room temperature **Gram + ve** = Gram positive bacteria, **Gram - ve** = Gram negative bacteria, **NCTC**= National Collection of Type Culture, Colindale, England, **ATCC**= American Type Culture Collection, Rockville, Maryland, USA.

3.2 Experimental Section (Methods)

3.2.1 Preparation of crude extracts

Fresh plant samples were dried in shades for 7 days, powdered and then used for extraction. Extractions were carried out for the different part of plants by using overnight maceration techniques according to the method described by Harbone (1984). About 50 g round material were macerated in 500 ml of ethanol (80%) for 3 days at room temperature. Occasional shaking for 24 h at room temperature was performed and, the supernatant was decanted. Thereafter, the supernatant was filtered under reduced pressure by rotary evaporation at 55°C. Each residue was weighed and the yields percentage were calculated and then stored at 4°C in tightly sealed glass vial ready for use. The remaining extracts which not soluble was successively extracted using ethanol and were kept in deep freezer for 48 h, (Virtis, USA) until they were completely dried. The extracts were kept and stored at 4 °C until required. The residue was weighed and the yield percentage was calculated as follow:

Yield % = (weigh of extract/weigh of sample) X 100

The extracts, were dried and concentrated by using hood extracts and the yield percentage was then calculated Table (4.1).

3.2.2 Fractionation of crude extracts

The most potent crude ethanolic extracts of selected medicinal plants were subjected to polarity based solvent-solvent fractionation as described by Ul-Haq *et al*. (2012). Extracts were dissolved in distilled water then partitioned between n-hexane, ethyl acetate, n-butanol and aqueous using separation funnel**.** As a result n-Hexane fraction (nHF), ethyl acetate fraction (EF), n-butanol fraction (nBF) and aqueous fractions (AF) were obtained and the yield percentage was then calculated. Each fraction was evaporated at 40 °C under reduced pressure in rotary apparatus and stored in air tight containers at 4 °C until further used. The quantity of each fraction obtained after drying was given in Table (4.2).

3.3 General experiment procedure for biological activities

3.3.1 Antimicrobial activity of the plants

A. Preparation of standard bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, to produce a suspension containing about (10^8- 10^9 C.F.U/ ml). The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles *et al*., 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

B. Preparation of fungal suspensions

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

C. *In vitro* testing of extracts for antimicrobial activity

C.1 Testing for antibacterial Activity of the plants

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial of the prepared extracts. 100 mg of each extract was weighed and dissolved in 1 ml of DMSO 10% separately and mixed well which was used to perform antimicrobial screening tests. One ml of the standardized bacterial stock suspension 10^8–10^9 C.F.U/ ml were thoroughly mixed with 100 ml

of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the oil using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Three replicates were carried out for the extracts against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Activity index for each extracts was calculated using following formula (Singariya *et al*., 2011):

Activity index (AI) = Inhibition Zone of the sample/Inhibition Zone of the standard

C.2 Testing for antifungal activity of the studied plants

The same method as for bacteria was adopted, instead of nutrient agar, sabouraud dextrose agar was used, and the inoculated medium was incubated at 25 º C for two days for *Candida albicans*. Three replicates were carried out for the extracts against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated. Activity index for each extracts was calculated as per previous equation (Singariya *et al*., 2011):

Activity index (AI) = Inhibition Zone of the sample/Inhibition Zone of the standard

D. Determination of Minimum Inhibitory Concentrations (MICs)

Determination of Minimum Inhibitory Concentrations (MIC) using different methods:

D.1 Minimum Inhibitory Concentrations (MICs) using solid media (Nutrient agar)

This test was carried out on the extracts which exhibited higher antimicrobial activity against more sensitive organisms. The minimum inhibitory concentrations of the extracts were determined on solid media (Nutrient agar) using the method adopted by Kavanagh (1972). The ranges of concentrations used were (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml).

D.2 Minimum Inhibitory Concentrations (MICs) using resazurin microtiter-plate assay

The minimum inhibitory concentration (MIC) of the isolated compounds were evaluated by a modified resazurin microtiter-plate assay. The broth microdilution method was used to determine the MIC according to the National Committee for Clinical Laboratory Standards Guidelines , 1999)(National, 1999), with modification. Twofold serial dilutions of isolated compounds were prepared directly in a microtiter plate containing Mueller Hinton broth (MHB) to obtain various concentrations. The bacterial inoculum was added to give a final concentration of 5×10^5 CFU/mL in each well. The positive control was used containing amikacin as a standard drug, and 10% DMSO (v/v) solution as a negative control. The plate was covered with a sterile sealer and incubated for 24 h at 37°C. Resazurin (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) was added in each well of the microtiter plate and was incubated at 37°C for 3-4 h. The wells containing the bacterial growth turned into pink color whereas the

well without bacterial growth remained blue. The MIC was considered as the lowest concentration of the isolated compounds that completely inhibits the bacterial growth.

E. Determination of Minimum Bactericidal Concentration (MBCs)

In addition to the solid medium diffusion procedure, the micro-plate bioassay (micro dilution) was used, as recommended by NCCLS, for determination of minimum bactericidal concentration (MBC). The MBC was defined as lowest concentration of crude extracts and isolated compounds kills bacteria after incubation for 18 - 24 h at 37°C. Into each well 100µl of Mueller Hinton broth (MHB) inoculated with the bacteria inoculum prior to the essay. Concentrations used (100 to 078 mg/ml) and (1000 to 31.25 µg/ml) of the crude extracts and isolated compounds, respectively. The MBC was determined by sub-culturing from each test concentrations in the plates were incubated at 37°C for 18 - 24h. All inoculated plates were then incubated for about 18 hours at 37^{o}C and investigated for bacterial growth. Plates with no visible growth were considered to be the MBC for the crude extracts and isolated compounds (Balekar et al., 2012).

F. Positive and negative controls (reference drugs) against standard microorganisms

Four antibiotics were used as reference drugs. These include, Ampicillin and Gentamicin as positive controls for the antibacterial tested. Clotrimazole and Nystatin were used as positive controls for the antifungal tested. Sterilized distilled water (DW) and DMSO 10% were used as negative control for the antibacterial and antifungal analyses.

F.1 Positive controls

F.1.1 Antibacterial activity of reference drugs

Antibacterial drugs were tested at different concentrations obtained by taking 0.1 g of each powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of (40, 20, 10 and 5 µg/ml). These drugs were tested against reference bacteria i.e. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

F.1.2 Antifungal activity of reference drugs

The antifungal drugs were also tested at different concentrations obtained by taking 0.1 g of each powdered drug and dissolved in 100 ml sterile distilled water to give a concentration of 1000 µg/ml followed by serial dilutions to give concentrations of (40, 20, 10 and 5 µg/ml) Clotrimazole and Nystatin against reference fungi *Candida albicans.*

F.2 Negative controls

Distilled water (DW) and DMSO 10% were tested against reference bacteria and fungi i.e. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.*

3.3.2 Anti-protozoal activity of the plants

A. Parasites collection and identification

Entamoba histolytica and *Gairdia lamblia* were used in the experiments were taken from patient with positive amoebiasis and giardiasis.

B. Culture Medium

The transport the samples of trophozoites of *E. histolytica* and *G. lamblia* from hospital to the research institute were maintained in CRPMI medium. Sub culturing of the parasite was performed at 37 ± 1°C in RPMI 1640 medium containing 5% bovine serum. Trophozoites for the assays were employed in the log phase of growth.

C. Parasites Counting

Parasites counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, cleaned carefully with distilled water and swapped with 70% ethanol, then dried. The liquid of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was completed with cell suspension. After parasites had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating parasites:

$$(\mathbf{Parasites/ml})\ \mathbf{N} = \frac{\mathbf{Number\ of\ cells\ counted\ X\ Dilution\ factor}}{\mathbf{4}}\ \mathbf{X}\ 10^4$$

D. *In-vitro* susceptibility assays

In-vitro susceptibility assays used the sub-culture method by Cedillo-Rivera *et al*. (2002). This is highly stringent and sensitive method for assessing the anti-protozoal effects gold standard particularly in *Entamoba histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* (Argüello-García *et al.*, 2004). 5 mg from each honey sample was dissolved in 50 µl of dimethyl sulfoxide (DMSO) in Eppendorf tube containing 950 µl distilled water in order to reach concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20 °C for further analysis. Sterile 96-well microtiter plate was used for different honeys as well as for positive and negative controls. Twenty µl of complete RPMI medium were placed in the wells-except the first three wells C-1 (which 40 µl of an extract solution (5 mg/ml) were added in the first three wells and the final concentrations were 1000 µg/ml. 20 µl of complete RPMI medium were placed in the wells in the following C-2 was 500µg/ml and C-3 which was 250 µg/ml. 80 µl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 µl. Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl5 nitroimidazole], a trichomonocide was used as positive control in concentration 312.5 ppm, whereas untreated cells used as a negative controls (culture medium plus trophozoites). Samples taken for counting at 24, 48, 72, and 96 hours, were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer in triplicate. The mortality % of parasite for each extracts activity was carried out according to the following formula:

$$\mathbf{Mortality\ of\ parasite}\ (\%) = \frac{(\mathbf{Control\ negative\ -\ tested\ sample\ with\ extract})}{\mathbf{Control\ negative}}\mathbf{X100}\%$$

Only 100% inhibition of the parasite considered, when there was no motile parasite observed.

E. Cryopreservation of Parasites

To avoid the loss of the *Entamoeba histolytica* and *Giardia lamblia*, excess *Entamoeba histolytica* and *Giardia lamblia* were preserved in liquid nitrogen as follows: Equal parts of the parasite suspension and freezing medium (10 % DMSO in complete media) were dispersed to cry tubes. The cry tubes were racked in appropriately labeled polystyrene boxes, gradually cooled till reaching -80 ^{o}C. Then the cry tubes were transferred to a liquid nitrogen (-196 ^{o}C).

3.3.3 Molluscicidal activity of the plants

A. Parasite collection and examination

Naturally infected Snails *Biomphlaria pfeifferi* had been collected from the minor canals of Al-Gazira agricultural scheme near Alhasahisa town (about 130 Km south to Khartoum). Snails were transported to the biological assay laboratory in the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), as shown in Appendix (3).

B. *In-vitro* molluscicidal activity

Exploratory experiments were conducted to detect the activity of the plant extracts. Three replications of serial concentrations (500, 250 and 125 ppm) of the plants extracts were used in the exploratory experiments. 50 ml of each concentration was put in same sized beakers. Then 5 snails were put in each beaker in addition to 5 snails in each of three beakers containing 50 ml of distilled water as a control. Readings for molluscicidal activity of the extracts were recorded after 24 and 48 hours of exposure. The mortality of the snail was detected by its motionlessness or discoloration of the shell. Dead snails were counted and then percentage mortality (%) and IC values (µg/ml).

C. Detection of snail death

A snail was considered dead when floating with one of its flat sides uppermost onto the surface of the water with its shell becoming translucent. Some snails settled motionless at the bottom of the dish. Confirmation of the snail death was ensured when it settled motionless into the bottom of the bowl with no response to photo stimulation and mechanostimulation.

3.3.4 Antioxidant activity (DPPH-assay) of the plants

A. Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method of Shimada *et al*. (1992) with slight modification. Active samples can reduce the stable radical DPPH to the yellow-colored diphenyl- picrylhydrazine.

B. Assay: Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37^{o}C in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate.

$$\textbf{DPPH radical scavenging}\ (\%) = \mathbf{100} - \{(\mathbf{Ac} - \mathbf{At})/\mathbf{Ac}\} \times \mathbf{100}$$

Where, At= Absorbance value of test compound; Ac=Absorbance value of control.

C. IC_{50} Calculations

The IC_{50} (the concentration of test material, which possess 50% inhibition of free radicals) of all the extracts and their fractions was determined by monitoring the effect of different concentrations. The IC_{50} of the extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U.S.A).

3.4 Cytotoxicity effect of the plants

3.4.1 Toxicity testing against the brine shrimp

A. Storage of *Artemia salina* eggs

Eggs of *Artemia salina* were stored at low temperatures (4º C), they will remain viable for many years.

B. Hatching shrimp

Brine shrimp eggs, *Artemia salina* were hatched in artificial seawater prepared by dissolving 38g of sea salt in one liter of distilled water. After 24-72h incubation at room temperature (37 °C), the larvae were attracted to one side of the vessel with a light source and then collected with pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing artificial seawater.

C. Brine shrimp assay

Bioactivity of the extract was monitored by the brine shrimp lethality test (Meyer *et al*., 1982a). Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts. 50 mg of *Artemia salina* (Leach) eggs were added to a hatching chamber containing artificial Sea water (75 ml). The hatching chamber was kept under an inflorescent bulb for 48h for the eggs to hatch into shrimp larvae. 20 mg of test extracts of the various plant species were separately dissolved in 2 ml of methanol, then 500, 50, and 5µl of each solution was transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dosage was tested in triplicate. 10 larvae of *A. salina* Leach (taken 48 – 72h after the initiation of hatching) were added to each vial. The final volume of solution in each vial was adjusted to 5ml with Sea water immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LD_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a "Finney Programme." The concentration at which it could kill 50% larvae (LD_{50}) was determined. LD_{50} values below 200ppm are generally considered as significant according to Oladimeji *et al*. (2006)**.**

3.4.2 Micro culture Tetrazolium (MTT-assay)

Micro culture tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of the studied plants**.**

A. Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel *et al*., 2009).

B. Preparation of Extracts, Solutions

Using a sensitive balance 5 mg of each extracts were weighed and put in Eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

C. Cell Line used and Culturing Medium

BHK (Baby Hamster Kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37^{o}C. The cells were subculture twice a week.

D. Cell counting

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

$$\text{(Cells/ml) } N = \frac{\text{Number of cells counted X Dilution factor X } 10^4}{4}$$

E. Procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96-well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete

medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37ºC for 96 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37ºC. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100-\{(Ac-At)/Ac\} \times 100$$

Where, **At** = Absorbance value of test compound; **Ac** =Absorbance value of control.

3.5 Phytochemical Analysis of the plants

3.5.1 Qualitative phytochemical analysis

A. Phytochemical screening

Phytochemical screening is of great importance in providing information about chemicals found in the plant in term of their nature and range of occurrence. This information would enable correlation between the nature and range of occurrence of these chemicals, and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening was conducted to determine the presence of natural products in the extracts samples using standard methods according to (Sofowora, 1996, Trease and Evans, 1983, Harborne, 1984).

A.1 Detection of Alkaloids

7.5 ml of (PE) was evaporated to dryness on a water bath. 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added. While to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harborne, 1984).

A.2 Detection of Flavonoids

A weigh of 0.5 g of the extract was dissolved in 15 ml of 96% ethanol and filtered. The filtrate was used for the following tests: (A) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of yellow color indicated the presence of Flavonoids, (Flavones and / or chalcone). (B) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of the Flavonoids compounds (flavones or flavanones) chalcone and/or flavonol. (C) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Producing of defiant

color to pink or red was taken as presumptive evidence that flavanones were present in the plant sample (Harborne, 1984).

A.3 Detection of Tannins

A weigh of 0.5 g of the extract was stirred with 10 ml of hot saline solution. The mixture was cooled and filtered. About 5 ml of this solution was treated with few drops of the Gelatin-salt reagents. Formation of an immediate precipitate was taken as evidence for the presence of tannins. Positive test confirmed by the addition of few drops of 1% $FeCl_3$. The formation of blue, black or green was taken as an evidence for the presence of tannins.

A.4 Detection of Saponins

A weigh of 0.5 g of the extract was placed in a clean test tube and 10 ml of distilled water was added. The test tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of Saponins.

A.5 Detection of Anthraquinone

A weigh of 0.5 g of the extract was boiled with 10 ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated when the alkaline layer was found to have assumed pink or red color.

A.6 Detection of Coumarins

A weigh of 0.5 g of the extract was dissolved in 10 ml distilled water in test tube and filter paper was attached to the test tube to be saturated with the vapour after a spot of 0.5N KOH was put on it. Then the filter paper was inspected under ultra violet (UV) light, the presence of coumarins was indicated when the spot absorbed the ultra violet (UV) light.

A.7 Detection of sterols and triterpenes

A weigh of 0.5 g of the extract was dissolved in 10 ml chloroform, 0.5 ml acetic anhydride (0.5 ml) was added and the solution was transferred into a dry test tube. Few drops of concentrated sulphuric acid were poured carefully down the walls of the test tube so as to form a lower layer. Brownish-red or violet ring at the zone of the contact with supernatant and green or violet coloration denoted the presence of sterols and /or triterpenes pink to purple.

A.8 Detection of Phenols

To 1ml of extract, 2 ml of distilled water were added followed by few drops of 10% ferric chloride ($FeC1_3$). Appearance of blue or green colour indicates the presence of phenols.

B. Chromatographic analysis for thin layer chromatography (TLC)

Phytochemical constituents of the crude extracts were analyzed by thin layer chromatography (TLC) as described by Eloff *et al*. (2007). The following developed in different solvent systems (eluent) were use (Bladt, 2009). Development of the chromatograms was done in a closed tank in which the atmosphere had been saturated with the eluent vapour. The plates were then dried overnight under a stream of air to remove excess solvent. Visible bands were viewed under daylight and under UV light at 245 nm and 365 nm respectively, as shown in Appendix (4).The plates were carefully heated at 105°C for optimal colour development (Masoko *et al*., 2008).

Table (3.3): Chromatographic analysis by thin layer chromatography (TLC) using different solvent system:

Compounds	Solvent system	Reagents
Anthraquinone	Ethyl acetate:methanol:water (**100:13.5:10**)	KOH reagent
Tannins	Ethyl acetate:methanol:water (**100:13.5:1.0**)	Berlin blue UV 365
Alkaloids	Toluene:ethyl acetate:diethylamine (**70:20:10**)	Dragendorff
Flavonoids	ethyl acetate:formicacid:glacial acetic acid:water (**100:11:11:26**)	NP/PEG reagent UV-365nm
Saponins	Chloroform:glacial acetic acid – methanol:water (**64:32:12:8**)	AS reagent

B.1 Rétention factor (R_f) values of compound

Characterization of the different compounds identified was done by calculating their R_f values in the different TLC system (Masoko *et al*., 2005) Briefly, the solvent front was marked on the TLC plates immediately after removing it from the chamber, and allowed to dry before visualizing the bands relating to different compounds. The R_f value was calculated by the following equation:

$$\textbf{Rf value} = \frac{\textbf{Distance moved by the compound from the origin to spot center}}{\textbf{Distance moved from origin to solvent}}$$

B.2 Preparation of spraying reagents for TLC

B.2.1 Potassium hydroxide: Potassium hydroxide (1 g) was dissolved in 100 ml éthanol used for détection of anthraquinone.

B.2.2 Berlin blue: A freshly prepared solution of 10 g iron Chloride and 0.5 g potassium hexacyanoferrate in 100 ml water. The plate is sprayed with 5-8 ml and evaluated in vis; used for détection of tannins.

B.2.3 Dragendorff' reagent: A/ 0.85 g bismuth sub nitrate was dissolved in 10 ml glacial acetic acid and 40 ml distilled water. **B/** 20 g potassium iodide was dissolved in 50 ml distilled water. Solutions (A and B) were left over night, equal volumes of them were mixed and 20 ml of glacial acetic acid added then completed to 10 ml with distilled water used for detection of alkaloids.

B.2.4 Natural produets-polyethylenglyeol reagent (NP/PEG): The plate is sprayed with 1 % methanolic diphenylboric acid-ß-ethylamino ester (= diphenylboryloxyethylamine) (NP), followed by 5% ethanolic polyethyleneglycol4000 (PEG) (10 ml and 8 ml, respectively) used for detection of flavonoids.

B.2.5 Anisaldehyde-sulphuric acid reagent (AS): 0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order. The re agent has only limited stability, and is no longer useable when the colour has turned to red-violet. The TLC plate is sprayed with ab out 10 ml, heated at 100 °C for 5-10 min, then evaluated in vis. or UV-365 nm, used for detection of saponins.

3.5.2 Quantitative phytochemical analysis

A. Determination of total contents

A.1 Determination of total phenolics content (TPC)

Total phenolic content was assessed approximately by using Folin-Ciocalteau Phenol reagent through the method by Wolfe *et al*. (2003), with slight modification (Ainsworth and Gillespie, 2007) using standard curve generated with gallic acid as a reference. 0.5 mL of extract (1 mg/mL) was mixed with 0.4 mL Folin-Ciocalteu reagent (diluted with water 1:10 v/v) and 0.910 mL (75%) sodium carbonate. The mixture was allowed to stand for 90 min at room temperature for colour development. Absorbance was measured at 765 nm using multiplate reader spectrophotometer. Total phenolic content is expressed as mg gallic acid equivalent (GAE) per g of dry weight, and was determined using the equation based on the calibration line: $Y = 0.0019x + 0.013$, where y is the absorbance and x is the gallic acid equivalent (mg/g) and $R^2 = 0.995$.

A.2 Determination of the total flavonoids content (TFC)

The total flavonoid content was measured using a modified colorimetric method by Kim *et al*. (2003) with some modifications. 1 mL of extract (1 mg/mL in methanol) received 0.3 mL of 5% $NaNO_2$, 0.3 ml of 10% $AlCl_3$ and 2 ml of 1 M NaOH at 5 min intervals. The absorbance was measured against the blank at 510 nm after incubation time of 15 minutes. The standard curve was prepared using different concentrations of Quercetin as a reference. The flavonoid content was expressed as mg Quercetin equivalents (QE) per g of dry weight, and was determined using the equation based on the calibration line: $Y = 0.00953x + 0.0024$, where y is the absorbance and x is the Quercetin equivalent (mg/g) and $R^2 = 0.997$.

A.3 Determination of the Total tannin content (TTC)

The tannins content was determined by using $FeCl_3$ and gelatin test method by Shivakumar *et al*. (2012), with some modification. One ml of extract (1 mg/ml) was transferred to vials, 1ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ were added, and the volume was made up to 10 ml with distilled water. After 5 min absorbance was measured at 510 nm against a reagent blank. The experiment was conducted in triplicate analysis. The total tannin content was determined using a calibration line prepared with tannic acid standard as a reference. The total tannin content was expressed as mg of tannic acid equivalent per gram dry weight of residues.

3.5.3 Quantitative and qualitative analysis

A. Gas Chromatography–Mass Spectrometry (GC-MS) analysis

GC-MS technique was used to identify the phytocomponents present in the crude extracts. The tested extracts were analyzed by GC-MS using Shimadzu (Japan) Mass Spectrometer-2010 series at International Center for Chemical and biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan. 1 µL of sample was injected in GC-MS equipped with a split injector. The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min. The analytical column connected to the system was an Rtx-5 capillary column (length-30 m × 0.25 mm i.d., 0.25 µm film thickness). The column head pressure was adjusted to 93.9 kPa. Column temperature programmed from 110 °C (7 min) to 200 °C at 10 °C/min and from 200-280 °C at 5 °C/min withhold time 0 and 9 min respectively. A solvent delay of 4.50 min was selected. The injector temperature was set at 250 °C, and the GC-MS interface was maintained at 280 °C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

3.6 Bio-guided isolation of active compounds

Based on the results of preliminary biological activities; antimicrobial, antiprotozoal, anti-schistosoma and antioxidant properties, only two fractions were selected for isolation and structural elucidation of bioactive principles. Based on results the most potent of ethyl acetate fractions from *M. indica* and *T. bakis* were selected for isolation of antimicrobial and antioxidant compounds which show superior antibacterial and antioxidant activity against all of the tested pathogens.

For bioactive principles isolated compounds from selected fractions chromatographic techniques were used. Major isolation compounds were carried out by using column chromatography (Normal and Reverse phase) and silica gel of different partial sizes, RP-C18 and Sephadex LH-20. Purification of compounds was done by preparative TLC. High Performance Liquid Chromatography (HPLC) was used as it required.

3.6.1 Thin layer chromatography (TLC)

The precoated TLC plates (TLC Silica gel F_{254}, Merck, Germany) were trimmed to desired size strips and the position of the origin (2 cm above the bottom of TLC strip) and end (1 cm below the top of TLC strip) was marked by a straight line.

The selected fractions were spotted on the origin and put in a lidded tank containing desired solvent system. The level of solvent system in the tank was about 1 cm beneath the origin. The solvent travelled up (move) the plate by capillary action till it reached the solvent front (end). TLC strip was removed from the solvent tank and dried. The TLC plates were visualized by spraying reagents solutions and under UV light at 254 nm, as shown in Appendix (4). The procedure was followed with other strips and various solvent-solvent

ratios were tried until good resolution was noticed. The retention factor (R_f) values of all the spots were determined by the following formula:

$$\textbf{Rf value} = \frac{\textbf{Distance moved by the compound from the origin to spot center}}{\textbf{Distance moved from origin to solvent}}$$

A. Spraying Reagents for TLC

TLC analyses were done on silica gel 60 F_{254} plates developed in the organic solvent. TLC spraying reagents used for identification of compounds were ceric sulphate reagent, vanillin reagent, Dragendorff"s reagent, 10% H_2SO_4 and aluminum chloride ($AlCl_3$) reagents.

3.6.2 Column Chromatography (CC)

The ethyl acetate fractions of *M. indica* and *T. bakis* were subjected to column chromatography to obtain purified bioactive compounds. The column was packed with a solution of silica gel (60-120 mesh) with DCM using wet slurry method, as shown in Appendix (5). This involves preparing a solution of silica gel, with DCM and subsequently adding this until the column was three-fourth filled. The solution was stirred for dispersal and quickly added to the column before get settled down. This method was used to prevent the trapping of air bubbles. A ball of cotton wool was pushed into the column to settle at for the packed silica gel. A substantial amount of DCM was poured continuously into the column and allowed to drain, but prevented from reaching the cotton wool. The collected solvent was poured back into the column. Fraction adsorbed silica gel was loaded into the pre-packed column and eluted with desired solvent system by increasing the polarity of solvent. All the fractions were eluted and collected in dry glass container. Each obtained fraction was dried and tested for required bioactivity using standard methods (as mentioned in the previous sections antimicrobial and antioxidant activities).

The fractions showing highly potential biological activity, was selected and monitored by TLC. Based on the TLC profile, further purifications of selected fractions were done by series of column chromatography using silica gel and eluted with desired solvent systems. All the column fractions were continuously examined for their required bioactivity and TLC profile until a single bioactive compound was obtained. Finally, the R_f values of purified bioactive fractions (single spotted) were measured in TLC. Those fractions with similar R_f values were combined together and kept for further biological screening and structural elucidation. The following separation systems were used:

A. Normal phase: Silica gel

Silica gel (silica gel 60, 230-400 mesh), as shown in Appendix (5-A). (Sigma-Aldrich) with pore different size were used as required for column chromatography using dichloromethane: methanol (1:0 to 0:1) mobile phase increasing polarity. Sufficient quantity of silica gel was added in dichloromethane (DCM) to make slurry and then loaded to column avoiding air bubbles. Sample was adsorbed on small amount of dry silica gel and this dry powder of silica with sample was loaded at the top of column. One table spoon of dry sand was added at the top of sample to maintain uniformity of sample layer. Mobile phase was used in order of increasing polarity starting from dichloromethane followed by methanol in different ratios**.**

B. Reverse phase: C-18

For reverse phase column chromatography, reverse phase silica was used, and the sample was loaded in silica by using methanol and water in different ratios. In reverse phase column chromatography, mobile phase was used in order of decreasing polarity started with methanol: water (20:80%) followed by methanol: water (40:60%), methanol: water (60:40%), methanol: water (80:20%) and finally 1000 ml of methanol (100%). Fractions were collected and TLC plates were developed as in normal phase column chromatography, as shown in Appendix (5-B).

C. Stationary Phase: Sephadex LH-20

Sephadex LH-20, as shown in Appendix (5-C), was dissolved in methanol for overnight and then it was packed in glass column with continuous flow of methanol by gravity (without applying pressure). The sample was dissolved in methanol and was loaded at the top of Sephadex LH-20. When sample moved into the Sephadex LH-20 then the top of the column was filled with methanol. Fractions were collected and subjected to TLC as described in normal phase column chromatography.

3.6.3 High Performance Liquid Chromatography (HPLC)

Analytical HPLC, as shown in Appendix (6), was used to identify the interesting peaks from fractions as well as to evaluate the purity of isolated compounds. The gradient used started with 10:90 (MeOH: H_2O to 100% MeOH) in 35 min. Peaks were detected by UV-Vis diode array detector. Semi-preparative HPLC was used for the isolation of pure compounds from fractions previously separated using column chromatography. Each injection consists of about 2-5 mg of the fraction dissolved in 1 ml of the solvent system. The solvent system consisting of MeOH was pumped through the column at a flow rate of 1 ml/min. The eluted peaks which were detected by the online UV detector were collected separately in vials.

3.7 Identification and structural elucidation of isolated compounds

The isolated compounds identification and structural elucidations were conformed using various techniques and spectral data such as 1D NMR (^{1}H, ^{13}C and DEPT), 2D NMR (HMBC, HSQC and COSY) and Mass Spectrum (MS) analysis, etc, are shown in Appendix (6 – 10).

3.7.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

Identification of isolated compounds of ethyl acetate fractions, was performed by using one and two dimensional technique NMR techniques at International Center for Chemical and biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan. And C-NMR is used to identify the types of carbon present in the compound. H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected. 1D NMR spectra (^{1}H and ^{13}C) were recorded from a Varian Unity 500 at 500 MHz for ^{1}H, and at 100 MHz for ^{13}C NMR. 2D NMR spectra (HSQC, HMBC and COSY) were recorded from a Varian Inova 600 at 600 MHz for ^{1}H and 150 MHz for ^{13}C. $CDCl_3$ and CD_3OD were used as NMR solvents used as an internal standard. Chemical shifts were measured in δ scale in ppm.

3.7.2 Mass Spectrometry (MS)

Identification of isolated compounds of ethyl acetate fractions were performed by using mass spectrometer (MS), at International Center for Chemical and biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan, determines the mass of a molecule and this is achieved by measuring the molecule`s m/z ratio. The molecular weight of sample can be determined by MS spectrum.

3.8 Statistical analysis

All the experiments were conducted in triplicates and results were shown as mean ± standard deviation. Statistical analysis for all the assays results were done using Microsoft Excel Program (2016). Regression line equations were used to calculate IC_{50} and LD_{50}. All the grouped data were statistically evaluated and the significance of various treatments were calculated using one-way ANOVA. Database management and statistical analyses were carried out using SPSS (Statistical Package for the Sociological Sciences) data analysis software version 26.0.

CHAPTER FOUR

RESULTS AND DISCUSSION

CHABTER FOUR

RESULTS AND DISCUSSION

This research was conducted on three medicinal plants with different families namely; *Mangifera indica* L. (Family: Anacardiaceae), *Rosmarinus officinalis* L. (Family: Lamiaceae) and *Tinospora bakis* (A. Rich) Miers (Family: Menispermaceae). They were investigated for their biological activities viz; antimicrobial, antiprotozoal, molluscicidal and antioxidant activities, and cytotoxicity viz; MTT-assay and Brine shrimps, and phytochemical analysis (Qualitative and quantitative), viz; (isolation, identification and characterization). The active fractions of ethyl acetate were subjected to chromatographic (TLC, CC and HPLC); and the chemical structures of isolated compounds were elucidated using (1D-NMR, 2D-NMR and MS) to their bioactive compound(s).

4.1 Extractions and fractionations of the plants

4.1.1 Yields of extraction

Extractable matter percentage is one of the parameters used for the characterization of botanical drugs (Gami and Parabia, 2010). Some selected medicinal plants were extracted by ethanol (80% v/v) using cold maceration method of extraction for all botanical samples at room temperature and after complete evaporation to dryness, samples showed the following yield percentages. The yields percentage obtained from crude ethanolic extracts ranging from (11.78 – 22.40%). The *T. bakis* had the highest yield percentage with (22.40 %), this high yield could be due to its content of highly polar compounds. This was followed by *R. officinalis* extraction yield (14.17%), and *M. indica* extract showed the lowest extraction yield (11.78%), as shown in Table (4.1). There were a significant difference in the yields obtained. The efficiency of ethanol in the extraction of phytochemicals has been reported in other studies (Siddhuraju and Becker, 2003). These results seem to be consistent with others confirming ethanol as a good solvent for extraction of bioactive compounds from plants as it gave the highest yield in plants studied. This variation in colour and texture could be attributed to the kinds of chemical components of each extract. The *M. indica* and *T. bakis* ethanolic extracts were brown in colour and their texture generally appeared to be gummy, while, *R. officinalis* extract varied in colour and texture from green and powder respectively. The ethanolic crude extracts were investigated for their biological activities, cytotoxicity and phytochemical analysis.

Table (4.1): Yield percentages of crude ethanolic extracts of the selected medicinal plants:

No.	Scientific Name of Plants	Family Name	Weigt of Plants (g)	Part Used	Weight of extracts (g)	Yield (%)	Color	Texture
1	***Mangifera indica***	Anacardiaceae	**50**	**S.**	5.890	11.78	Brown	Gummy
2	***Rosmarinus officinalis***	Lamiaceae		**P.**	7.085	14.17	Green	Powder
3	***Tinospora bakis***	Menispermaceae		**WP.**	**11.20**	**22.40**	Brown	Gummy

Key: g = Gram, **(%) =** Percentage, **S. =** Seeds**, P. =** Pods, **WP. =** Whole plant, **the highly yield of extract was written in bold.**

4.1.2 Yields of fractionations

The crude ethanolic extracts of the selected medicinal plants, after complete evaporation to dryness, were fractionated with different solvents with gradual polarity including; hexane (polarity index = 0.1), ethyl acetate (polarity index = 4.4), n-butanol (polarity index = 4.0) and water. The yield percentages obtained from fractions were ranging from (0.2– 54.25%), and the ethyl acetate fractions produced the highest yields compared to the other solvents tested. The ethyl acetate fractions of *M. indica, R. officinalis* and *T. bakis* were 54.25, 47.10 and 40.75% respectively. The n-hexane fractions of *M. indica, R. officinalis* and *T. bakis* were 0.2, 14.05 and 10.25% respectively. The n-butanol fractions of *M. indica, R. officinalis* and *T. bakis* were 16, 12.50 and 30.50% respectively. And the aqueous fractions of *M. indica, R. officinalis* and *T. bakis* were 28.50, 24.90 and 18.50% respectively (Table 4.2). The fractions were evaluated for some biological activities and cytotoxicity.

Table (4.2): Yield percentages of fractions of crude ethanolic extracts of the selected medicinal plants:

No	Scientific Name of Plants	Solvents	Weight of Extracts (g)	Weight of Fractions (g)	Yield percentage (%)	Colour
1.	***Mangifera indica***	**n-hexane**	**20**	0.04	0.2	White
2.		**Ethyl acetate**		**10.85**	**54.25**	Yellow
3.		**n-Butanol**		3.2	16	Yellow
4.		**Aqueous**		5.70	28.5	Yellow
5.	***Rosmarinus officinalis***	**n-hexane**		2.81	14.05	Green
6.		**Ethyl acetate**		**9.42**	**47.1**	Green
7.		**n-Butanol**		2.50	12.5	Yellow
8.		**Aqueous**		4.98	24.9	Yellow
9.	***Tinospora bakis***	**n-hexane**		2.05	10.25	Yellow
10.		**Ethyl acetate**		**8.15**	**40.75**	Yellow
11.		**n-Butanol**		6.10	30.50	Brown
12.		**Aqueous**		3.70	18.50	Brown

Key: g = Gram, **(%) =** Percentage, **the highly amount yield of fractions was written in bold.**

4.1.3 Yields of fractionations by column chromatographic technique

A. *M. indica* (seeds)

The fraction of ethyl acetate from *M. indica* (seeds) has shown good biological activities; it was selected for further investigation on the basis of the bioactivity guided approach. The yields percentage obtained from fractions were ranging from (0.10 – 27.30 %). TLC was used with gradient mixtures of Dichloromethane: Methanol (DCM: MEOH) to obtain other known compounds. The yields percentage, as shown in Table (4.3), all fractions collected (F_1–F_{19}) were subjected to column chromatography using silica gel, C-18 and sephadex as purification compounds. The good amount (weight) of fractions collected (F_1–F_{19}) were obtained and evaluated for their antimicrobial and antioxidant activities. Some fractions collected (F_1–F_{19}) were subjected to column chromatography used silica gel, C-18 and sephadex as purification compounds. The fraction 16 had the highest yield percentage with (27.30%).

Table (4.3): Yields percentage (%) of fractionation of the ethyl acetate fraction from *Mangifera indica* (seeds) by column chromatographic technique:

No. of Fractions	Weight of Fraction (g)	Weight of Compounds (g)	Yield percentage (%)	Colour	Texture
F_1	**28**	0.027	0.10	Yellow	Gummy
F_2		0.271	0.97	Yellow	Oil
F_3		0.025	0.09	White	Powder
F_4		0.145	0.52	Brown	Gummy
F_5		0.009	0.03	Brown	Gummy
F_6		0.003	0.01	Brown	Gummy
F_7		0.092	0.33	Brown	Gummy
F_8		0.089	0.32	Brown	Gummy
F_9		2.678	9.56	Brown	Gummy
F_{10}		0.226	0.81	White	Powder
F_{11}		0.153	0.55	Brown	Gummy
F_{12}		0.213	0.86	Brown	Gummy
F_{13}		0.187	0.67	Brown	Gummy
F_{14}		0.112	0.40	Brown	Gummy
F_{15}		0.318	1.14	Brown	Gummy
F_{16}		7.644	27.30	Brown	Powder
F_{17}		5.674	20.26	Brown	Powder
F_{18}		0.236	0.84	Brown	Gummy
F_{19}		1.311	4.68	Brown	Gummy

B. *T. bakis* (whole plant)

The fraction of ethyl acetate from *T. bakis* (whole plant) has shown better biological activities; it was selected for further investigation on the basis of the bioactivity guided approach. The yields percentage obtained from fractions were ranging from (0.05 – 14.52 %). TLC was used with gradient mixtures of Dichloromethane: Methanol (DCM: MEOH) to obtain other known compounds. The yields percentage are shown in Table (4.4). The fraction 8 had the highest yield percentage with (14.52%).

Table (4.4): Yields percentage (%) of fractionation of the ethyl acetate fraction from *T. bakis* (whole plant) by column chromatographic technique:

No. of Fractions	Weight of Fraction (g)	Weight of Compounds (g)	Yield percentage (%)	Colour	Texture
F 1	**560**	1.0595	0.66	Yellow	Gummy
F 2		0.0791	0.05	Yellow	Gummy
F 3		0.01243	0.008	Brown	Gummy
F 4		0.2403	0.150	Brown	Gummy
F 5		0.8513	0.532	Brown	Gummy
F 6		7.1113	4.44	Brown	Gummy
F 7		10.2702	6.42	White	Powder
F 8		23.2394	14.52	White	Powder
F 9		17.2258	10.77	Brown	Gummy
F 10		1.9798	1.237	Brown	Gummy
F 11		2.0581	1.286	Brown	Gummy
F 12		2.5021	1.564	Brown	Gummy
F 13		3.637	2.273	Brown	Gummy
F 14		2.3758	1.485	Brown	Gummy
F 15		2.0986	1.312	Brown	Gummy
F16		10.4809	6.550	Brown	Gummy
F 17		5.7726	3.608	Brown	Gummy
F 18		3.3149	2.072	Brown	Gummy
F 19		5.3116	3.319	Brown	Gummy
F 20		3.2578	2.036	Brown	Gummy
F 21		1.0637	0.665	Brown	Gummy

Key: F= Fraction.

4.2 Biological activities

4.2.1 Antimicrobial activity

The antimicrobial activity of ethanolic (80%) crude extracts, fractions and compounds of some selected medicinal plants used medicinally in Sudanese traditional medicine: (viz; *Mangifera indica* (seeds), *Rosmarinus officinalis* (pods) and *Tinospora bakis* (whole plant)) were investigated for their antibacterial and antifungal activities against standard microorganisms against two Gram positive (+) bacteria (*Bacillus subtilis* (NCTC 7596)), and (*Staphylococcus aureus* (ATCC 25923)), two Gram negative (-) bacteria (*Escherichia coli* (ATCC 25922)) and (*Pseudomonas aeruginosa* (ATCC 27853)), as well as one fungi against (*Candida albicans* (ATCC 9763)) comparable with antibiotics (reference drugs), against antibacterial and antifungal drugs. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using the cup plate agar diffusion method and resazurin microtiter-plate assays.

B. Interpretation of results

All average of the diameters of the growth inhibition zones obtained in the experiments has been shown in Table (4.5). The results were interpreted in terms of the commonly used terms " Sensitive intermediate and resistant. On the basis of the results obtained with standard chemotherapeutic agents against the same

standard tested organisms Table (4.5), extracts resulting in more than (18 mm) growth inhibition zone are considered to possess high antimicrobial activity, those resulting in (14-18 mm), are of intermediate activity and those resulting in zone below (14 mm) are inactive (Barry, 1976, Cruickshank, 1975).

C. Screening of antimicrobial activity of the plants and antibiotics

The results revealed that, the extracts showed the highest inhibition zone more than (18 mm) diameter against all tested standard microorganisms, with the inhibition zone ranging from (20 to 37 mm) at concentration (100 mg/ml). While, the negative controls showed that solvents (DMSO 10% and Distil water) without extracts had no inhibitory effect (-) on bacterial and fungal growth.

The ethanolic extract of *M. indica* exhibited inhibitory effects against all standard microorganisms tested at different concentrations used, with the inhibition zone ranging from (24 to 37 mm) in length. It showed high activity inhibition zone against *Ps. aeruginosa* (37 mm), followed by *S. aureus* (36 mm), *E. coli* (31 mm), *C. albicans* (25 mm) and *B. subtilis* (24 mm) against the microorganisms tested, as shown in Table (4.5) and Figure (4.1). Therefore this result showed that the extract tested inhibited the growth of all microorganisms with their varied sensitivities whereas; the Gram negative and Gram positive bacteria are more active than the fungi tested. The ethanolic extract with the highest activity against *P. aerugonosa*, *S. aureus* and *E. coli* were less sensitive and *B. subtilis* more susceptible. This result similarly have been obtained by scholars (El-Mahmood *et al*., 2008, Sanusi *et al*., 2011), and by El-Gied *et al*. (2012) except that in their study, *B. subtilis* was not tested with the extract of *M. indica*.

The finding is in line with the findings of Khammuang and Sarnthima (2011), where they reported that different mango kernel extract showed interesting antibacterial activity against both Gram positive and Gram negative bacteria as determine by disc diffusion method and the most sensitive strain inhibited by all extracts was *Pseudomonas aeruginosa*. In one of another study conducted in USA, ethanolic and methanolic extract of mango leaf showed relatively high zones of inhibition against *S. typhi*. Both ethanolic and methanolic extract of mango leaf showed inhibition zones of >13.0 mm and <16.0 mm at 50 and 100 mg/ml (Zakaria *et al*., 2006). And Ouf *et al*. (2020) found similar result with the oil of *M. indica* except that in their study, *B. subtilis* and *C. albicans* were not tested. Several reports on the antimicrobial activity of extract *M. indica* have been made in the literature (Mushore and Matuvhunye, 2013, Vaghasiya *et al*., 2011, Bbosa *et al*., 2007).

The antimicrobial activity of the extracts may be attributed to the presence of phytoconstituents such as triterpenoids, flavonoids and tannins. Similarly (Passi *et al*., 2009) showed that plant containing triterpenoids exhibit antimicrobial activity, and Cushnie and Lamb (2005) work reviewed the antimicrobial activity of flavonoids. Thus, the antibacterial activity of the extracts may be attributed to the presence of various phytoconstituents present in the extracts.

In addition, in the present study for GC-MS was percentage 1, 2, 3-Benzenetriol (68.61 %) highly amount. 1, 2, 3-Benzenetriol (Pyrogallol) is known as one of phenolic (hydrolysable tannin) (Khanbabaee and Van

Ree, 2001). 1, 2, 3-Benzenetriol (Pyrogallol) phenolic known to possess antimalarial, antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, anticancer and cytotoxic activities (Beulah *et al*., 2018).

The *R. officinalis* extract was found to have high activity against all of the standard microorganisms tested, with the inhibition zone ranging from (20 to 33 mm) in length. The extract showed higher activity in more than (18 mm) growth inhibition zone against all microorganisms tested, showed high activity inhibition zone against *E. coli* (33 mm), followed by *S. aureus* (27 mm), *B. subtilis* (23 mm), *Ps. aeruginosa* and *C. albicans* (20 mm) against the standard microorganisms tested, as shown in Table (4.5). Therefore this result showed that the extract tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied. Therefore this result showed that the Gram negative and Gram positive organisms are more active that the fungi tested.

This result was similar by Sekretar *et al*. (2004) except that in their study, *E. coli* and *Ps. aeruginosa* were not sensitive to the extract and that *C. albicans* was not tested. On the other study, similar to that reported by Lahlou *et al*. (2019) except that in their study, *B. subtilis* and *C. albicans* were not tested. The results indicated that the rosemary extracts showed antibacterial activity, according to Weckesser *et al*. (2007), mainly against the Gram-positive bacteria (*S. aureus* and *B. subtilis*). The extract also exhibited an effect against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi against *C. albicans*.

The *T. bakis* extract showed high activity against all of the tested standard microorganisms with the inhibition zone ranging from (20 to 25 mm) in length. The extract high activity in more than (18 mm) growth inhibition zone against all microorganisms tested, showed high active inhibition zone against *C. albicans* (25 mm), followed by *B. subtilis* (22 mm), *Ps. aeruginosa* (21 mm), *S. aureus* and *E. coli* (20 mm) against the standard microorganisms tested (Table 4.5). Therefore this result showed that the extract tested inhibited the growth of all microorganisms though the sensitivities of microorganisms varied. Therefore this result showed that the fungi and Gram positive organisms are more active than the Gram negative tested. There were no reports this plant on the antimicrobial investigations of this species.

This results may be due to the increasing in the amount of antimicrobial compounds for these plant such as triterpenes and phenolic compounds, according to the results obtained from the analysis of extract using GC-MS.

All tested medicinal plants contain phytochemicals like saponins, steroids, glycosides and flavonoids, which indicated that plants rich in tannin and phenolic compounds, may possess antimicrobial activities against a number of microorganisms (Riaz *et al*., 2010, Parekh and Chanda, 2007).

Compared to reference antibiotics, the selected plants exhibited a broader spectrum of antibacterial activity and were found to be clearly superior in all tested microorganisms. The *M. indica* seed extract showed highest potent than the all tested antibiotics. The *M. indica* extract showed inhibition against all tested bacteria higher than 40 µg/ml Ampicillin, and inhibited *S. aureus* and *Ps. aeruginosa* higher than 40 µg/ml Gentamicin. The ethanolic crude extract of *M. indica* inhibited against *C. albicans* tested higher more than

50 µg/ml Nystatin. The ethanolic crude extract of *R. officinalis* pods dissolved in DMSO (10%) inhibited all bacteria tested more higher than 40 µg/ml Ampicillin, *E. coli* higher than 40 µg/ml Gentamicin and *C. albicans* higher than 50 µg/ml Nystatin. The whole plant extract of *T. bakis* dissolved in DMSO (10%) inhibited all bacteria higher than 40 µg/ml Ampicillin except *S. aureus* and *C. albicans* higher than 50 µg/ml Nystatin (Table (4.5).

The percentage activity of tested plant extracts demonstrates the total antimicrobial potency of particular extract. It shows numbers of bacterial and fungi susceptible to one particular extract. Ethanolic crude extracts of *M. indica*, *R. officinalis* and *T. bakis* in particular showed noticeable efficiency (100% activity) against the different microorganisms tested (Table 4.6).

Table (4.5): Antimicrobial activity of ethanolic extracts and reference drugs (controls) against standard microorganisms used cup plate method at concentration (100 mg/ml):

No.	Name of drugs	Families/controls	MZID* (mm) ± SD				
			Bacteria*				Fungi**
			Gram (+ve)		Gram (-ve)		
			B. s	***S. a***	***E. c***	***Ps. a***	***C. a***
1.	***Mangifera indica***	**Anacardiaceae**	24 ± 0.08	**36 ± 0.01**	**31 ± 0.08**	**37 ± 0.05**	25 ± 0.01
2.	***Rosmarinus officinalis***	**Lamiaceae**	23 ± 0.05	27 ± 0.02	**33 ± 0.01**	20 ± 0.08	20 ± 0.05
3.	***Tinospora bakis***	**Menispermaceae**	22 ± 0.02	20 ± 0.05	20 ± 0.04	21 ± 0.05	25 ± 0.01
4.	**DMSO 10%**	**Control -ve**	-	-	-	-	-
5.	**Water**	**Control -ve**	-	-	-	-	-
6.	**Ampicillin**	**Control +ve**	15 ± 0.01	25 ± 0.02	-	16 ± 0.02	ND
7.	**Gentamicin**	**Control +ve**	29 ± 0.01	**35 ± 0.01**	**32 ± 0.01**	23 ± 0.02	ND
8.	**Clotrimazole**	**Control +ve**	ND	ND	ND	ND	**42 ± 0.01**
9.	**Nystatin**	**Control +ve**	ND	ND	ND	ND	17 ± 0.02

Key: ***B.s***= *Bacillus subtilis*, ***S.a***= *Staphylococcus aureus*, ***E.c***= *Escherichia coli*, ***Ps.a***= *Pseudomonas aeruginosa* and ***C.a***= *Candida albicans.* MDIZ* (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition zone, ND: not determined, concentration used 100 mg/ml of 0.1ml/cup and 40 µg/ml for extracts and drugs, respectively. **The active extracts and drugs was highest than (> 30 mm) written in bold,** Values expressed as Mean ± standard error (SEM); **n**=3 in each group, Inhibition zones were measured in mm and cup diameter was included.

Table (4.6): Percentage activity values of the crude extracts:

No.	Name of plants	NO. of microorganisms tested	No. of active microorganisms tested	Percent activity (%)
1.	***Mangifera indica***	5	5	**100**
2.	***Rosmarinus officinalis***		5	**100**
3.	***Tinospora bakis***		5	**100**

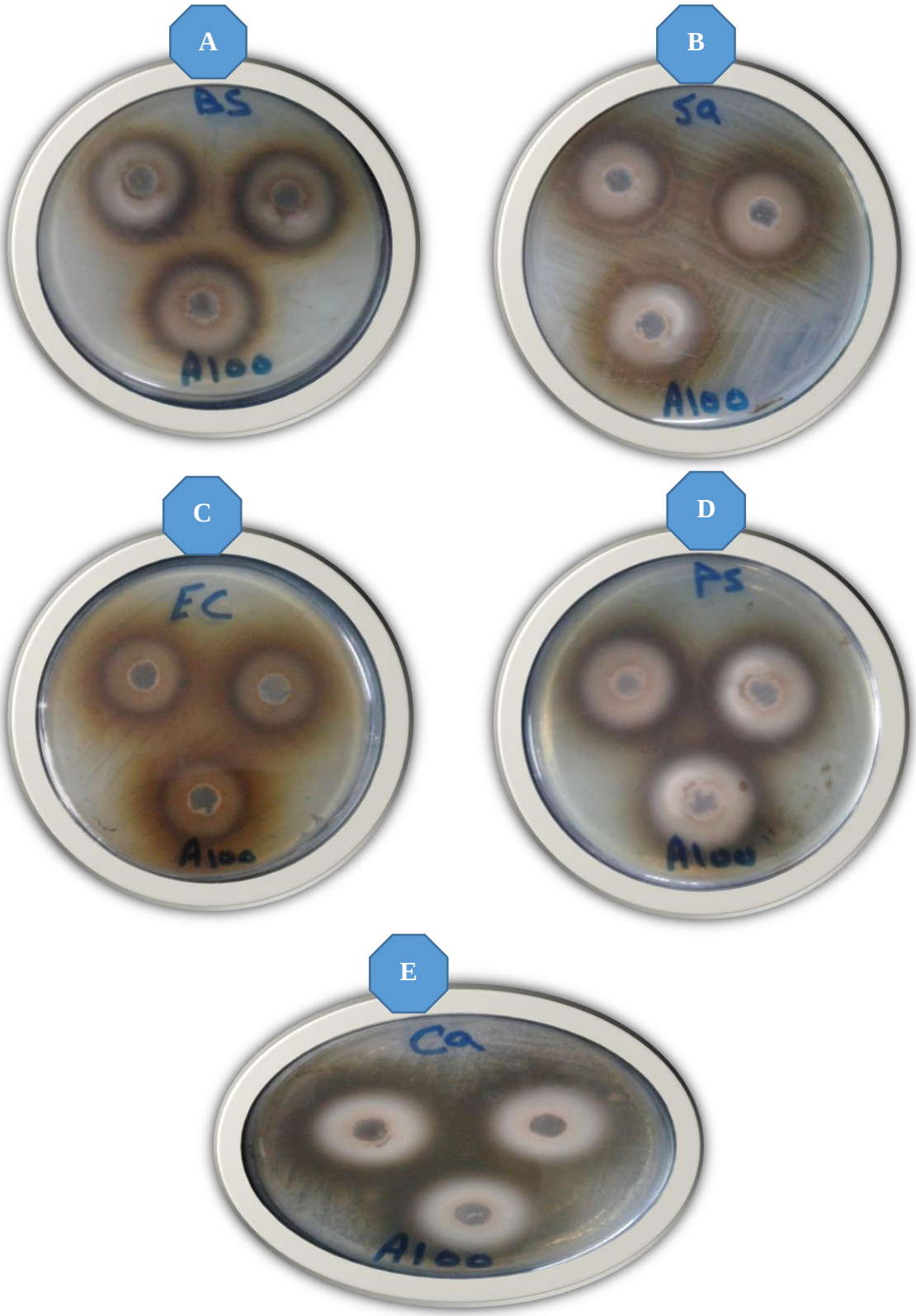


Figure (4.1): Antimicrobial activity of ethanolic crude extract of *M. indica* against Gram-positive bacteria **(A)** ***Bacillus subtilis*** and **(B)** ***Staphylococcus aureus,*** Gram-negative bacteria **(C)** ***Escherichia coli*** and **(D)** ***Pseudomonas aeruginosa*** and fungi against **(E)** ***Candida albicans*** at concentration (100 mg/ml).

Bacterial and fungal susceptibility index, are shown in Table (4.7) is useful in evaluating the susceptibility of the different bacteria and fungi towards the plant extracts investigated. The all microorganisms tested found to be of high susceptibility to the all ethanolic crude extracts investigated (100 %), in Table (4.7).

Table (4.7) Bacterial and fungal Susceptibility Index (BSI) of the plants:

Test organisms	No. of active extracts	Percent activity (%)
Bacillus subtilis	3	100
Staphylococcus aureus		100
Escherichia coli		100
Pseudomonas aeruginosa		100
Candida albicans		100

D. Activity index (AI) for studied plants and reference drugs (Antibiotics)

The antimicrobial nature of ethanolic extracts of medicinal plants were estimated by the activity index values. The plant extracts at the concentration (100 mg/ml) showed significant antimicrobial activity compared to standard drugs at the concentration (40 µg/ml) (antibacterial and antifungal) in dose dependent manor, as shown in Table (4.8). The (AI) values are helpful in estimating the potential of antimicrobial activity quantitatively compared to the respective standards.

The significant use of ethanolic extracts of selected medicinal plants with standard antibiotics were calculated through activity index. More than 1 activity index value indicated the considerable role of herbal extracts which showed the strong effect against tested pathogens, as shown in Table (4.8), which support the traditional use of medicinal herbs against infectious pathogens.

Generally, bacteria were more susceptible to the plant extracts than the yeast *Candida*, with activity index ranging between (0.57-2.31 and 0.48-1.47), for bacteria and fungi, respectively. The *M. indica* extract highest activity against Gram negative bacteria (*Ps. aeruginosa*) with an activity index (2.31). The *T. bakis* extract lowest activity against Gram positive bacteria (*S. aureus*) with an activity index (0.57) compared to other bacterial species, as shown in Table (4.7). However, the maximum zone of inhibition values are observed for Gram negative bacteria compared to Gram positive bacteria. The activity index is an expression of the degree of potency of the plant extract in relation to the standard drug (Moshi *et al*., 2006). The ethanolic extracts have shown higher AI values against Gram positive bacteria which means that the extracts are having good activity against the Gram positive bacteria compared to Ampicillin, except *T. bakis*. The ethanolic extracts have shown higher values against Gram negative bacteria against *P. aeruginosa* compared to Ampicillin. The extracts have shown higher activity index values against fungi compared to Nystatin, as shown in Table (4.8).

Table (4.8): Activity index (AI) for crude extracts with reference drugs (Antibiotics):

Name of plants	Gram (+ve)				Gram (-ve)				Fungi	
	B. s		*S. a*		*E. c*		*Ps. A*		*C. a*	
	Amp.	**Gen.**	**Amp.**	**Gen.**	**Amp.**	**Gen.**	**Amp.**	**Gen.**	**Clot.**	**Nyst.**
	Activity index (AI)									
Mangifera indica	**1.6**	0.83	**1.44**	**1.03**	**-**	0.97	**2.31**	**1.61**	0.59	**1.47**
Rosmarinus officinalis	**1.53**	0.79	**1.08**	0.77	**-**	**1.03**	**1.25**	0.87	0.48	**1.18**
Tinospora bakis	**1.47**	0.76	0.80	0.57	**-**	0.62	**1.31**	0.91	0.59	**1.47**

Key: AI= Activity Index (IZ developed by extract/IZ developed by standard), **Amp. =** Ampicillin**, Gen. =** Gentamicin**, Clot. =** Clotrimazole**, Nyst. =** Nystatin**, (-) =** no inhibition zone of antibiotic, tested concentration of extracts: 100 mg/ml (0.1 ml/well) and concentration of antibacterial and antifungal (40 µg/ml), **the inhibition zone of extract more active than antibiotic was written in bold**.

E. Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MBC) of *M. indica*, *R. officinalis* and *T. bakis* extract against standard microorganisms (*B. subtilis, S. aureus, E. coli, P. aeruginosa*) as well as one fungi (*C. albicans*) were determined using concentrations range (100 - 0.78 mg/ml) and compared with antibacterial and antifungal selected standard drugs (antibiotics) against standard microorganisms which determined using range (40-5 µg /ml). The result of the MIC of the extracts and standard drugs (antibiotics) are presented in Tables (4.9 and 4.10) and Figure (4.2). The results of minimum inhibitory concentration values have shown good correlation with IZ values.

The extracts were active against the all microorganisms tested with MIC values of less than 0.78, 12.5 and 1.56 mg/ml for *M. indica*, *R. officinalis* and *T. bakis* extract respectively. The MICs of *M. indica* and *T. bakis* extract were lowest of less than 0.78 and 1.56 mg/ml), respectively, and moderate (12.5 mg/ml) against all microorganisms tested. The range of MBC of *M. indica* extract showed less than 0.78 to 1.56 mg/ml), as shown in Tables (4.9 and 4.10). Results obtained showed that MIC values were lower than MBC values, suggesting that extracts were bacteriostatic at lower concentration.

On the other hand, the range of MBC of *M. indica* extract was (≤ 0.78 to 1.56 mg/ml), as shown in Tables (4.9 and 4.10) and Figure (4.2). These results were similar to Mushore and Matuvhunye (2013), and Singh *et al*., (2010) extracted stem park of *M. indica* and excellent results have been generated with significant antibacterial and anti-fungal activities (MIC = 0.08 mg/mL) against *Streptococcus pneumonia*, *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Candida albicans*. In the present study encouraging results have been produced with ethanolic extract but interestingly with the seeds of mango.

The *R. officinalis* extract showed that 12.5 mg/ml was the lowest concentration to inhibit the growth of all microorganisms tested, as shown in Table (4.9). The maximum AI values are observed against *B. subtilis* (1.53) and low AI value for *C. albicans* (0.48) compared to other bacterial species, as shown in Table (4.8). However, the maximum zone of inhibition values are observed for Gram negative bacteria compared to Gram positive bacteria. The Minimum inhibitory concentration values have shown good correlation with IZ values. The Minimum inhibitory concentration (MIC) of *R. officinalis* extract was 12.5 mg/ml against

all bacteria tested. The range of Minimum Bactericidal Concentration (MBC) of *R. officinalis* extract was (12.5 to 25 mg/ml), as shown in Tables (4.9 and 4.10). Similar to our result Petrolini *et al*. (2013) found that the *S. aureus.* This result agree with Oluwatuyi *et al*. (2004) found that *S. aureus*.

The *T. bakis* extract showed that 1.56 mg/ml was the lowest concentration to inhibit the growth of all microorganisms tested as shown in Table (4.9). The maximum AI values are observed against *B. subtilis* and *C. albicans* (1.47) and low AI value for *S. aureus* (0.57) compared to other bacterial species (Table 4.8). However, the maximum zone of inhibition values are observed for Gram positive bacteria compared to Gram negative bacteria. The minimum inhibitory concentration values have shown good correlation with IZ values. The minimum inhibitory concentration of *R. officinalis* extract showed 1.56 mg/ml against all bacteria tested. The range of minimum bactericidal concentration (MBC) of *R. officinalis* extract showed (1.56 to 6.25 mg/ml), as shown in Tables (4.9 and 4.10). Thus, the results of these studies suggest that different organisms react differently to the same extract, as well as fractions and pure compounds; similarly to the work of (Rios and Recio, 2005) on the plant *R. officinalis* furnished promising results, particularly for Gram-positive bacteria, and compared with published data, these findings justify the considerable interest in the development of credible alternatives for the treatment of urinary tract infections.

H. Screening of antimicrobial activity of fractionation of the plants and antibiotics

The *M. indica* fractions exhibited inhibitory effects against standard microorganisms tested with the inhibition zone ranging from (11 to 29 mm) in length. The *M. indica* fractions exhibited inhibitory effects against all of the tested standard microorganisms, while, n-hexane fraction was in-active against *Ps. aeruginosa*. In addition, the ethyl acetate *M. indica* fraction inhibition zone ranging from (23 to 29 mm) in length. The highest activity inhibition zone against Gram positive bacteria, *B. subtilis* and *S. aureus* (29 and 27 mm), respectively. While, the inhibition zone against Gram negative bacteria *Ps. aeruginosa* and *E. coli* (25 and 22 mm), respectively, and the *C. albicans* 23 mm. The n-butanol fraction of *M. indica* exhibited inhibitory effects against all of the tested standard microorganisms with the inhibition zone ranging from (19 to 22 mm) in length. The highest activity inhibition zone against Gram negative bacteria *Ps. aeruginosa* (22 mm), in addition, the Gram positive bacteria inhibition zone (21 mm) against *B. subtilis* and *S. aureus*, whereas, the lowest inhibition zone (19 mm) was against *C. albicans.* The aqueous fraction of *M. indica* exhibited inhibitory effects against all tested standard microorganisms with the inhibition zone ranging from (12 to 19 mm) in length. The highest activity inhibition zone of against Gram positive bacteria against *B. subtilis* and *S. aureus* (19 and 16 mm), respectively, the inhibition zone (16 mm) against *C. albicans*, while, the lowest inhibition zone (15 and 12 mm) against Gram negative bacteria *Ps. aeruginosa* and *E. coli*. And the n-hexane fraction of *M. indica* exhibited inhibitory effects against all tested standard microorganisms except in-active against Gram negative bacteria *Ps. aeruginosa*, with the inhibition zone ranging from (11 to 16 mm) in length. The highest activity inhibition zone against Gram positive bacteria against *B. subtilis* (16 mm), and the inhibition zone (15 mm) against *E. coli* and *C. albicans*, while, the

lowest inhibition zone (11 mm) against Gram positive bacteria *S. aureus*. In addition, the ethyl acetate and n-butanol fractions were highly activity in more than (18 mm) growth inhibition zone against all microorganisms tested, as in Table (4.11) and Figure (4.3). On the other hand, the ethyl acetate showed a higher

Table (4.9): Determination of the Minimum Inhibitory Concentrations (MICs) by (mm) of medicinal plants and reference drugs against the standard microorganisms used cup plate method:

Name of plants/drugs	Concentrations (mg/ml)	MZID* (mm)				
		Bacteria*				Fungi**
		Gram (+ve)		Gram (- ve)		
		B. s	***S. a***	***E. c***	***Ps. a***	***C. a***
Mangifera indica	**100**	24 ± 0.08	36 ± 0.01	31 ± 0.08	37 ± 0.05	25 ± 0.01
	50	22 ± 0.01	23 ± 0.05	30 ± 0.05	27 ± 0.02	23 ± 0.06
	25	19 ± 0.08	20 ± 0.02	25 ± 0.04	25 ± 0.04	21 ± 0.08
	12.5	18 ± 0.05	19 ± 0.08	22 ± 0.02	24 ± 0.06	20 ± 0.02
	6.25	16 ± 0.02	18 ± 0.05	20 ± 0.01	19 ± 0.08	18 ± 0.04
	3.125	15 ± 0.08	16 ± 0.06	17 ± 0.04	15 ± 0.05	16 ± 0.02
	1.56	14 ± 0.04	15 ± 0.02	15 ± 0.08	14 ± 0.01	15 ± 0.08
	0.78	14 ± 0.05	15 ± 0.08	13 ± 0.01	13 ± 0.05	14 ± 0.01
Rosmarinus officinalis	**100**	23 ± 0.05	27 ± 0.02	33 ± 0.01	20 ± 0.08	20 ± 0.05
	50	20 ± 0.01	24 ± 0.05	25 ± 0.05	16 ± 0.05	17 ± 0.02
	25	17 ± 0.05	23 ± 0.04	16 ± 0.02	14 ± 0.08	15 ± 0.01
	12.5	13 ± 0.04	21 ± 0.08	12 ± 0.05	12 ± 0.06	13 ± 0.06
	6.25	-	-	-	-	-
	3.125	-	-	-	-	-
	1.56	-	-	-	-	-
	0.78	-	-	-	-	-
Tinospora bakis	**100**	22 ± 0.02	20 ± 0.05	20 ± 0.04	21 ± 0.05	25 ± 0.01
	50	21 ± 0.05	18 ± 0.08	19 ± 0.05	20 ± 0.06	24 ± 0.04
	25	20 ± 0.04	15 ± 0.02	18 ± 0.03	18 ± 0.04	22 ± 0.05
	12.5	18 ± 0.01	13 ± 0.05	16 ± 0.02	17 ± 0.02	20 ± 0.03
	6.25	17 ± 0.05	12 ± 0.01	15 ± 0.04	15 ± 0.03	19 ± 0.01
	3.125	15 ± 0.01	11 ± 0.02	13 ± 0.06	12 ± 0.01	17 ± 0.04
	1.56	14 ± 0.04	11 ± 0.01	12 ± 0.02	11 ± 0.06	14 ± 0.01
	0.78	-	-	-	-	-
Ampicillin	**40**	15 ± 0.01	25 ± 0.02	-	16 ± 0.02	-ve
	20	14 ± 0.05	20 ± 0.01	-	13 ± 0.01	-ve
	10	13 ± 0.01	18 ± 0.02	-	12 ± 0.05	-ve

	5	12 ± 0.04	15 ± 0.01	-	-	-ve
Gentamicin	**40**	29 ± 0.01	3 5± 0.01	32 ± 0.01	23 ± 0.02	-ve
	20	22 ± 0.02	33 ± 0.04	30 ± 0.02	22 ± 0.01	ND
	10	20 ± 0.00	30 ± 0.01	17 ± 0.01	21 ± 0.03	ND
	5	17 ± 0.01	28 ± 0.02	-	19 ± 0.02	ND
Clotrimazole	**40**	ND	ND	ND	ND	42 ± 0.01
	20	ND	ND	ND	ND	40 ± 0.00
	10	ND	ND	ND	ND	33 ± 0.01
	5	ND	ND	ND	ND	30 ± 0.02
Nystatin	**50**	ND	ND	ND	ND	17 ± 0.01
	25	ND	ND	ND	ND	14 ± 0.01
	12.5	ND	ND	ND	ND	-

Key: ***B.s****= Bacillus subtilis,* ***S.a****= Staphylococcus aureus,* ***E.c****= Escherichia coli,* ***Ps.a****= Pseudomonas aeruginosa* and ***C.a****= Candida albicans.* MDIZ* (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm): >18 mm: Sensitive, 14 to 18 mm: Intermediate: <14 mm: Resistant. (-): No inhibition zone, ND: not determined, concentrations used 100 – 0.78 mg/ml of 0.1ml/cup.

Inhibition zone for *E. coli* and *P. aeruginosa*; similar findings were shown by Singh *et al*. in (2015) from *M. indica* steam bark. And a higher antibacterial susceptibility was observed for the ethyl acetate with *P. mirabilis*, in fact, higher than that reported by Singh *et al.* (2015).

The *R. officinalis* fractions in-active against all standard microorganisms tested except n-hexane fraction against *E. coli* and *S. aureus* (16 and 15 mm), respectively, (Table 4.11).

The ethyl acetate fraction of *T. bakis* exhibited inhibitory effects against standard microorganisms tested with inhibition zone ranging from (16 to 20 mm) in length, respectively. The highest activity inhibition zone against *C. albicans* (20 mm), followed by *E. coli* (19 mm), *Ps. aeruginosa* (18 mm), *B. subtilis* and *S. aureus* (17 and 16 mm), respectively. The aqueous fraction of *T. bakis* exhibited inhibitory effects against all standard microorganisms tested except *S. aureus* was in-active with the inhibition zone ranging from (13 to 15 mm) in length. The highest activity inhibition zone of aqueous fraction against *Ps. aeruginosa* and *C. albicans* (15 mm), *B. subtilis* (14 mm), while, the lowest inhibition zone against *E. coli* (13 mm). The n-hexane fraction of *T. bakis* exhibited inhibitory effects against all standard microorganisms tested except in-active against *E. coli*. In addition, the inhibition zone ranging from (11 to 13 mm) in length. The highest activity inhibition zone against *C. albicans* (13 mm), *S. aureus* (12 mm) and *B. subtilis* and *Ps. aeruginosa* (11 mm). The n-butanol fraction of *T. bakis* exhibited inhibitory effect only against *Ps. aeruginosa* (12 mm), (Table 4.11). In addition, the ethyl acetate fraction showed more inhibitory activity rather than other fraction on the tested microorganisms. This plant there is no study.

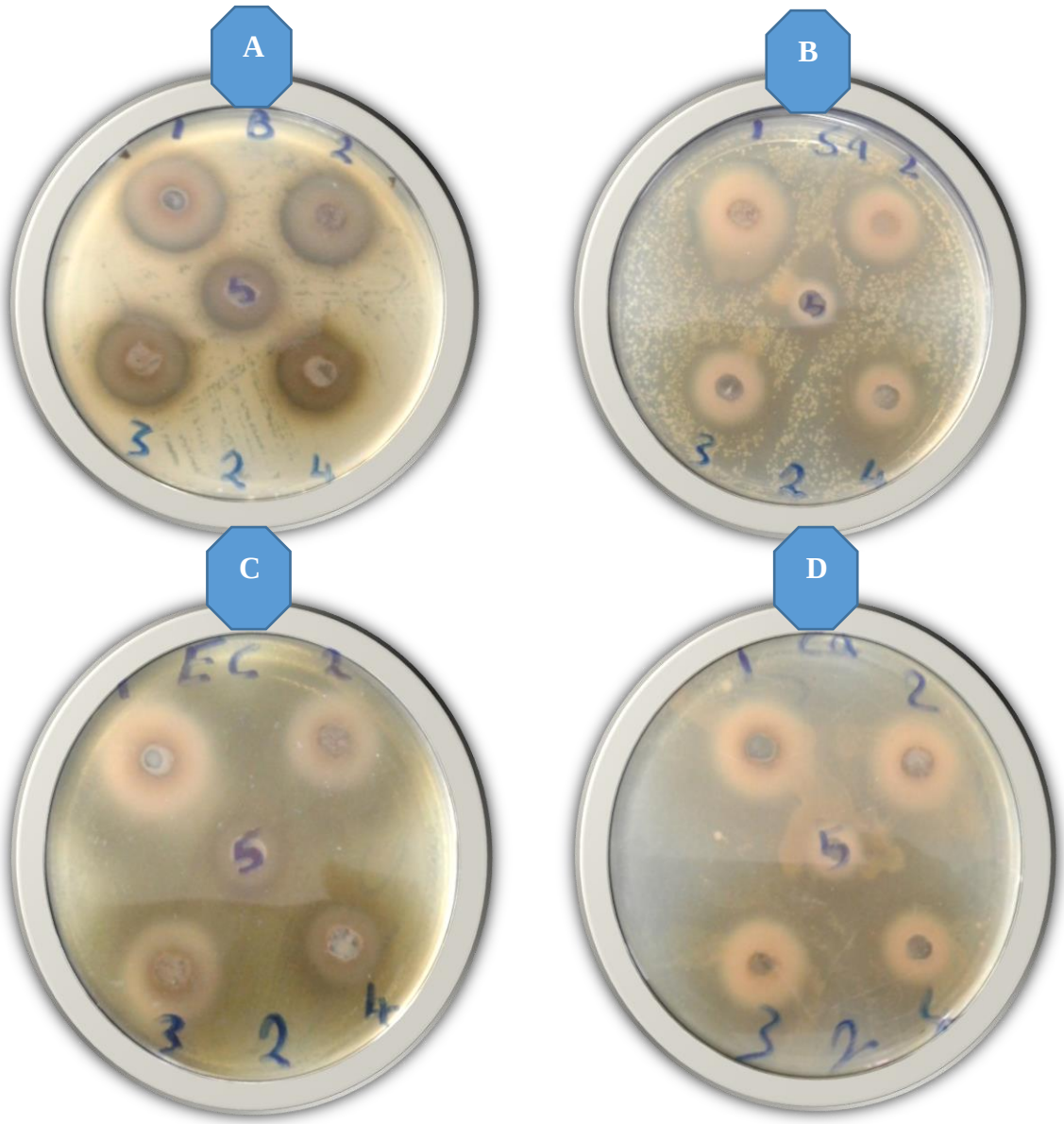


Figure (4.2): Minimum inhibitory concentrations of crude extract *Mangifera indica* (seeds) against Gram positive bacteria **(A)** ***Bacillus subtilis*** **and (B)** ***Staphylococcus aureus,*** Gram negative bacteria against **(C)** ***Escherichia*** and fungi against **(D)** ***Candida albicans.***

Table (4.10): The Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (mg/ml) of ethanolic crude extracts against tested bacteria:

Name of plants	Gram (+ve)				Gram (- ve)			
	B. s		***S. a***		***E. c***		***Ps. a***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Mangifera indica	≤ 0.78	1.56	≤ 0.78	≤ 0.78	≤ 0.78	1.56	≤ 0.78	≤ 0.78
Rosmarinus officinalis	12.5	25	12.5	12.5	12.5	25	12.5	25
Tinospora bakis	1.56	1.56	1.56	6.25	1.56	3.125	1.56	3.125

Key: MIC= Minimum Inhibition Concentration**, MBC=** Minimum Bactericidal Concentration.

Table (4.11): Antimicrobial activity of fractionation of crude extracts against the standard microorganisms used cup-plate method at concentration 50 mg/ml:

Name of drugs	Concentrations (mg/ml)	MZID* (mm)				
		Bacteria				Fungi
		Gram (+ve)		Gram (- ve)		
		B. s	***S. a***	***E. c***	***Ps. a***	***C. a***
Mangifera indica	**n-hexane**	16 ± 0.08	11 ± 0.02	15 ± 0.08	-	15 ± 0.02
	Ethyl acetate	**29 ± 0.02**	**27 ± 0.05**	**22 ± 0.04**	**25 ± 0.01**	**23 ± 0.05**
	n-butanol	21 ± 0.08	21 ± 0.02	20 ± 0.05	22 ± 0.02	19 ± 0.08
	Aqueous	19 ± 0.05	16 ± 0.08	12 ± 0.02	15 ± 0.05	16 ± 0.06
Rosmarinus officinalis	**n-hexane**	-	15 ± 0.01	16 ± 0.05	-	-
	Ethyl acetate	-	-	-	-	-
	n-butanol	-	-	-	-	-
	Aqueous	-	-	-	-	-
Tinospora bakis	**n-hexane**	11 ± 0.08	12 ± 0.05	-	11 ± 0.01	13 ± 0.02
	Ethyl acetate	**17 ± 0.02**	**16 ± 0.01**	**19 ± 0.08**	**18 ± 0.01**	**20 ± 0.05**
	n-butanol	-	-	-	12 ± 1.0	-
	Aqueous	14 ± 0.05	-	13 ± 1.0	15 ± 0.05	15 ± 0.01
DMSO 10%	**Control -ve**	-	-	-	-	-
Water	**Control -ve**	-	-	-	-	-
Ampicillin	**Control +ve**	15 ± 0.01	25 ± 0.02	-	16 ± 0.02	-ve
Gentamicin	**Control +ve**	29 ± 0.01	3 5± 0.01	32 ± 0.01	23 ± 0.02	-ve
Clotrimazole	**Control +ve**	-ve	-ve	-ve	-ve	42 ± 0.01
Nystatin	**Control +ve**	-ve	-ve	-ve	-ve	17 ± 0.02

Key: ***B.s****= Bacillus subtilis,* ***S.a****= Staphylococcus aureus,* ***E.c****= Escherichia coli,* ***Ps.a****= Pseudomonas aeruginosa* and ***C.a****= Candida albicans.* **MDIZ* (mm)** = Mean diameter of growth inhibition zone in mm. **Interpretation of results:** MDIZ (mm): **>18 mm**: Sensitive, **14 to 18** mm: Intermediate: **<14 mm**: Resistant**. (-):** No inhibition zone, **-ve:** Not tested, concentration used 50 mg/ml, and 40 µg/ml for fractions and drugs. **The highly activity of fractions were written in bold.**

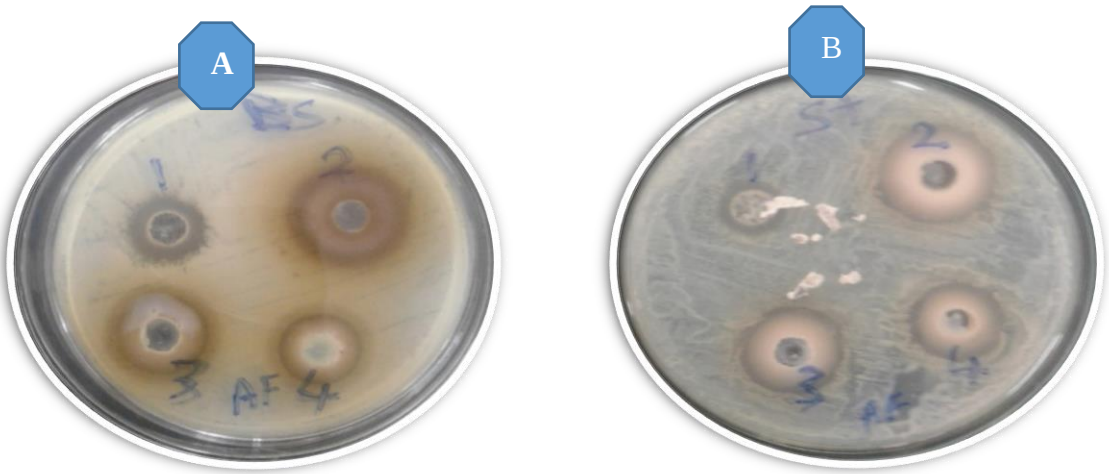


Figure (4.3): Antimicrobial activity of fractions of *M. indica* (seeds) against gram positive bacteria **(A)** ***Bacillus subtilis*** and **(B)** ***Staphylococcus aureus***.

I. Antimicrobial activity of fractionation of the ethyl acetate from *M. indica* seeds by column chromatographic technique

The antimicrobial activity of fractionations of ethyl acetate from *M. indica* seeds by column chromatographic technique against standard microorganisms were determined. To perform a rapid screening study of potential antimicrobial activity, using cup-plate agar diffusion method, and the inhibition zone (IZ) results, are presented in Table (4.12) and Figure (4.4).

The results showed that fractionations of ethyl acetate from *M. indica* (seeds) exhibited strong growth inhibition activity of fractions (F_{10} and F_{15}) were showed inhibition zone values ranging from (20 to 25 mm) against all tested standard microorganisms. While, they showed strong activity of inhibition zone (25 mm) against Gram-negative bacteria *E. coli* from fractions 10 and 15 (F_{10} and F_{15}). In addition, all fractionations tested showed more than (18 mm) growth inhibition zone were considered to possess highest antimicrobial activity. The fractions from (F_{16} to F_{22}) showed inhibition zone values ranging from (12 to 24 mm) against all standard microorganisms tested. While, the showed highest activity of inhibition zone (25 mm) against Gram-positive bacteria against *B. subtilis* from fraction 20 (F_{20}).

Table (4.12): Antimicrobial activity of fractionations of the ethyl acetate fractions from *M. indica* (seeds) by column chromatographic technique:

No. of Fractions	MZID* (mm)				
	Bacteria				Fungi
	Gram (+ve)		Gram (- ve)		
	B. s	***S. a***	***E. c***	***Ps. a***	***C. a***
F 1	ND	ND	ND	ND	ND
F 2	ND	ND	ND	ND	ND
F 3	ND	ND	ND	ND	ND
F 4	ND	ND	ND	ND	ND
F 5	ND	ND	ND	ND	ND
F 6	ND	ND	ND	ND	ND
F 7	ND	ND	ND	ND	ND
F 8	ND	ND	ND	ND	ND
F 9	ND	ND	ND	ND	ND
F 10	**20 ± 0.05**	**22 ± 0.02**	**25 ± 0.03**	**21 ± 0.01**	**22 ± 0.04**
F 11	ND	ND	ND	ND	ND
F 12	ND	ND	ND	ND	ND
F 13	ND	ND	ND	ND	ND
F 14	ND	ND	ND	ND	ND
F 15	**21 ± 0.01**	**20 ± 0.02**	**25 ± 0.05**	**21 ± 0.03**	**22 ± 0.01**
F16	**22 ± 0.02**	18 ± 0.04	12 ± 0.01	**20 ± 0.02**	**21 ± 0.05**
F 17	**21 ± 0.01**	18 ± 0.03	**20 ± 0.05**	**20 ± 0.03**	19 ± 0.02
F 18	**22 ± 0.05**	15 ± 0.01	**22 ± 0.02**	**23 ± 0.04**	19 ± 0.05
F 19	**21 ± 0.02**	18± 0.02	**2 5± 0.04**	**2 1± 0.02**	1 8± 0.01
F 20	**24 ± 0.01**	16± 0.01	**2 3± 0.06**	18± 0.05	1 4± 0.02
F 21	17 ± 0.02	15 ± 0.05	**22 ± 0.06**	**22 ± 0.03**	15 ± 0.05
F 22	16 ± 0.01	14± 0.03	**2 1± 0.05**	**2 0± 0.01**	**20± 0.02**

Key: F: Fraction, ND; not determined**, the inhibition zone of fractionation ≥ 20 mm against all microorganisms tested were written bold.**

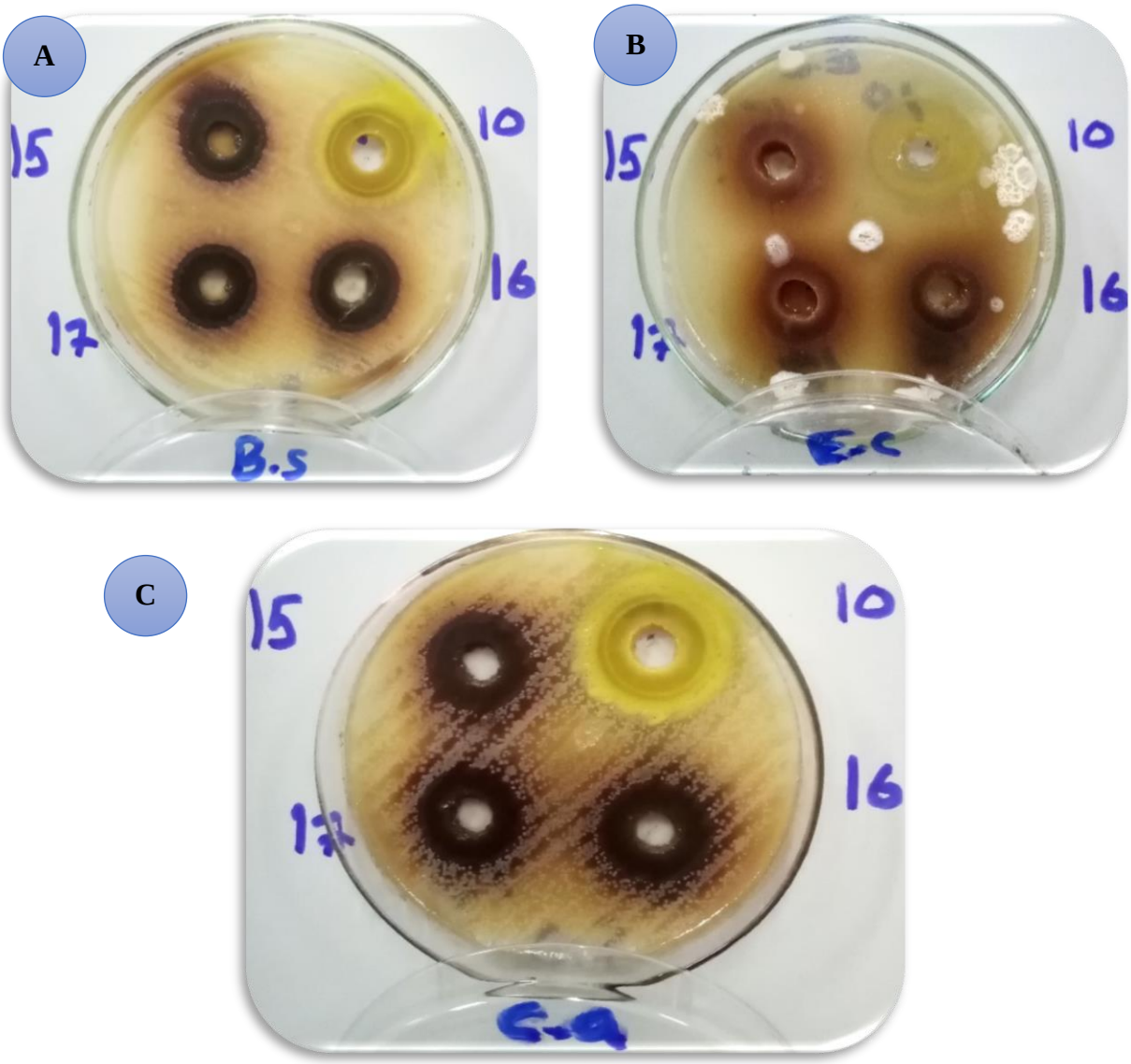


Figure (4.4): Antimicrobial activity of fractionations (**10, 15, 16 and 17**) of the ethyl acetate fraction from *Mangifera indica* (seeds) by column chromatographic technique against Gram positive bacteria **(A)** ***Bacillus subtilis,*** Gram negative bacteria against **(B)** ***Escherichia coli*** and fungi against **(C)** ***Candida albicans.***

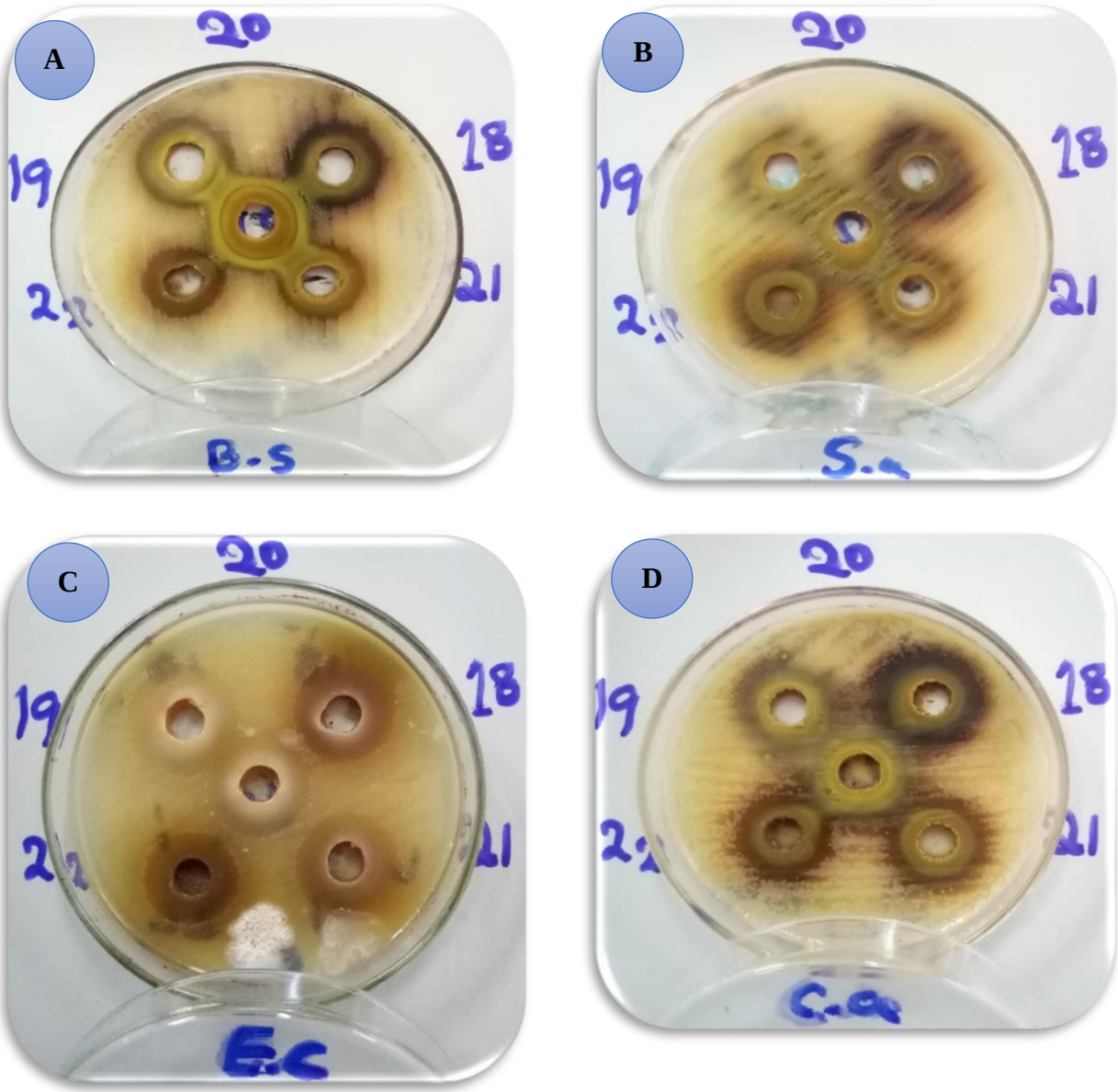


Figure (4.5): Antimicrobial activity of fractionations (**18 to 22**) of the ethyl acetate fraction from *Mangifera indica* (seeds) by column chromatographic technique against Gram positive bacteria **(A)** ***Bacillus subtilis*** and **(B)** ***Staphylococcus aureus***, Gram negative bacteria against **(C)** ***Escherichia coli*** and fungi against **(D)** ***Candida albicans.***

J. Antimicrobial activity of isolated compounds from *M. indica* and *T. bakis*

The *in-vitro* study to determine the antibacterial activity of isolated compounds from *M. indica* and *T. bakis* were measured against selected Gram-positive and Gram-negative bacterial specie. Minimum inhibitory concentration values were determined by microdilution method including resazurin dye. Different activities were tested and the result of their MIC and MBC values, were shown in Table (4.13).

The isolated compounds from *M. indica* with MIC and MBC values ranging from (15.62 500 µg/ml) and (31.25 – 1000 µg/ml), respectively, And the isolated compounds from *T. bakis* with MIC and MBC values ranging from (≤ 1.95 - 500 µg/ml) and (3.91 – 1000 µg/ml), respectively, as shown in Table (4.10). In addition, all isolated compounds tested showed a good activity against bacteria used, except AS-15-9-8 was the least effective against Gram-positive bacteria against *B. subtilis* and *S. aureus*. The four compounds

(AK-10, AS-5-11-5, AS-5-12-3 and AS-8) showed high active against all microorganisms tested with MIC value ranging from (1.95 – 250 µg/ml), and MBC value ranging from (3.91 – 250 µg/ml). In addition, all isolated compounds tested showed strong activity against Gram-negative bacteria against *E. coli* and *Ps. aeruginosa*. The MIC and MBC values ranging from (≤ 1.95 - 250 µg/ml) and (3.91 – 500 µg/ml) against *E. coli*

Table (4.13): The Minimum Inhibition Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) by (µg/ml) of isolated compounds against tested bacteria:

Name of plants	Code of isolated compounds	Gram (+ve)				Gram (- ve)			
		B. s		***S. a***		***E. c***		***Ps. a***	
		MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Mangifera indica	**AK-45-3**	250	500	250	750	**≤ 1.95**	3.91	500	750
	AK-11-9	500	1000	500	1000	250	500	62.5	125
	AK-10	**31.25**	**62.5**	**15.62**	**31.25**	**31.25**	**62.5**	**31.25**	**250**
	AK-8-4	ND	ND	ND	ND	ND	ND	ND	ND
Tinospora bakis	**AS-48-4**	ND	ND	ND	ND	ND	ND	ND	ND
	AS-5-11-5	**62.5**	**125**	**125**	**250**	**≤ 1.95**	**7.81**	**62.5**	**125**
	AS-5-12-3	**125**	**250**	**125**	**250**	**≤ 1.95**	**3.91**	**31.25**	**62.5**
	AS-8	**31.25**	**62.5**	**62.5**	**125**	**≤ 1.95**	**7.81**	**125**	**250**
	AS-10-8-2	125	250	250	500	≤ 1.95	3.91	31.5	62.5
	AS-9-4-2	62.5	125	125	500	≤ 1.95	7.81	31.5	125
	AS-9-3-7	125	500	250	500	≤ 1.95	3.91	31.5	250
	AS-10-4-18-6	ND	ND	ND	ND	ND	ND	ND	ND
	AS-13-2-6	ND	ND	ND	ND	ND	ND	ND	ND
	As-45-5	250	500	250	750	≤ 1.95	3.91	500	750
	AS-15-9-8	500	1000	500	1000	250	500	62.5	125
	AS-1-1	ND	ND	ND	ND	ND	ND	ND	ND
	AS-11-9-2	ND	ND	ND	ND	ND	ND	ND	ND

Key: MIC= Minimum Inhibition Concentration**, MBC=** Minimum Bactericidal Concentration, **ND;** not determined**, the strong activity of MIC and MBC were written in bold.**

4.2.2 Antiprotozoal activity

A. Anti-amoebic activity

The result of the *in-vitro* effect of ethanolic extracts on *E. histolytica* reading after 24, 48 and 72 hours of incubation was summarized in Figures (4.6 to 4.11) respectively. The concentrations showed significant mortality percentage (%) as Figures (4.6, 4.9 & 4.11), and shows the inhibition concentrations (IC) that killed 50, 90, 95 and 99% ($IC_{50,}$ $LC_{90,}$ IC_{95} and IC_{99}) as Figures (4.7, 4.9 & 4.11) were against *E. histolyica* trophozoites *In-vitro* method, used Microsoft Excel (2016) analysis data.

This condition may be due to infection with *E. histolytica*. Our finding confirms the traditional therapeutic claims for these herbs. Little is known about the nature of the compounds with anti-amoebic activity from the plants investigated. Furthermore, these plant extracts were also active against another intestinal protozoan parasite pathogenic to humans, *G. intestinalis* growing *in-vitro* (Sawangjaroen and Sawangjaroen, 2005). Many of the plant extracts tested exhibited amoebicidal activity mainly *Codiaeum variegatum* and *Voacanga africana*. In addition their *in-vitro* activity were higher than that of metronidazole, the reference drug (Moundipa *et al*., 2005).

The ethanolic extract of *M. indica* exhibit (100%) mortality at concentration 500 µg/ml after 72 hours, this was compared with metronidazole powder (reference drug) which gave (90.4%) mortality at concentration 312.5 µg/ml at the same time, as shown in Figure (4.6). Furthermore the study revealed that the extract of *M. indica* has infectious anti-amoebic activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values were 3, 146, 242 and 362 µg/ml, respectively after 72 h, against *E. histolyica* trophozoites *in-vitro*, as shown in Figure (4.7). In addition there was no cyst in the medium. This result obtained was similar to what was observed by Tona *et al*. (1998). At 500 µg/ml and after three days of incubation the best anti-amoebic activity was obtained with the extract of *M. indica*. This plant is a good potential candidate for future studies, mainly to confirm the true amoebicidal activity in axenical culture, and biochemical mechanism of anti-amoebic inhibition.

The ethanolic extract of *R. officinalis* exhibit (90%) mortality at concentration 500 µg/mL after 72 hours, this was compared with metronidazole powder (reference drug) which gave (90.4%) mortality at concentration 312.5 µg/ml at the same time, as shown in Figure (4.8). The study revealed that the extract of *R. officinalis* has infectious anti-amoebic activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values were 19, 525, 794 and 1106 µg/ml, respectively after 72 h, against *E. histolyica* trophozoites *in-vitro,* as shown in Figure (4.9). The result is in agreement with Anacarso *et al*. (2019) who found a highest and lowest concentration of 4 and 0,1 mg/ml for *R. officinalis* essential oil against *A. polyphaga*.

The ethanolic extract of *T. bakis* exhibit (95%) mortality at concentration 500 µg/mL after 72 hours, this was compared with metronidazole powder (reference drug) which gave (90.4%) mortality at concentration 312.5 µg/ml at the same time, as shown in Figure (4.10). The study revealed that the extract of *T. bakis* has infectious anti-amoebic activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values were 44, 394,

519 and 647 µg/ml, respectively after 72 h, against *E. histolyica* trophozoites *in-vitro*, as shown in Figure (4.11). Although there are no reports on the use of extract of *T. bakis* against *E. histolytica*.

For the other extracts tested, their activities were similar to what was observed by Tona *et al*. (1998) and may be due to their harmful effect on bacteria living in amoeba *histolytica* culture (Ibrahim and Osman, 1995, Nakamura *et al*., 1999, Moundipa *et al*., 2005). This finding would be an advantage in initiating therapy to reduce the morbidity and mortality among such patients due to these pathogens.

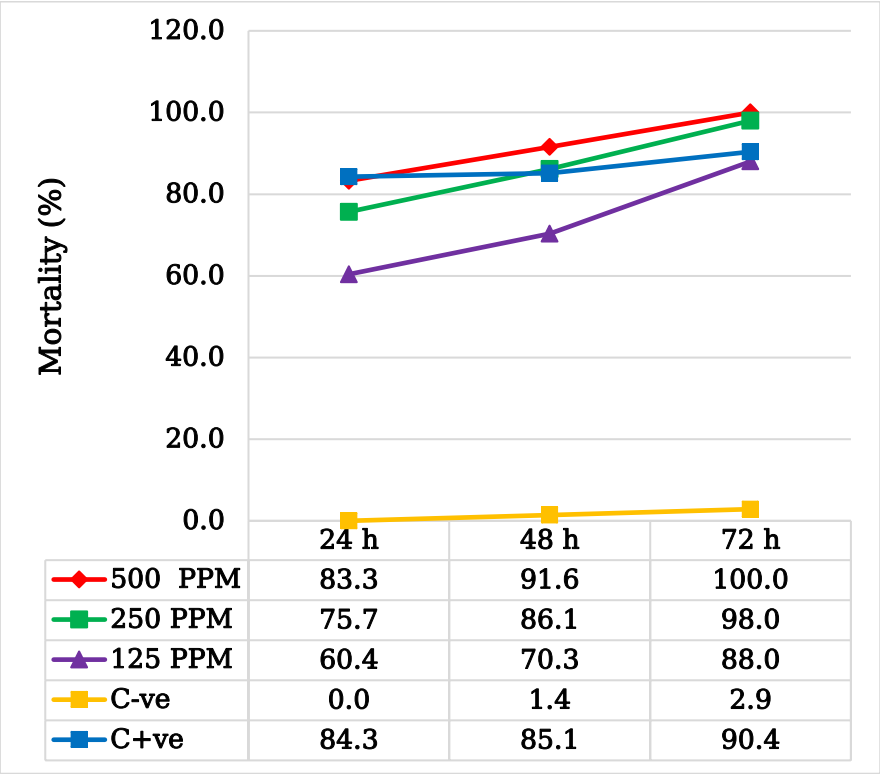


Figure (4.6): *In-vitro* amoebicidal activity of ethanolic extract from *Mangifera indica* (seeds) against *Entamoeba histolytica* (trophozoites).

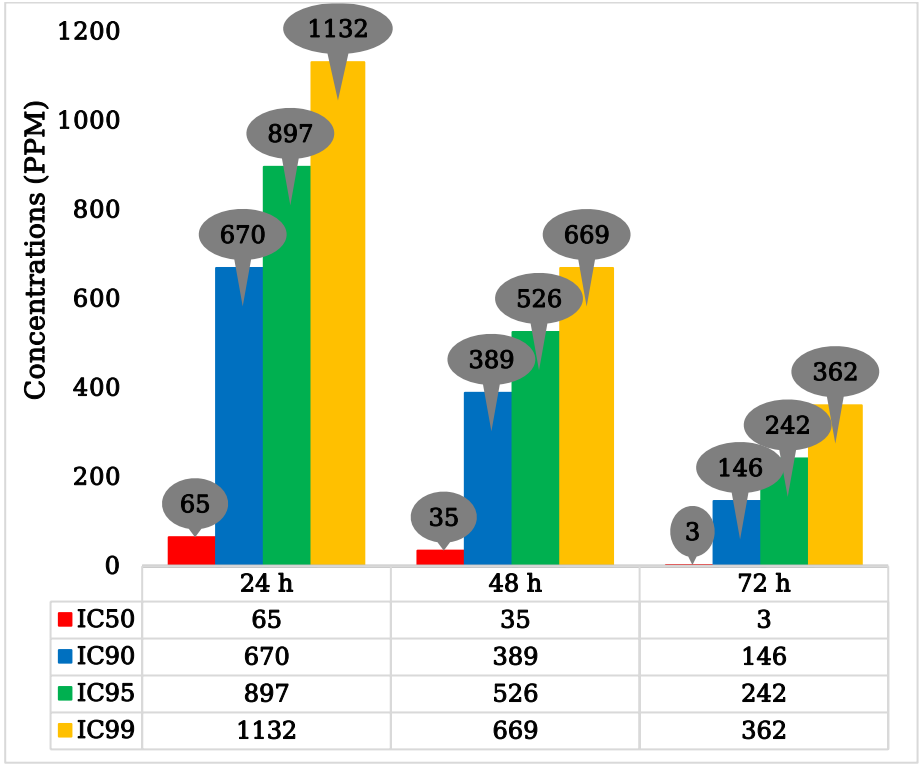
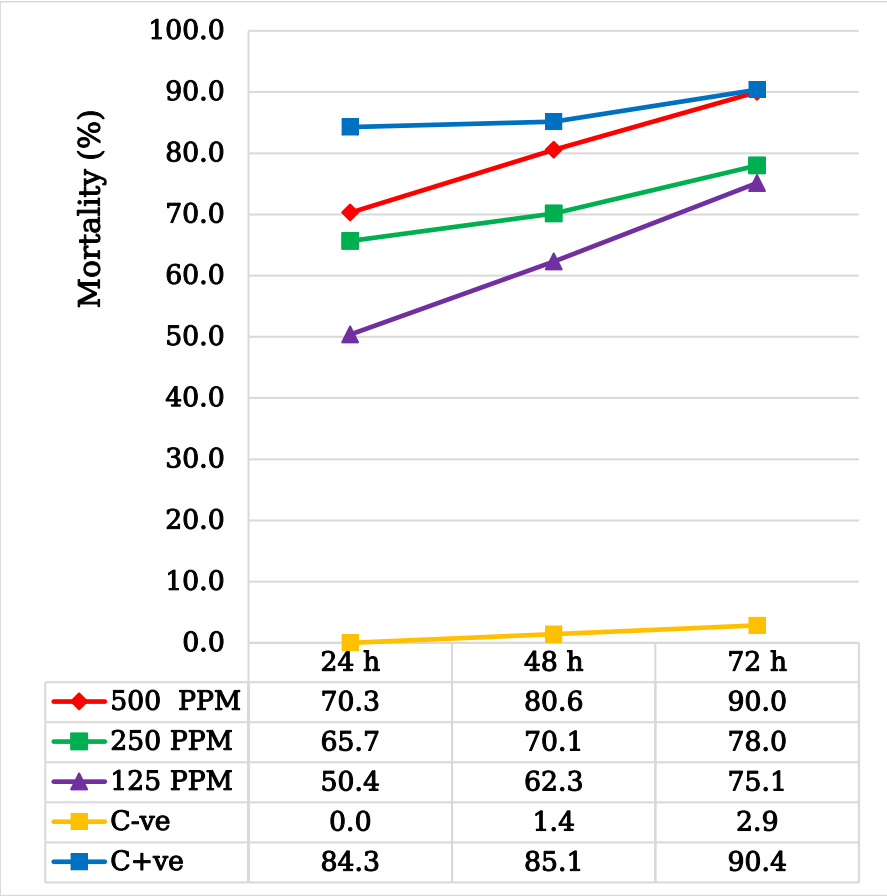
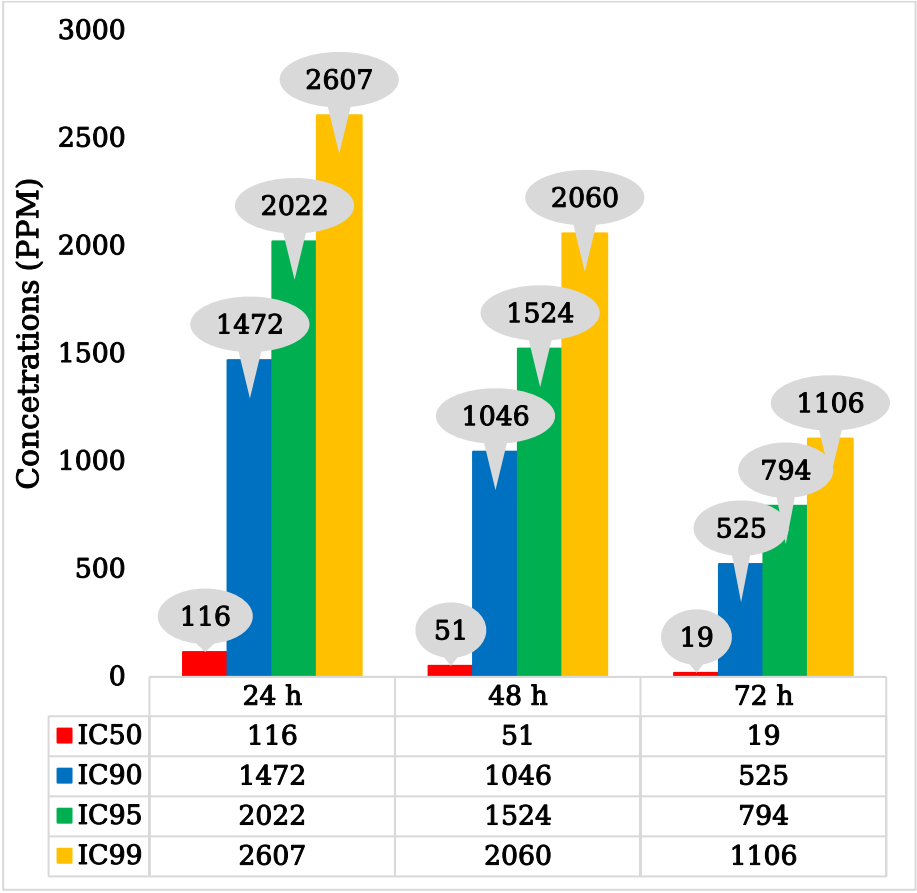


Figure (4.7): Inhibition concentrations (IC_{50}, IC_{90}, IC_{95} and IC_{99}) of ethanolic extract from *Mangifera indica* against *Entamoeba histolyica* (trophozoites) *in-vitro*.



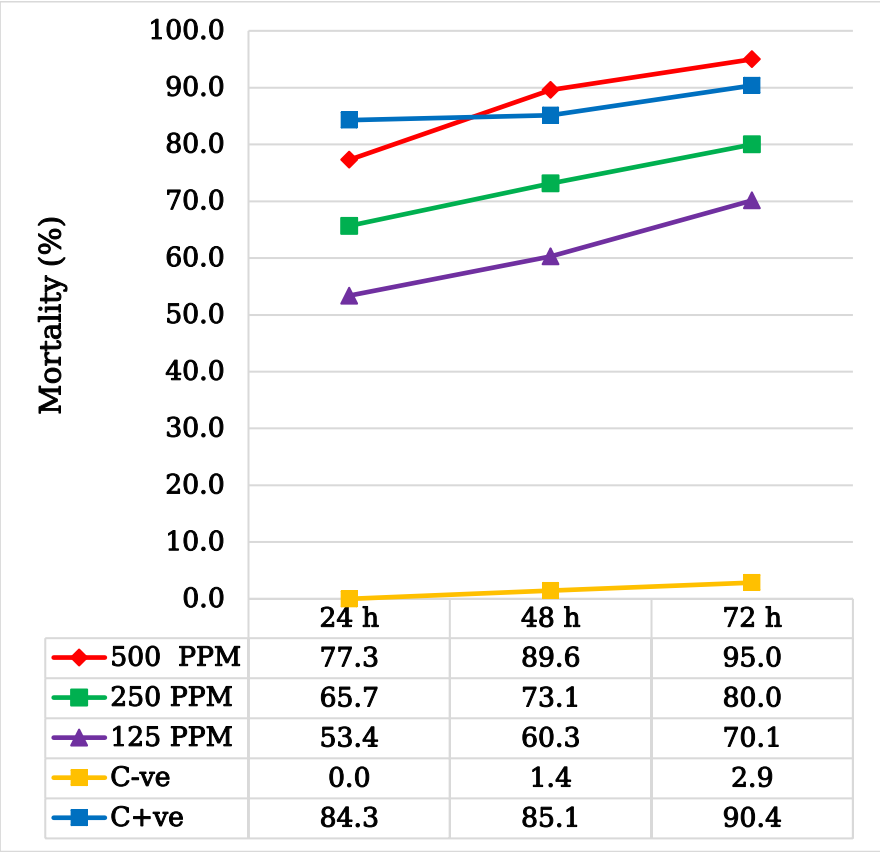
	24 h	48 h	72 h
500 PPM	70.3	80.6	90.0
250 PPM	65.7	70.1	78.0
125 PPM	50.4	62.3	75.1
C-ve	0.0	1.4	2.9
C+ve	84.3	85.1	90.4

Figure (4.8): *In-vitro* amoebicidal activity of ethanolic extract from *Rosmarinus officinalis* (pods) against *Entamoeba histolytica* (trophozoites).



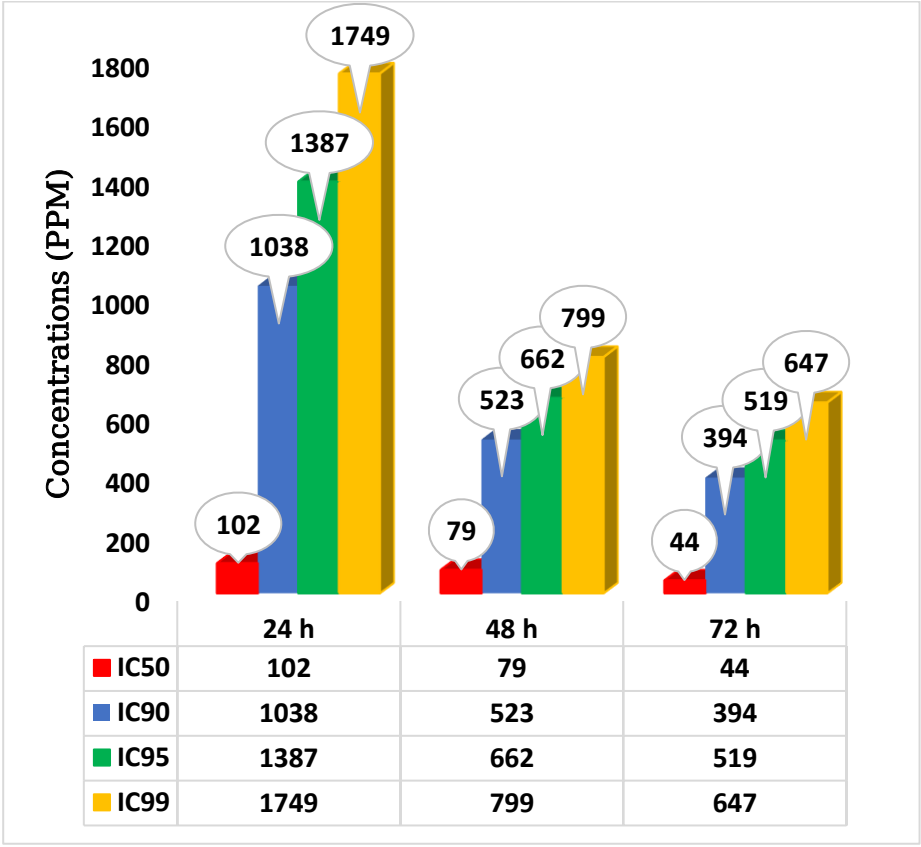
	24 h	48 h	72 h
IC50	116	51	19
IC90	1472	1046	525
IC95	2022	1524	794
IC99	2607	2060	1106

Figure (4.9): Inhibition concentrations (IC_{50}, LC_{90}, IC_{95} and IC_{99}) of ethanolic extract from *Rosmarinus officinalis* against *Entamoeba histolyica* (trophozoites) *in-vitro*.



	24 h	48 h	72 h
500 PPM	77.3	89.6	95.0
250 PPM	65.7	73.1	80.0
125 PPM	53.4	60.3	70.1
C-ve	0.0	1.4	2.9
C+ve	84.3	85.1	90.4

Figure (4.10): *In-vitro* amoebicidal activity of ethanolic extract from *Tinospora bakis* (whole plant) against *Entamoeba histolytica* (trophozoites).



	24 h	48 h	72 h
IC50	102	79	44
IC90	1038	523	394
IC95	1387	662	519
IC99	1749	799	647

Figure (4.11): Inhibition concentrations (IC_{50}, LC_{90}, IC_{95} and IC_{99}) of ethanolic extract from *Tinospora bakis* against *Entamoeba histolyica* (trophozoites) *in-vitro*.

B. Antigiardial activity

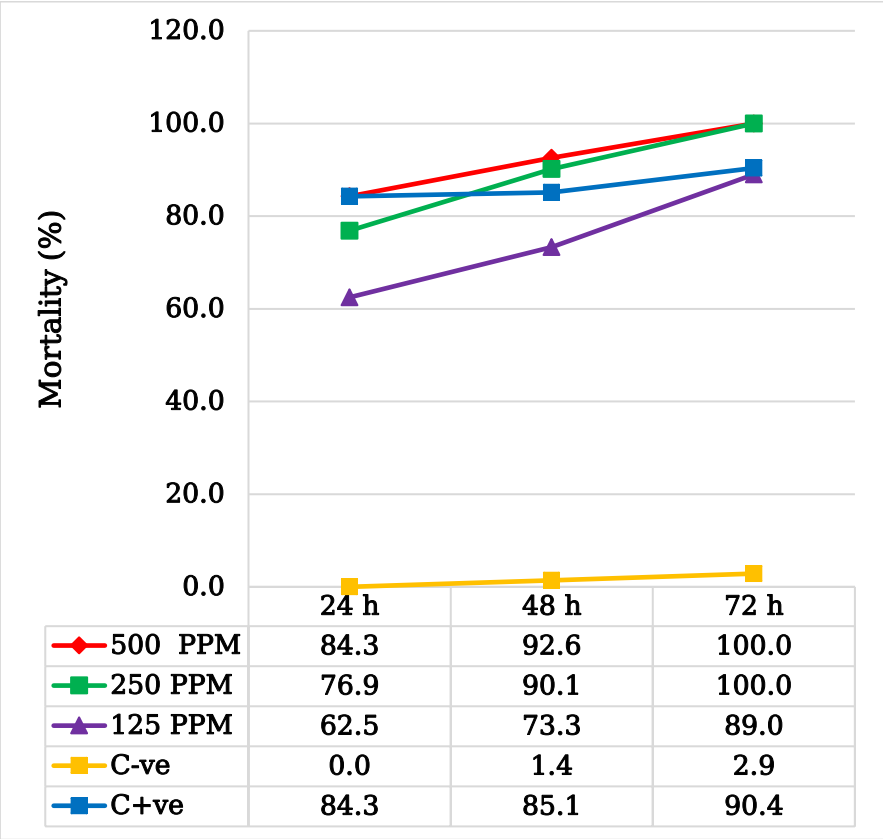
The results are summarized in Figures (4.13 to 4.18), respectively. The other concentrations showed significant mortality percentage as Figures (4.12, 4.14 & 4.16), and shows the inhibition concentrations, (IC) that killed 50, 90, 95 and 99% ($IC_{50,}$ $LC_{90,}$ IC_{95} and IC_{99}) as Figures (4.13, 4.15 & 4.17) were against *G. lamblia* trophozoites *In-vitro* method used Microsoft Excel (2016) analysis data. The antigiardial effects of extracts are clearly dose-dependent. Several results were reported about antigiardial activity of different plant extracts (Najumudin *et al*., 2018, Mohammed *et al*., 2015, Hassan *et al*., 2011, Garbi *et al*., 2018).

In the present study *in-vitro* investigation reveals promising results for the use of the plant for cultivated parasites to formulate such ingredient of the plant extract as a drug. However, the IC_{50} of *M. indica*, *R. officinalis* and *T. bakis* are 3, 20 and 42 µg/ml, respectively after 72 h, against *G. lamblia* trophozoites *in-vitro*, in addition, the highest activity against *G. lamblia* was from *M. indica.* The ethanolic crude extract of *M. indica* exhibit (100%) mortality at concentrations 500 and 250 µg/ml after 72 h, comparable compared with metronidazole powder (reference drug) which gave 90.4% mortality at concentration 312.5 µg/mL at the same time, as shown in Figure (4.12). The study revealed that the extract of *M. indica* has infectious anti-giardial activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values were 3, 137, 220 and 322 µg/ml, respectively after 72 h, against *G. lamblia* trophozoites *in-vitro*, as shown in Figure (4.13).

The ethanolic extract of *R. officinalis* exhibit (94%) mortality at concentrations 500 and 250 µg/mL after 72 hours, this was compared with metronidazole powder (reference drug) which gave (90.4%) mortality at concentration 312.5 µg/ml at the same time, as shown in Figure (4.14). The study revealed that the crude extract of *R. officinalis* has infectious anti-giardial activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values were 20, 378, 545 and 731 µg/ml, respectively after 72 h, against *G. lamblia* trophozoites *in-vitro*, as shown in Figure (4.15). The result is in agreement with Elbadr *et al*. (2013a) who found strong activity against *G. lamblia* when used methanol extract from *R. officinalis*. In this study antigiardial activity of *R. officinalis* extract may be attributed to their phytoconstituents content.

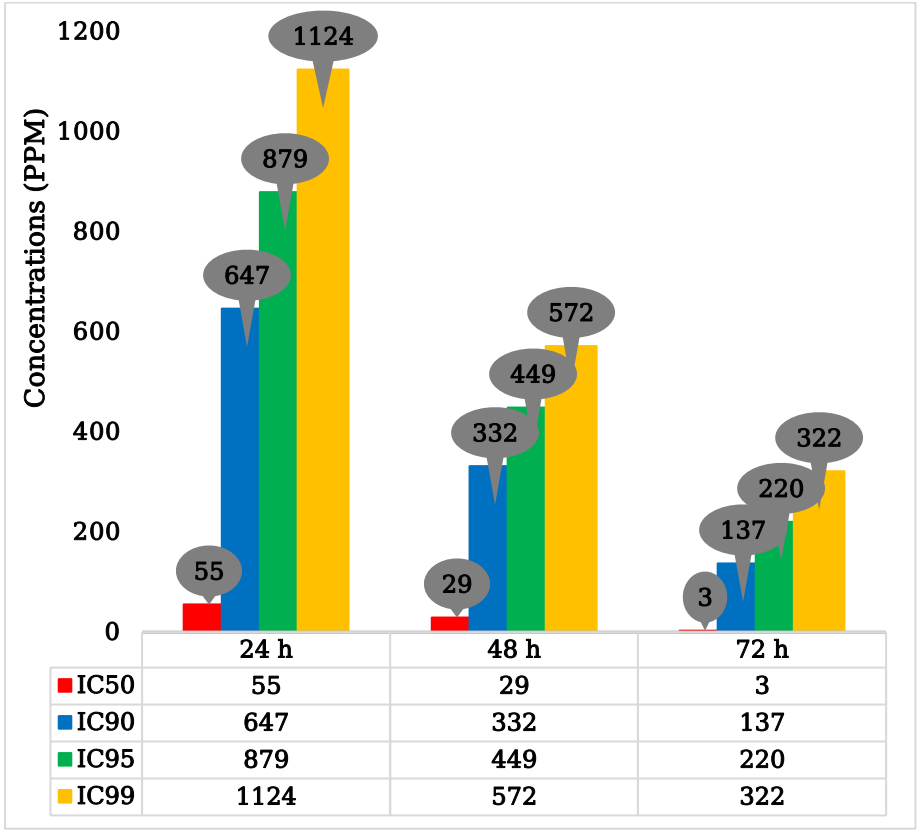
And ethanolic extract of *T. bakis* exhibit (96%) mortality at concentrations 500 and 250 µg/mL after 72 hours, this was compared with metronidazole powder (reference drug) which gave (90.4%) mortality at concentration 312.5 µg/mL at the same time, as shown in Figure (4.16). The study revealed that the crude extract of *T. bakis* has infectious anti-giardial activity at inhibition concentrations $IC_{50,}$ IC_{90}, IC_{95} and IC_{99} values 42, 350, 455 and 562 µg/ml, respectively after 72 h, against *G. lamblia* trophozoites *in-vitro*, as shown in Figure (4.17). In addition, there are no reports on the use of extract of *T. bakis* against *G. lamblia*. This study shows that the selected medicinal plant extracts reduce the severity of *G. lamblia* infection *in-vitro*. Similarly several studies have proven that plants and their isolates can be a source of anti-parasitic agents. Also, Barbosa *et al*. (2007) reported that epicatechin, a flavonoid isolated from the *Geranium mexicanum* exhibited potent activity against *G. lamblia* more than metronidazole which is widely used as

the main therapy. Moreover, McGaw *et al*. (2000) work on plant extracts and compounds containing tannins and alkaloids possess activity against diarrhea-causing parasites such as *G. lamblia* and *E. histolytica*.



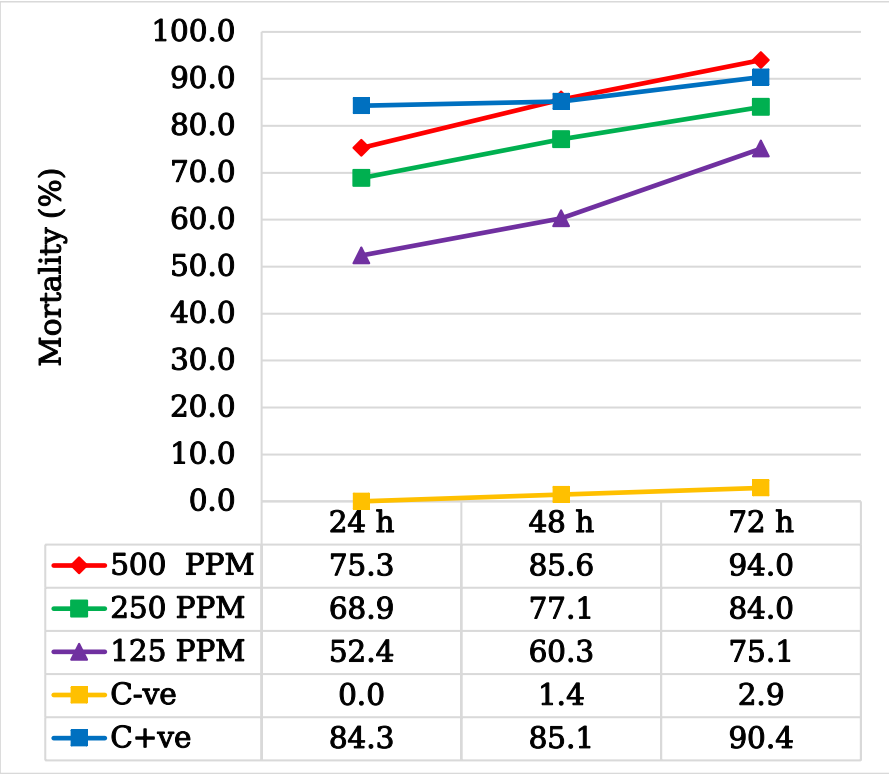
	24 h	48 h	72 h
500 PPM	84.3	92.6	100.0
250 PPM	76.9	90.1	100.0
125 PPM	62.5	73.3	89.0
C-ve	0.0	1.4	2.9
C+ve	84.3	85.1	90.4

Figure (4.12): ***In-vitro*** **gardicidal activity of ethanolic extract from** ***Mangifera indica*** **(seeds) against** ***Giardia lamblia*** **(trophozoites).**



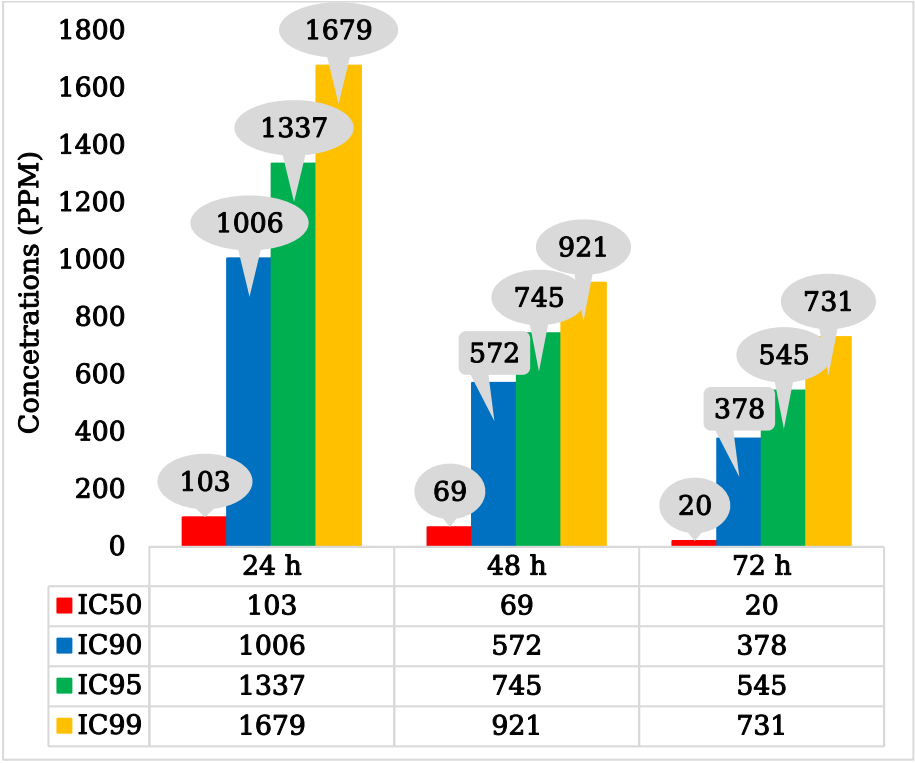
	24 h	48 h	72 h
IC50	55	29	3
IC90	647	332	137
IC95	879	449	220
IC99	1124	572	322

Figure (4.13): Inhibition concentrations (IC_{50}, LC_{90}, IC_{95} and IC_{99}) of ethanolic extract from ***Mangifera indica*** **against** ***Giardia lamblia*** **(trophozoites)** ***in-vitro.***



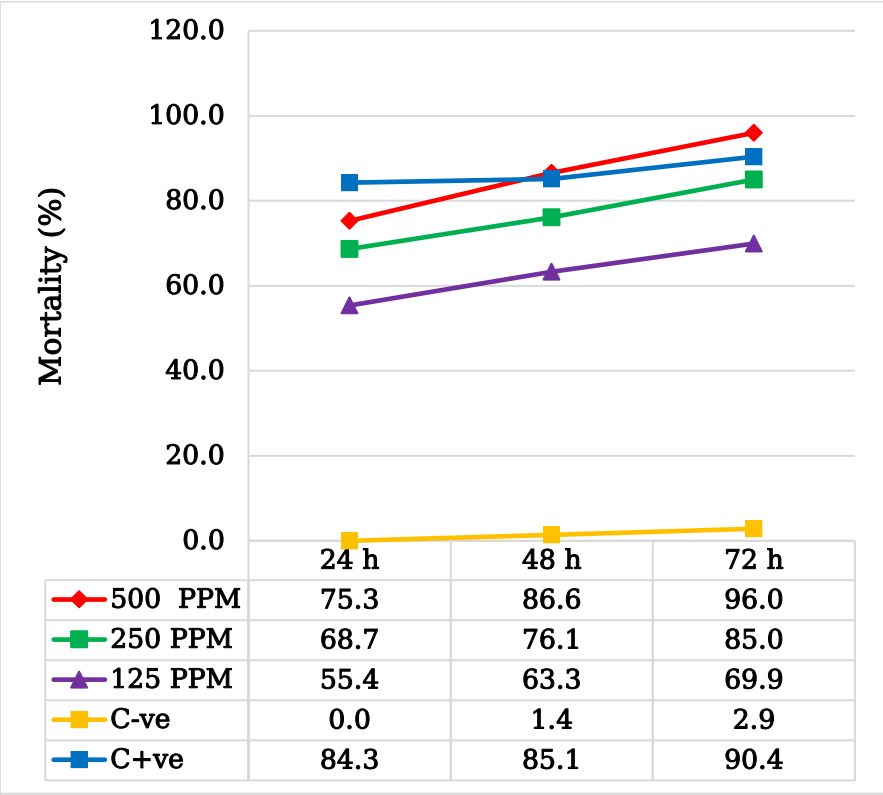
	24 h	48 h	72 h
500 PPM	75.3	85.6	94.0
250 PPM	68.9	77.1	84.0
125 PPM	52.4	60.3	75.1
C-ve	0.0	1.4	2.9
C+ve	84.3	85.1	90.4

Figure (4.14): ***In-vitro*** **gardicidal activity of ethanolic extract from** ***Rosmarinus officinalis*** **(pods) against** ***Giardia lamblia*** **(trophozoites).**



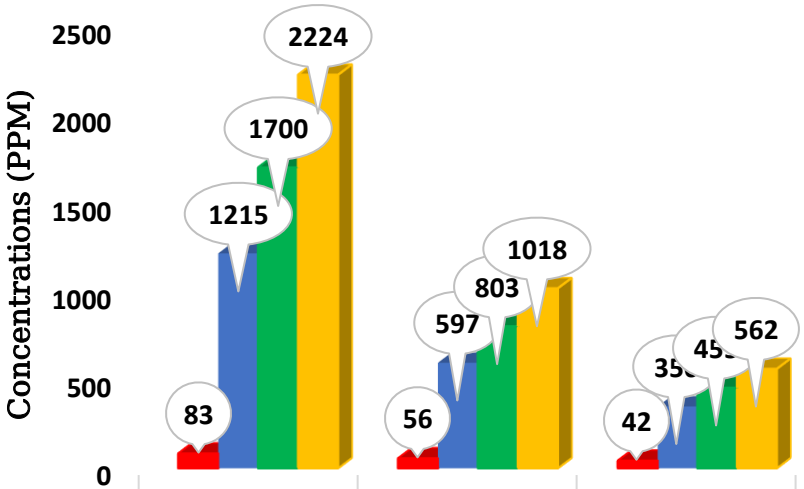
	24 h	48 h	72 h
IC50	103	69	20
IC90	1006	572	378
IC95	1337	745	545
IC99	1679	921	731

Figure (4.15): Inhibition concentrations (IC_{50}, LC_{90}, IC_{95} and IC_{99}) of ethanolic extract from ***Rosmarinus officinalis*** **against** ***Giardia lamblia*** **(trophozoites)** ***in-vitro.***



	24 h	48 h	72 h
500 PPM	75.3	86.6	96.0
250 PPM	68.7	76.1	85.0
125 PPM	55.4	63.3	69.9
C-ve	0.0	1.4	2.9
C+ve	84.3	85.1	90.4

Figure (4.16): *In-vitro* gardicidal activity of ethanolic extract from *Tinospora bakis* (whole plant) against *Giardia lamblia* (trophozoites).



	24 h	48 h	72 h
IC50	83	56	42
IC90	1215	597	350
IC95	1700	803	455
IC99	2224	1018	562

Figure (4.17): Inhibition concentrations (IC_{50}, LC_{90}, IC_{95} and IC_{99}) of ethanolic extract from *Tinospora bakis* against *Giardia lamblia* (trophozoites) *in-vitro*.

3.2.3 Molluscicidal activity

The results in figures 4.19, 4.21 and 4.23 showed that the molluscicidal potency of all extracts tested against *B. pfeiffer* was concentration dependent. Generally, mortality increased with the increase in concentration of the extracts. This scientific work emphasized the potential effects of the plants against the snail vector as had been investigated by Abdelsalam *et al*. 2016).

The ethanolic extract of *M. indica* was exhibited (100%) mortality within 24 h, at a concentration 1000 ppm; and exhibited 100% mortality within 48 h, at concentrations 1000, 500 and 250 ppm, as shown in Figure (4.18). The IC_{50}, IC_{90}, IC_{95} and IC_{99} values of ethanolic extract of *M. indica* against *B. pfeiffer* were 0.03, 69.01, 185.75 and 410.17 µg/ml, respectively after day two, as shown in Figure (4.19). The ethanolic extract of *R. officinalis* was exhibited (100%) mortality within 24 h, at a concentration 1000 ppm; and exhibited 100% mortality within 48 h, at concentrations 1000 and 500 ppm, as shown in Figure (4.20). The IC_{50}, IC_{90}, IC_{95} and IC_{99} values of ethanolic extract of *R. officinalis* against *B. pfeiffer* were 2.52, 231.47, 407.17 and 639.74 µg/ml, respectively after day two, as shown in Figure (4.21). And the ethanolic extract of *T. bakis* was exhibited (100%) mortality within 48 h, at a concentrations 1000, 500 and 250 ppm, as shown in Figure (4.22). The IC_{50}, IC_{90}, IC_{95} and IC_{99} values of ethanolic extract of *T. bakis* against *B. pfeifferi* were 4.92, 198.43, 314.98 and 455.86 µg/ml, respectively after day two, as shown in Figure (4.23). The result was confirmed by Ouattara *et al*. (2006a) who found that aqueous extract of *T. bakis* roots had *in vitro* activity against *Plasmodium falciparum* chloroquine resistant strain. The results were similarly confirmed by the work by Farah *et al*. (2013) on aqueous extract of *T. bakis* roots against *Theileria lestoquardi*. The result also showed clear difference in the efficiency between plants extract tested whereas; the *M. indica*, proved to be more effective than the *R. officinalis* and *T. bakis*. In addition, comparing the IC_{50} values of *M. indica* seeds and *R. officinalis* pods they showed the highest molluscicidal activity 0.03 and 2.52 µg/ml, respectively, followed by *T. bakis* whole plant 4.92 µg/ml, against snail.

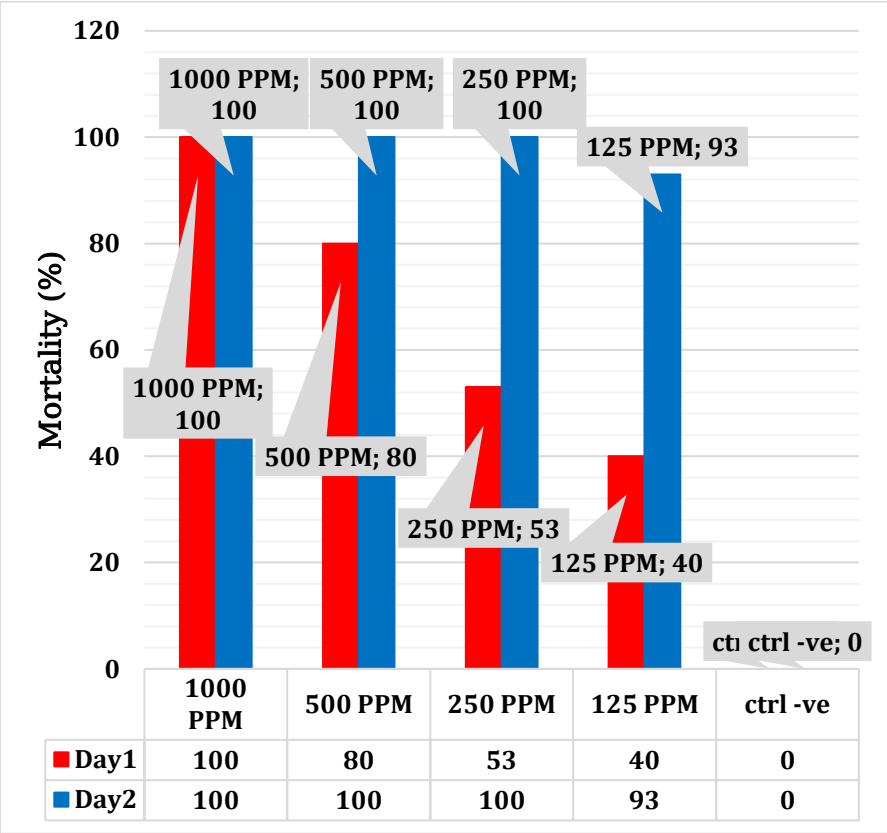


Figure (4.18): The molluscicidal activity of ethanolic extract from *Mangifera indica* against *Biomphalaria Pfeifferi* (snail).

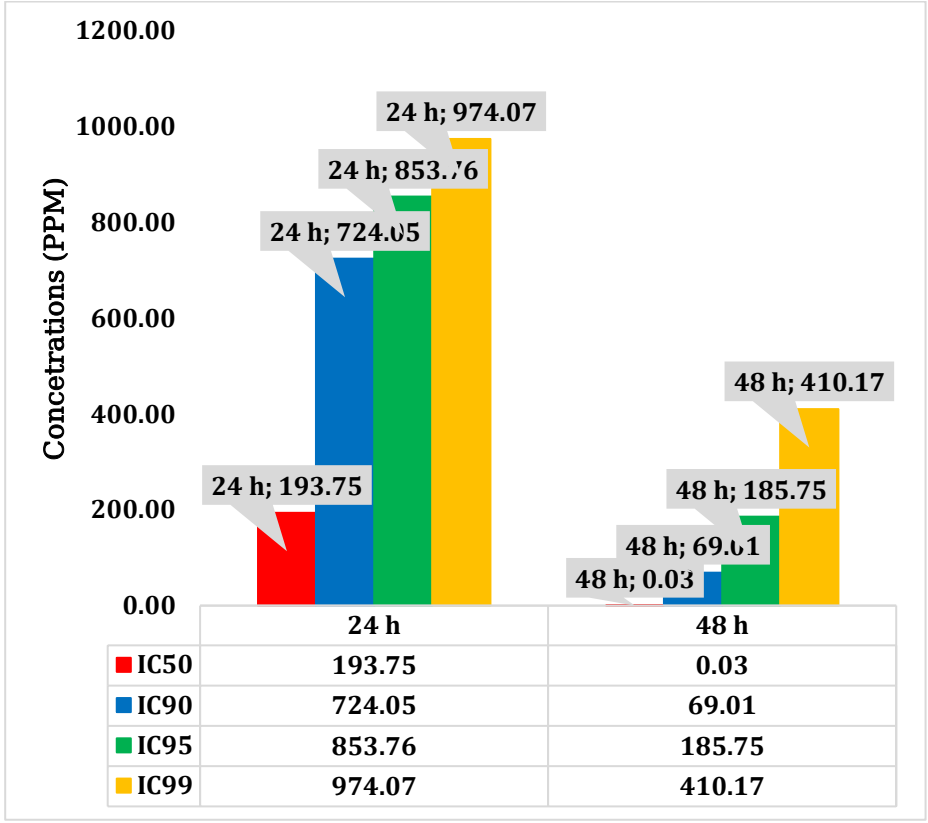


Figure (4.19): Inhibition concentrations (IC) of ethanolic extract from *Mangifera indica* of molluscicidal activity against *Biomphalaria Pfeifferi* (snail).

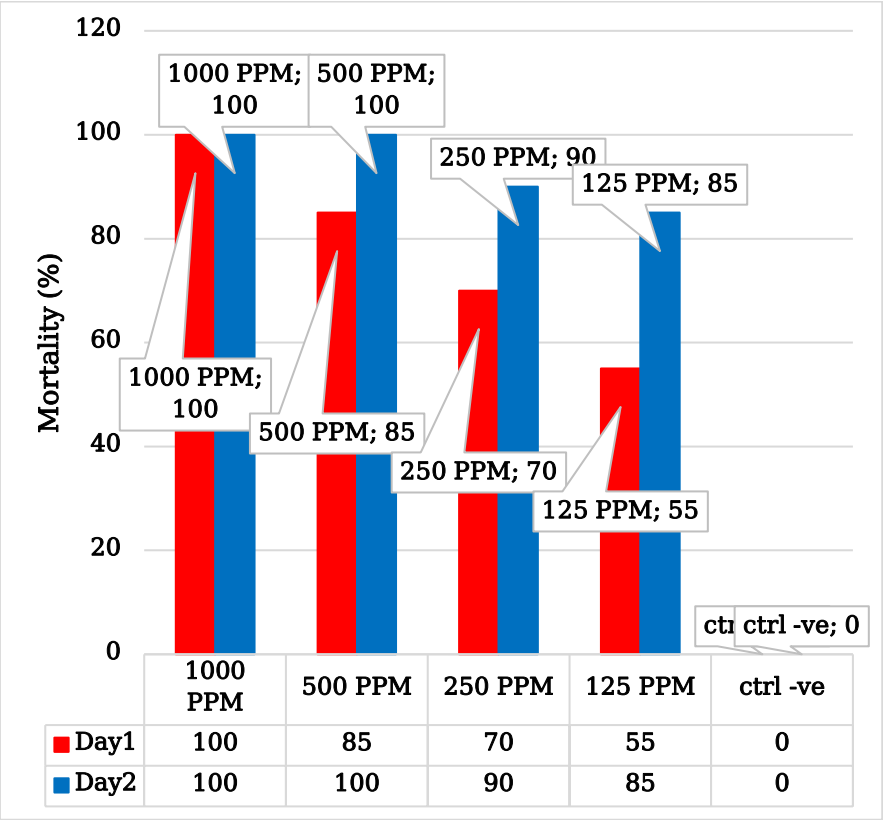


Figure (4.20): The molluscicidal activity of ethanolic extract from *Rosmarinus officinalis* against *Biomphalaria Pfeifferi* (snail).

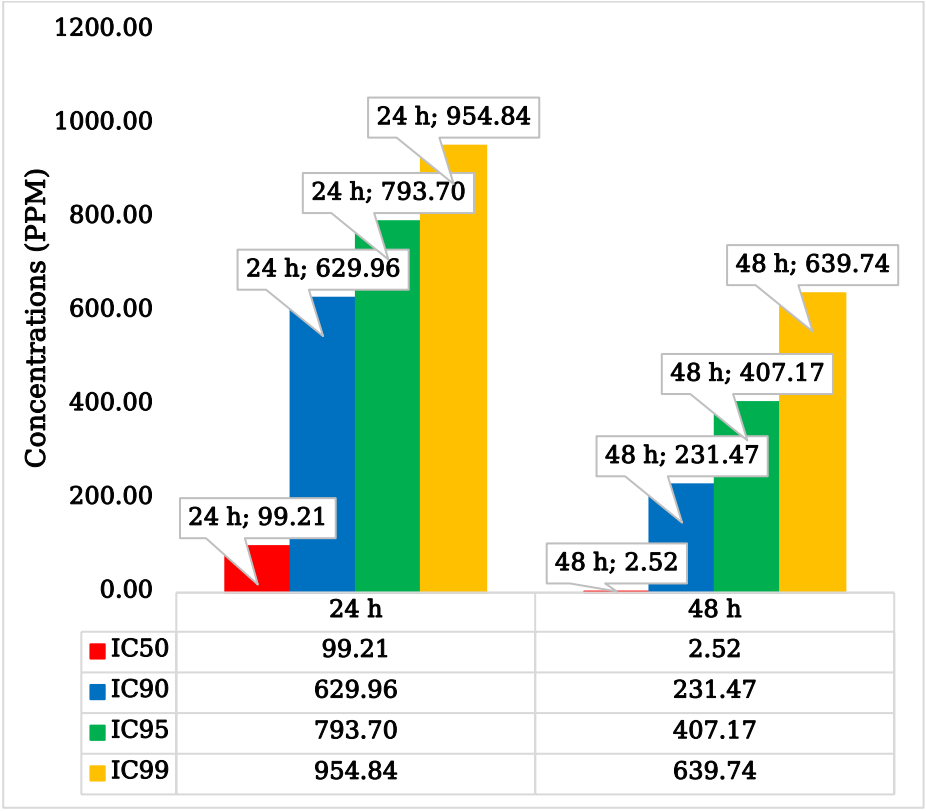


Figure (4.21): Inhibition concentrations (IC) of ethanolic extract from *Rosmarinus officinalis* of molluscicidal activity against *Biomphalaria Pfeifferi* (snail).

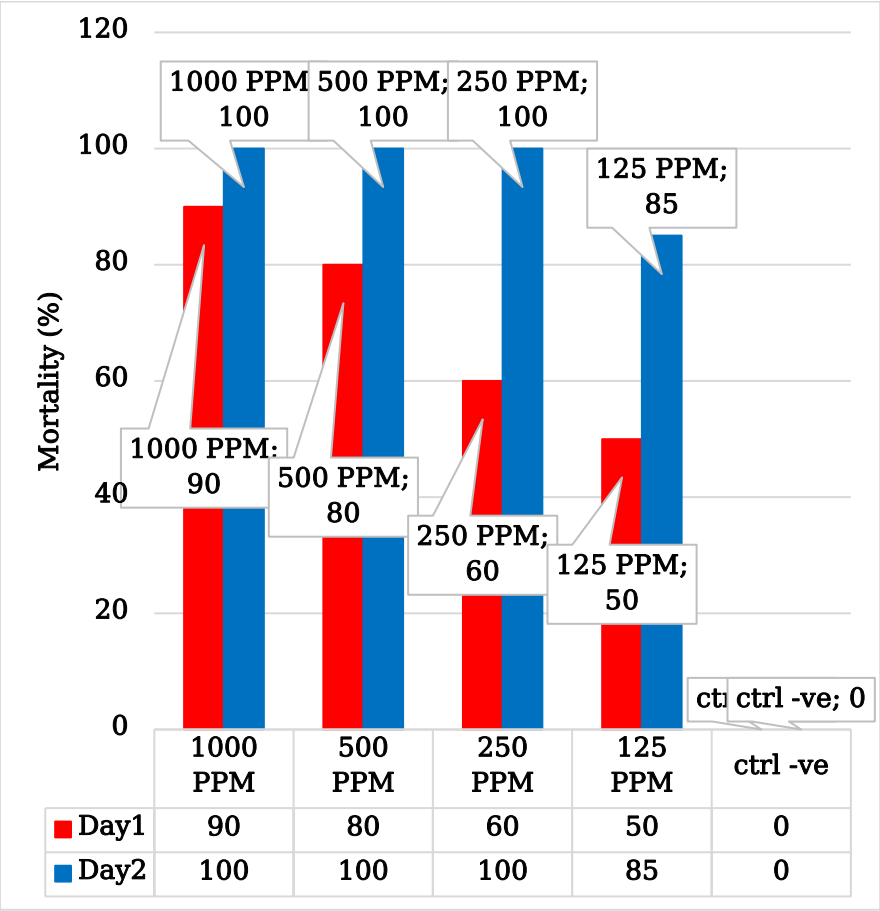


Figure (4.22): The molluscicidal activity of ethanolic extract from *Tinospora bakis* against *Biomphalaria Pfeifferi* (snail).

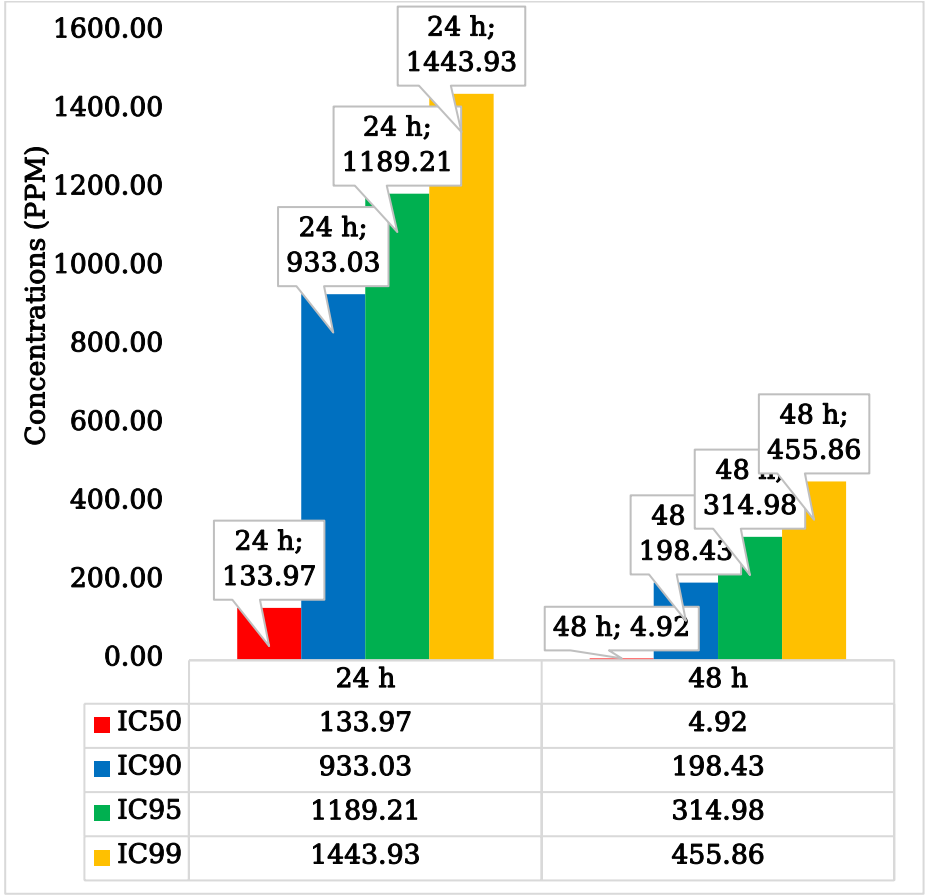


Figure (4.23): Inhibition concentrations (IC) of ethanolic extract from *Tinospora bakis* of molluscicidal activity against *Biomphalaria Pfeifferi* (snail).

4.2.4 Anti-oxidant Activity

Secondary metabolites of plants, specifically phenols, can adjust the concentration of ROS, thus activating a network of biochemical events to increase tolerance, hence the importance of studying the antioxidant activity of typical plant species. This study was screened for their free radical scavenging properties using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) of three medicinal plants namely: *M. indica*, *R. officinalis* and *T. bakis*, fractions and compounds to compare propyl gallate was used as standard antioxidant.

B. Antioxidant activity of the plants

Extracts and fractions subjected to the evaluation of antioxidant activity by using (DPPH-assay) *in-vitro*. DPPH radical scavenging activity was observed in all the extracts and fractions tested. The extracts and fractions showed dominant activity percentage and IC_{50} values (the concentration of the sample tested required to reduce 50% of the DPPH radical) were calculated and are depicted in Table (4.14).

Table (4.14), indicates the antioxidant DPPH-assay of ethanol extracts and fractions of some selected medicinal plants to compare propyl gallate as standard antioxidant. The percentage inhibitions of extracts and fractions for DPPH-assay was ranged from (36.0 to 94.0 %). The extracts and fractions tested showed the highest anti-oxidant activity more than 90 (> 90 %), except n-hexane and aqueous fractions of *T. bakis* showed the lowest 46 ± 0.02 and 36 ± 0.05 %, respectively. The IC_{50} values of the tested extracts, fractions and reference standard are shown in Table (4.14).

The ethanol extract and fractions of *M. indica* had the strongest antioxidant activity followed by *R. officinalis* and *T. bakis* in DPPH- assay *in-vitro*. The *M. indica* extract showed the strongest antioxidant activity with (92 ± 0.03 %), and IC_{50} (0.0083 ± 0.0001 mg/ml), while, *R. officinalis* was showed with (85 ± 0.02 %), and IC_{50} (0.00225 ± 0.001 mg/ml), *T. bakis* extract was showed moderate antioxidant activity with (71 ± 0.01 %), and in comparison to the standard antioxidant (propyl gallate) levels gave (91 ± 0.01 RSA %) and IC_{50} (0.0141 ± 0.01 mg/ml). The strong antioxidant activity of *M. indica* is consistent with phytochemical constituents of this plant, alkaloid and phenolics (Silva, 2003; Itoh *et al*., 2009). Although there are no reports on the use of extract of T. bakis against DPPH-assay.

The active crude extracts have been subjected to bio-guided fractionation. The hexane, ethyl acetate, butanol and aqueous fractions of *M. indica, R. officinalis* and *T. bakis* have been evaluated for DPPH radical scavenging activity. These results are illustrated in Table (4.14).

The IC_{50} values of the active fractions were determined. The ethyl acetate of *M. indica, R. officinalis* and *T. bakis* which showed highest antioxidant activity with 94 ± 0.02, 92 ± 0.03 and 82 ± 0.06 %, respectively. The n-hexane fraction of *R. officinalis* was showed high antioxidant activity whit (92 ± 0.02 %). In addition, ethyl acetate of *M. indica* was gave (0.0074 ± 0.0001 mg/ml**),** while, n-hexane and ethyl acetate of *R. officinalis* were gave (0.0082 ± 0.0001 and 0.0083 ± 0.0005 mg/ml**),** respectively**,** which showed the very strongest activities. The n-butanol and aqueous of *M. indica* were showed strongest activities gave (0.0126 ± 0.0002 and 0.0331 ± 0.001 mg/ml), respectively, is comparable values to the standard antioxidant PG

(0.0414 ± 0.11 mg/ml). Whereas, the n-hexane and aqueous fraction of *T. bakis* were showed less activity (IC_{50}) with (46 ± 0.02 and 36 ± 0.05 %), respectively, as shown in Table (4.14) illustrates these results.

The high RSA percentage exhibited by the plants may be due to the presence of phenolic compounds (tannins, flavonoids, and anthraquinone glycosides) and terpenes as was found by Paduraru *et al*., (2008). This study reveals that tested plant materials have strongest significance to antioxidant activity and free radical scavenging activity, and that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

Table (4.14): Percentage radical scavenging activity (%RSA) and IC_{50} (mg/ml) of selected medicinal plant extracts and fractions using DPPH-assay:

No.	Name of Plants & control	solvents	%RSA* ± SD (DPPH)	IC_{50} ± SD (mg/ml)
1.	***Mangifera indica***	Ethanol	**92 ± 0.03**	0.0083 ± 0.0001
2.		Ethyl acetate	**94 ± 0.02**	0.0074 ± 0.0001
3.		n-butanol	**91 ± 0.01**	0.0126 ± 0.0002
4.		Aqueous	84 ± 0.05	0.0331 ± 0.001
5.	***Rosmarinus officinalis***	Ethanol	85 ± 0.02	0.00225 ± 0.001
6.		n-hexane	**92 ± 0.02**	0.0082 ± 0.0001
7.		Ethyl acetate	**92 ± 0.03**	0.0083 ± 0.0005
8.		n-butanol	87 ± 0.06	0.1430 ± 0.003
9.		Aqueous	67 ± 0.03	0.3293 ± 0.002
10.	***Tinospora bakis***	Ethanol	71 ± 0.01	ND
11.		n-hexane	46 ± 0.02	ND
12.		Ethyl acetate	82 ± 0.06	0.1635 ± 0.005
13.		n-butanol	68 ± 0.04	0.3195 ± 0.002
14.		Aqueous	36 ± 0.05	ND
15.	**Std.**	Propyl gallate	**91 ± 0.01**	0.0141 ± 0.01

Key: RSA* = Radicals scavenging activity, (**n;** 3), **DPPH=** 2, 2, Diphenyl -1- Picrylhydrazyl, **SD=** standard Division, **Std. = Standard =** Propyl gallate, **ND=** not determined. **The radicals scavenging activity was high than 90% (> 90 %) written in bold.**

C. Antioxidant activity of compounds

The free radical-scavenging activity of isolated compounds evaluated from *M. indica* and *T. bakis* ethyl acetate fractions. The isolated compounds from *M. indica* and *T. bakis* displayed weak antioxidant activity less than 50% except Methyl gallate showed highly active inhibition percentage with (92 ± 0.00 RSA %), and IC_{50} (0.03 ± 0.01 mg/ml) isolated from *M. indica*. Comparable to the standard antioxidant (propyl gallate) levels gave (91 ± 0.01 RSA %), IC_{50} (0.0141 ± 0.01 mg/ml). in addition the Methyl gallate isolated had shown higher inhibition than standard antioxidant (propyl gallate) (Table 4.15).

Table (4.15): Percentage radical scavenging (%) activity (%RSA) and IC_{50} (mg/ml) of isolated compounds using DPPH-assay:

Name of plants & control	Code of isolated compounds	%RSA* ± SD (DPPH)	IC_{50} ± SD (mg/ml)
Mangifera indica	**AK-45-3**	Inactive	ND
	AK-11-9	Inactive	ND
	AK-10	**92 ± 0.00**	**0.03 ±0.002**
	AK-8-4	**ND**	**ND**
Tinospora bakis	**AS-48-4**	ND	ND
	AS-5-11-5	18 ± 0.07	ND
	AS-5-12-3	14 ± 0.01	ND
	AS-8	07 ± 0.10	ND
	AS-10-8-2	15 ± 0.06	ND
	AS-9-4-2	14 ± 0.01	ND
	AS-9-3-7	39 ± 0.25	ND
	AS-10-4-18-6	ND	ND
	AS-13-2-6	ND	ND
	As-45-5	19 ± 0.19	ND
	AS-15-9-8	Inactive	ND
	AS-1-1	ND	ND
	AS-11-9-2	ND	ND
Propyl gallate	Standard	**91 ± 0.01**	**0.0141 ± 0.01**

Key: RSA* = Radicals scavenging activity, (**n;** 3), **DPPH=** 2, 2, Diphenyl -1- Picrylhydrazyl, **SD=** standard Division, **Std. = Standard =** Propyl gallate, **ND=** not determined. **The radicals scavenging activity was high than 90% (> 90 %) written in bold.**

4.3 Cytotoxicity effect

Medicinal plants continue to provide humanity with new remedies. It is therefore important to explore medicinal plants for their safety, quality, toxicity, appropriate amount of plant materials to use, and efficacy. Investigated the cytotoxicity of crude extracts and fractions *in-vitro* against brine shrimps (*Artemia salina* L.) lethality bioassay at concentrations used (1000, 100 and 10 µg/ml) and against normal cell line at concentrations used (500, 250 and 125 ppm) to compare Tritonx-100 (reference drug) by using Micro-culture tetrazolium test (MTT-assay).

4.3.1 *In-vitro* cytotoxicity

A. Cytotoxicity of extracts using the Brine shrimps assay

There are a few methods to determine whether a substance has anticancer activity. One of those method is brine shrimp lethal test (BSLT) using *Artemia salina* larvae. This test is considered as a useful tool in preliminary assessment of biological activities of plant extracts. The cytotoxicity of plants was confirmed by using Brine shrimps (*Artemia salina* L.) lethality bioassay at different concentrations (1000, 100 and 10 µg/ml). The results as shown in Table (4.16), indicated that all examined plants are non-toxic with LD_{50} > 1000 µg/ml. LD_{50} values below 249 µg/ml were considered as highly toxic, 250–499 µg/ml as moderately

toxic and 500–1000 µg/ml as lightly toxic, and values above 1000 µg/ml were regarded as non-toxic (McLaughlin *et al*., 1998).

The brine shrimp lethality bioassay is found to have a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity, and lead to the discovery of new class of natural pesticides and active anti-tumor agents (McLaughlin *et al*., 1998). Cytotoxic action of a drug is believed to be provided by disturbing the fundamental mechanisms associated with cell growth, mitotic activity, differentiation and function (Goodman *et al*., 1980). The observed cytotoxic activity for these extracts may be due to one of these mechanism. The result obtained showed that the activity of all the plant extracts and fractions were concentration-dependent with the death-rate of brine shrimp, are shown in Table (4.16).

All the extracts showed moderately and lightly toxic lethality concentration between 400 and > 1000 µg/ml. The extract of *M. indica* exhibited the highest lethality activity with LD_{50} value of 420.10 µg/ml. Lethal dose concentration (LD_{50}) from the regression and probit analysis in 24 h of our study showed that LD_{50} value moderately toxic and lightly toxic of *M. indica* crude extract and fractions, respectively. The results were lightly toxic (LD_{50}: 500-1000 µg/ml) to most of the studied extracts and fractions tested, except moderately toxic (LD_{50}: 420.10 µg/ml) of *M. indica* extract, and non-toxic ethyl acetate of *M. indica* and aqueous fraction of *T. bakis*, comparable to the standard drug (Etoposide 7.4625 µg/ml) as control.

Table (4.16): Cytotoxicity of selected medicinal plants of crude extracts and fractions on Brine shrimp:

No.	Name of Plants/Drug	solvents	Concentrations (µg/ml), n (3)						LD_{50} (µg/ml)	The degree toxicity
			1000	100	10	1000	100	10		
			Number of dead			Number of survive				
1.	*Mangifera indica*	crude	23	09	01	07	21	29	420.10	Moderately toxic
2.		n-hexane	ND							
3.		Ethyl acetate	19	07	01	11	23	29	590.20	Lightly toxic
4.		n-butanol	07	03	01	23	27	29	525.35	Lightly toxic
5.		Aqueous	04	02	00	26	28	30	836.03	Lightly toxic
6.	*Rosmarinus officinalis*	crude	18	10	05	12	20	25	607.00	Lightly toxic
7.		n-hexane	20	09	03	10	21	27	580.01	Lightly toxic
8.		Ethyl acetate	13	09	05	17	21	25	1210.0	Non toxic
9.		n-butanol	14	04	02	16	26	28	975.00	Lightly toxic
10.		Aqueous	05	03	00	25	27	30	856.00	Lightly toxic
11.	*Tinospora bakis*	crude	25	02	01	05	28	29	590.20	Lightly toxic
12.		n-hexane	25	03	01	05	27	29	435.05	Moderately toxic
13.		Ethyl acetate	06	02	00	24	28	30	578.73	Lightly toxic
14.		n-butanol	20	04	01	10	26	29	654.72	Lightly toxic
15		Aqueous	04	01	00	26	29	30	3625.43	Non toxic
16	Etoposide	Control							7.463	Highly toxic

Interpretation: Key: n: number of replicates, **ND:** not determined, **LD_{50} < 249 µg/ml:** were considered as **highly toxic**; **250- 499 µg/ml:** as **moderately toxic**; **500-1000 µg/ml:** as **lightly toxic**; **> 1000 µg/ml**: **Non Toxic** (McLaughlin *et al*., 1998).

B. Cytotoxicity of extracts using the MTT-assay:

The results in Table (4.17), indicated that both plants determined against BHK cell line were non-toxic with IC_{50} > 100 µg/ml, whereas the positive control exhibited the highest toxicity with IC_{50} < 30 µg/ml. In addition, compared with the previous results for brine shrimp screening, there are the same results, where all of the plants were nontoxic. The ethanolic extract of *M. indica* showed that IC_{50} of cytotoxic activity was 300.34 µg/ml, and this result was similar to Fitriasih *et al*. (2019) cytotoxic activity was 308.12 µg/ml.

Table (4.17): Cytotoxicity of crude ethanolic extracts of selected medicinal plants on normal cell line (BHK cell line) as measured by the MTT-assay:

No.	Name of Plants	Name of solvents	Concentrations (µg/ml) Inhibition (%) ± SD			IC_{50} (µg/ml)	IC_{50}
			500	250	125		
1.	***Mangifera indica***	Ethanolic	62.06 ± 0.04	45.76 ± 0.02	29.09 ± 0.06	**300.34**	> 100
2.	***Rosmarinus officinalis***	Ethanolic	54.53 ± 0.02	44.15 ± 0.05	29.23 ± 0.09	**372.65**	> 100
3.	***Tinospora bakis***	Ethanolic	ND				
4.	Triton-x**100**	**Control**	**96.28 ± 0.00**				**< 30**

Key: (**n;** 3), **IC_{50}** <30 µg/ml: High toxic. **ND** = Not determined, *Control = Triton-x100 was used as the control positive at 0.2 µg/ml. The maximum concentration used was 500µg/ml. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated. **IC_{50}** <30 µg/ml: High toxic.

4.4 Chemical qualitative and quantitative analysis

4.4.1 Qualitative analysis

A. Qualitative phytochemical analysis

The qualitative screening of plant extracts were carried out according to the standard procedures as mentioned in Table (4.18). Phytochemical screening was performed for all that extracts. The qualitative phytochemicals screening revealed the presence of phenols, tannins, flavonoids, alkaloids, triterpenes, steroids, coumarins and saponins in all plants tested. While anthraquinone was present from *R. officinalis* whole plant only, as seen in Table (4.18). The phytochemical analysis conducted on *M. indica* seeds extract showed the presence of phenols, tannins, flavonoids, alkaloids, triterpenes, steroids, coumarins and saponins. On the other hand, anthraquinone was not detected in ethanol extract of *M. indica*. The *M. indica* seeds extract was strongly (+++) consist of tannins, flavonoids and phenols, and moderately (++) of saponins and alkaloids. While, *R. officinalis* was moderately (++) of flavonoids. And *T. bakis* was strongly (+++) of triterpenes, while, moderately (++) of saponins, tannins, alkaloids and flavonoids. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to against microbial infections. Flavonoids and phenols are considered as potent anti-agents for cancer, microbes and tumors (Rahman *et al*., 2021). Phenolics and flavonoids possess antiallergic, anti-inflammatory, antimicrobial and anticancer activity (Karak, 2019). Tannins are reported to have a strong antitumors, antioxidant, cytotoxic, anti-irritant, anti-secretolytic antiphlogistic, antimicrobial and antiparasitic effects (Sulaiman *et al*., 2015, Bladt, 2009). Saponnins Saponins possess hypocholesterolemic, antidiabetic, antimicrobial properties and also enhance immune system (OBODE *et al*., 2020).

Table (4.18): Qualitative phytochemical analysis of the plants:

No.	Name of test compounds	Reagent tested	Results		
			Mangifera indica	*Rosmarinus officinalis*	*Tinospora bakis*
1	Saponins	Frothing	++	+	++
2	Coumarins	KOH	+	+	+
3	Tannins	Ferric Chloride	+++	+	++
4	Alkaloids	Mayer's	++	+	++
5	Sterols	Salkowski	+	+	+
6	Triterpenes	Salkowski	+	+	+++
7	Flavonoids	Sodium hydroxide	+++	++	++
8	Anthraquinone	hydrogen peroxide	-	+	-
9	Phenols	Ferric Chloride	+++	+	+

Key: (+++): Strongly present, **(++):** Moderately present, **(+):** Poorly present, **(-):** Absent.

B. Qualitative thin layer chromatography (TLC)

The constituents are responsible for most pharmacological activities of plants. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analyzing the R_f values of compounds in different solvent system (Alebiosu and Yusuf, 2015).

TLC technique was used for preliminary phytochemical screening and to separate various constituents of crude extracts of plants under study. TLC profiling of plant extracts in different solvent system and different spray reagent confirms the presence of diverse group of phytochemicals, R_f values obtained from thin layer chromatographic analysis are listed in the Table (4.19). TLC studies of the ethanol extracts of *M. indica* and *R. officinalis* were used different Solvent system. The distance traveled by solvent (6.9).

Thin layer chromatography for alkaloids of crude ethanol extracts of *M. indica* and *R. officinalis* were using solvent system of Toluene: ethyl acetate: dimethylamine (70:20:10) and spray reagent (Dragendorff and UV 365 nm) were revealed that the presence of one compound from *R. officinalis* having R_f values was 0.62. While, did not found compound from *M. indica.*

The TLC for tannins of ethanolic crude extracts of *M. indica* and *R. officinalis* using solvent system of ethyl acetate: methanol: water (100:13.5:10) and spray reagent (Berlin blue and UV-365 nm) revealed the presence of four compounds from *R. officinalis* having R_f values; 0.36, 0.59, 0.84 and 0.97, while, the two compounds from *M. indica* having R_f values 0.28 and 0.96.

TLC of flavonoids of ethanol crude extracts of *M. indica* and *R. officinalis* using solvent system of ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) and reagent (NP/PEG reagent and UV- 365) revealed the presence of three compounds from *M. indica* having R_f values 0.42, 0.75 and 0.93, the presence of one compound from *R. officinalis* having R_f values 0.70**.**

The TLC for anthraquinone of ethanol extracts of *M. indica* and *R. officinalis* using solvent system of ethyl acetate: methanol: water (100:13.5:10) and spray reagent (KOH and UV-365nm) revealed the presence of three compounds from *R. officinalis* having R_f values of 0.42, 0.75 and 0.93, and the presence of two compounds from *M. indica* having R_f values 0.43 and 0.73.

TLC for saponins of ethanol crude extracts of *M. indica* and *R. officinalis* using solvent system of chloroform: glacial acetic acid: methanol: water (64:32:12:8) and reagent (AS reagent and UV- 365) revealed the presence of two compound from *R. officinalis* having R_f values 0.33 and 0.89, and the presence of one compounds from *M. indica* of R_f values 0.26.

Table (4.19): R_f values of TLC analysis of solvent systems of the studied plants:

No.	Name of components	R_f value	
		Mangifera indica	***Rosmarinus officinalis***
1.	**Alkaloids**	**-**	0.62
2.	**Tannins**	0.28, 0.96	**0.36, 0.59, 0.84, 0.97**
3.	**Flavonoids**	**0.63, 0.80, 0.94**	0.70
4.	**Anthraquinone**	0.43, 0.73	**0.42, 0.75, 0.93**
5.	**Saponins**	0.26	0.33, 0.89

Key: R_f: Retention factor, **the number of spots ≥ 3 written in bold.**

4.4.2 Quantitative Analysis

A. Determination of total phenolic content

As one of the most important antioxidant plant components, phenolic compounds were widely investigated in many medicinal plant and vegetables (Djeridane *et al*., 2006). Total phenolics content was determined by the preparation of Gallic acid calibration curve. Gallic acid was taken as standard in different concentration (µg/ml) and measured the absorbance of Gallic acid at 765 nm. According to this calibration curve, phenolics content of the extracts were determined by using following equation: $Y = 0.019x + 0.0013$, where $R^2 = 0.995$. The total phenolic contents (Gallic acid equivalents, mg GAE /g) in the ethanol crude extract of *M. indica* and *R. officinalis* were calculated to be 933 ± 0.31 and 732 ± 0.09 mg GAE /g, respectively, as shown in Table **(**4.20**)**. The highest Phenolic content of *M. indica* was (933 ± 0.31 mg GAE /g). These results could give a clue interpreting the observed high bioactivities of this plant. The results from several studies also showed that mango is a good source of antioxidants (Lin and Tang, 2007). Phenolic compounds have redox properties, which allow them to be active as antioxidants (Soobrattee *et al*., 2005). As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity (Baba and Malik, 2015).

B. Determination of total flavonoid content

Total flavonoids content was determined by the preparation of Quercetin calibration line. Quercetin was taken as standard in different concentration (µg/ml) and measured the absorbance of quercetin at 510 nm. According to this calibration curve, flavonoids content of the extracts were determined by using following equation: $Y = 0.0953x - 0.0024$, where R^2 value was obtained = 0.997. The quercetin calibration line

equation can be used as a comparison to determine the total concentration of flavonoids in the sample extract. The total flavonoids contents (Quercetin equivalents, mg QE/g) in the *M. indica* and *R. officinalis* were calculated to be 190 ± 0.07 and 41.5 ± 0.01 mg QE/g, respectively, as results shown in Table **(**4.20**)**. The higher flavonoid content of 190 ± 0.07 mg QE/g followed by *M. indica*. It's possible that could be the reason of this highly biological activities. The total flavonoid content of *M. indica* was found to be 190 ± 120 0.07 mg QE/g. The obtained results were much higher than that reported for peel and kernel of other mango cultivars (10 – 1170 mg/100 g) (Ribeiro *et al*., 2008, Dorta *et al*., 2014, Ajila and Rao, 2013). Our experiment revealed flavonoids were present in the highest amount in the *R. officinalis* contain hydroxyls is responsible for the radical scavenging effect in plants (Middleton *et al*., 2000, Amić *et al*., 2003). Flavonoids, including flavones, flavonols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in-vitro* and also activity as antioxidants *in vivo* (Geetha *et al*., 2003, Shimoi *et al*., 1996).

C. Determination of total Tannin content

Tannin has potential as antiseptics, astringent, antioxidants, anti-mites, anti-fungi, and metal chelating agent (Astiti *et al*., 2019). Total tannin content was using $FeCl_3$ and gelatin test and determined by the preparation of Tannic acid calibration line. Tannic acid was used as a standard in different concentration (µg/ml) and the total tannin content were expressed as tannic acid equivalents (TAE). Absorbance was measured using a spectrophotometer at 510 nm. According to this calibration line, tannin content of the extracts were determined by using following equation: The total tannins contents (Tannic acid equivalents, mg) in the *M. indica* and *R. officinalis* were calculated to be 1322.5 ± 0.20 mg and 367.5 ± 0.08 mg, respectively, as results shown in Table **(**4.20**)**. The higher tannin content of 1322.5 ± 0.20 mg followed by *M. indica*. Tannin is an excellent antioxidant compounds in plants and their activity depends of their quantity. Tannins are polyphenolic compounds which are reported to possess antiviral, anti-parasitic, anti-oxidant, anti-inflammatory activities. They are also used in the treatment of hemorrhoids, infections, skin ulcer (Boy *et al*., 2018).

Table (4.20): Quantitative phytochemicals of total Phenolic (mg GAE/g), total Flavonoid (mg QE/g) and total Tannin Content (mg/100 g) of medicinal plants:

No.	Name of Plants	Name of Extracts	Total polyphenols		
			Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Tannin (mg/100 g)
			Means ± SD		
1.	***Mangifera indica***	Ethanol crude	**933 ± 0.31**	**190 ± 0.07**	**1322.5 ± 0.20**
2.	***Rosmarinus officinalis***	Ethanol crude	732 ± 0.09	41.5 ± 0.01	367.5 ± 0.08
3.	***Tinospora bakis***	Ethanol crude	ND	ND	ND

Key: All data analysis used Excel (2013), Mean of triplicate determinations ± Standard Deviation (SD), **ND** = not determined**.**

4.4.3 Qualitative and Quantitative Analysis using GC-MS:

A. Identification of compounds

The results pertaining to GC-MS analysis lead to the identification of number of compounds. These compounds were identified through mass spectrometry attached with GC. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of these compounds. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The name, molecular weight and structure of the components of the test materials were ascertained, the components identified by the GC-MS are illustrated in Tables (4.21 to 4.29), respectively and Figures (4.25 to 4.37), respectively. Now a day, the study of bioactive components from medicinal plants and their activity has increased. These phytochemicals are responsible for various pharmacological actions like antimicrobial and antioxidant activities, Tapiero *et al*. (2002).

B. Chemical composition

The qualitative and quantitative analyses of the extracts of *M. indica*, *R. officinalis*, and *T. bakis* were showed components as detected in the GC-MS profile (11, 26 and 33.4), respectively, are illustrated in Tables (4.21 to 4.29) and Figures (4.24 to 4.36), respectively.

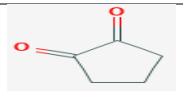
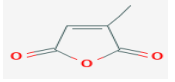
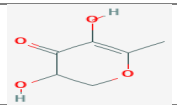
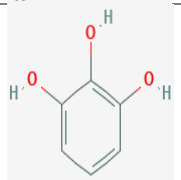
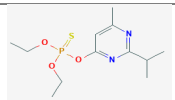
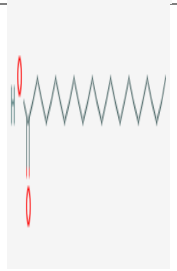
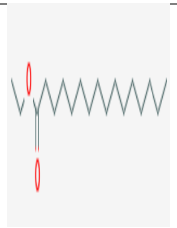
Gas chromatography and mass spectroscopy analysis of compounds was carried out in ethanolic extract of *M. indica*, shown in Table (4.21). It was subject to several phytochemical studies. The GC-MS chromatogram of the Eleven (11) known compounds of the extract detected was shown in Figures (4.25 and 4.26). The first compound identified with less retention time (3.607 min) was 1, 2-Cyclopentanedione, whereas Octadecanoic acid, ethyl ester was the last compound which took longest retention time (19.136 min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table (4.21). The major compounds were identified with high percentage (%) like 1, 2, 3-Benzenetriol (68.61 %) was found as a main components included Octadecanoic acid (15.34 %), 9-Octadecenoic acid, (E) - (5.57 %), n-Hexadecanoic acid (4.36 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1.61 %), Octadecanoic acid, ethyl ester (1.28 %) and 9,12-Octadecadienoic acid (Z,Z) (1.17 %), as shown in Figure (4.25), showed a wide range of potent bioactivity. *M. indica* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *M. indica* can be useful.

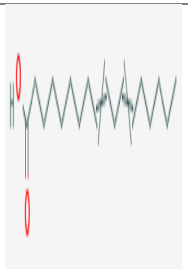
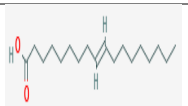
Of the eleven (11) compounds identified from the GC-MS result, 1, 2, 3-Benzenetriol (68.61 %) highly amount. 1, 2, 3-Benzenetriol (Pyrogallol) is known as one of phenolic (hydrolysable tannin) (Khanbabaee and Van Ree, 2001), and has been found as the major compound of *M. indica* in this study. Present study reports phytoconstituents in the spectrum which are known for various biological properties like that 1, 2, 3-Benzenetriol (Pyrogallol) phenolic known to possess antimalarial, antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, anticancer and cytotoxic (Beulah *et al*., 2018). The presence of hydroxyl

groups and alpha-beta double bonds in a phenolic compound plays an important role towards the antimicrobial activity (Ultee *et al*., 2002). Similarly Diazinone alkaloid compound as anti-cancer activity (Duke and Bogenschutz, 1994).

In this study, the GC-MS analysis showed the nature of compounds including fatty acid 4(37%) of the compounds identified in the investigated extract, among which ester 3(27%), alkaloids, ketone, phenolic and heterocyclic 1(9%), are shown in Table (4.22) and Figure (4.27). The fatty acid (4) compounds including 2, 5-Furandione, 3-methyl- (1), N-Hexadecanoic acid (2), Hexadecanoic acid, ethyl ester (3) and Octadecanoic acid (4). The ester (3) compounds showed 9, 12-Octadecadienoic acid (Z, Z) (1), 9-Octadecenoic acid, (E)- (2) and Octadecanoic acid, ethyl ester (3). Alkaloid (1) compound showed Diazinone (1), ketone (1) compound showed as 1,2-Cyclopentanedione (1), phenolic (1) compound showed as 1,2,3-Benzenetriol (1) and heterocyclic (1) compound showed as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (1), are shown in Table (4.22). In addition, the extract had highly compounds as fatty acids 4(37 %) and ester 3(27 %).

Table (4.21): Major phytochemical compounds identified in ethanolic extract of *Mangifera indica* by using GC-MS analysis:

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds (References)
1	1,2-Cyclopentanedione	3.607	0.24	$C_5H_6O_2$	98.1	Ketone		Anti-inflammatory agent (Chung *et al.*, 2007).
2	2,5-Furandione, 3-methyl-	3.802	0.57	$C_5H_4O_3$	112.08	Fatty acid		Anticancer and Antimicrobial activities (Mohammed et al., 2016a).
3	**4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-**	**6.662**	**1.61**	**$C_6H_8O_4$**	**144.12**	**Heterocyclic**		Antioxidant activity (Čechovská *et al.*, 2011).
4	**1,2,3-Benzenetriol**	**10.390**	**68.61**	**$C_6H_6O_3$**	**126.11**	**Phenolic**		Antimalarial, Antimicrobial, Anti-inflammatory, Antioxidant, Analgesic, Insecticide, Anticancer activities and Cytotoxic (Beulah *et al.*, 2018).
5	Diazinone	15.298	0.57	$C_{12}H_{21}N_2O_3PS$	304.35	Alkaloid		Anti-cancer activity (Duke and Bogenschutz, 1994).
6	**n-Hexadecanoic acid (Palmitic acid)**	**16.903**	**4.36**	**$C_{16}H_{32}O_2$**	**256.42**	**Fatty acid**		Antioxidant, Pesticide, Flavor, Hemolytic, Lubricant, and anti-cancer (Harada *et al.*, 2002, Ponnamma and Manjunath, 2012). Anti-inflammatory, antiandrogenic and hypocholestrolemic (Aparna *et al.*, 2012, Kumar *et al.*, 2010).
7	Hexadecanoic acid, ethyl ester	17.180	0.79	$C_{18}H_{36}O_2$	284.5	Fatty acid		Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Anti-androgenic, Hemolytic and 5- Alpha reductase inhibitor activities (Sudha *et al.*, 2013).

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds (References)
8	**9,12-Octadecadienoic acid (Z,Z)**	**18.670**	**1.17**	**$C_{18}H_{32}O_2$**	**280.4**	**Ester**		Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Anti-acne, Anti-arthritic, 5-Alpha reductase inhibitor, Anti-androgenic and Anti-coronary activities (Sermakkani and Thangapandian, 2012)
9	**9-Octadecenoic acid, (E)-**	**18.703**	**5.57**	**$C_{18}H_{34}O_2$**	**282.5**	**Ester**		Anticancer, Antibacterial and Antiviral activities (Duke and Bogenschutz, 1994)
10	**Octadecanoic acid**	**18.916**	**15.24**	**$C_{18}H_{36}O_2$**	**284.5**	**Fatty acid**		Antioxidant and cancer preventive activities (Duke and Bogenschutz, 1994).
11	**Octadecanoic acid, ethyl ester**	**19.136**	**1.28**	**$C_{20}H_{40}O_2$**	**312.5**	**Ester**		Anticancer activity (Duke and Bogenschutz, 1994).
	Total percentage		**100**					

Key: R. time= Retention time**, M. Weight**= Molecular Weight, **g/mol**= gram/molar. Main components (≥1%) marked in **bold**. **Activity Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases.

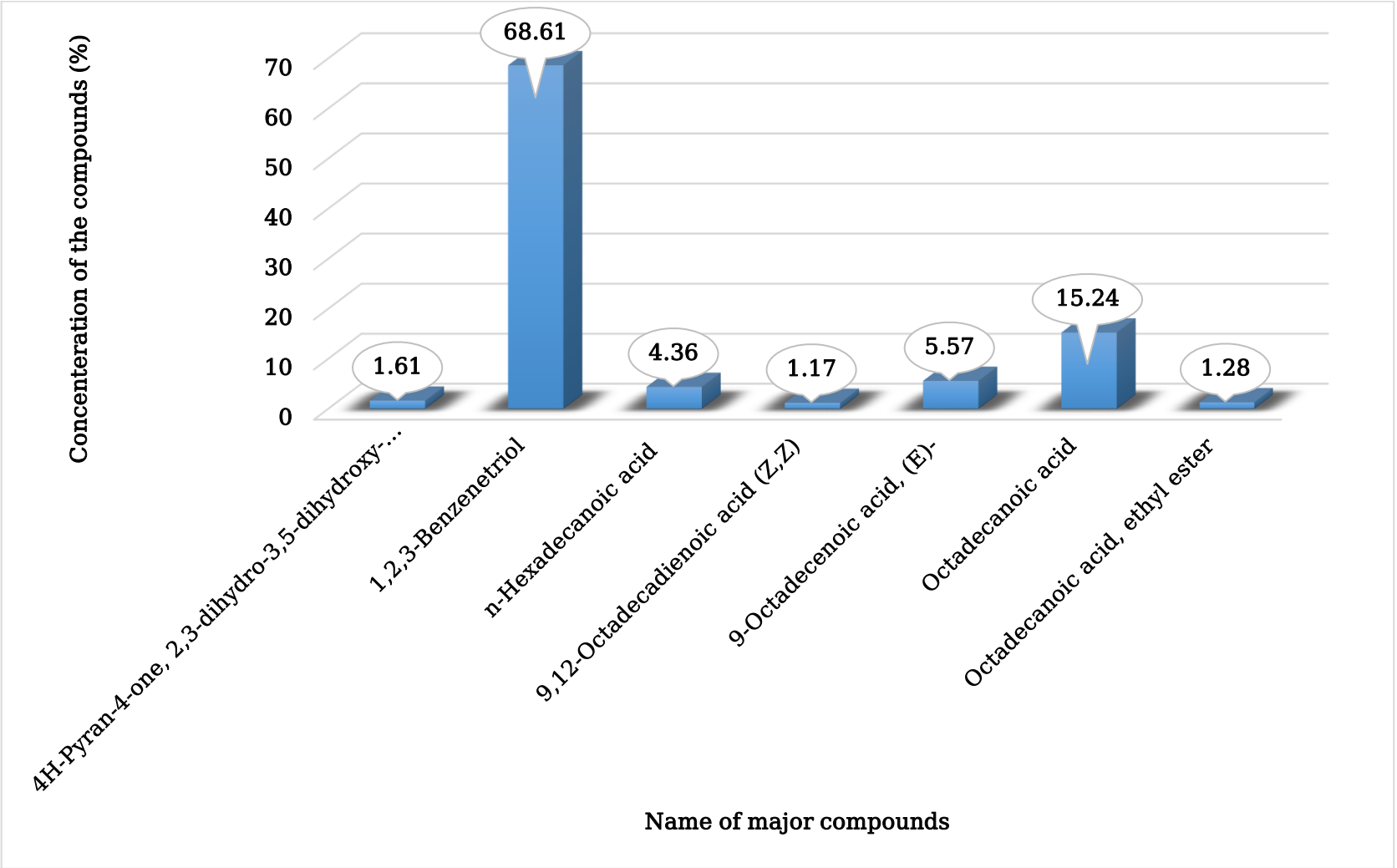


Figure (4.24): Sevan major compounds of extract from *Mangifera indica* (seeds).

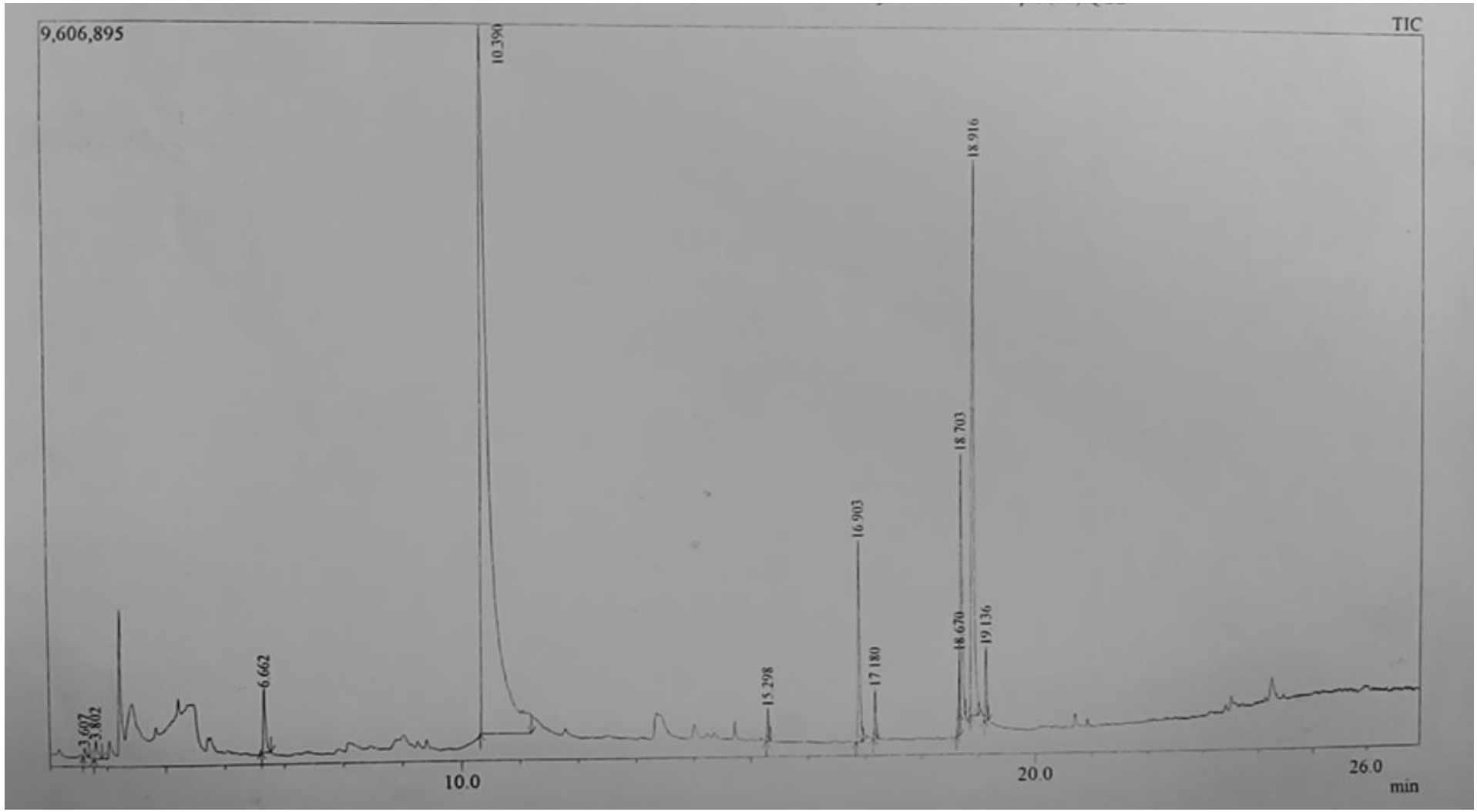


Figure (4.25): GC/MS analysis of ethanolic extract *Mangifera indica* (Seeds).

Table (4.22): Nature of compounds, number and name of compounds of *Mangifera indica* seeds:

Nature of compounds	Number of compounds	Name of compound (1)	Name of compound (2)	Name of compound (3)	Name of compound (4)
Alkaloids	**1**	Diazinone			
Ester	**3**	9,12-Octadecadienoic acid (Z,Z)	9-Octadecenoic acid, (E)-	Octadecanoic acid, ethyl ester	
Fatty acid	**4**	2,5-Furandione, 3-methyl-	N-Hexadecanoic acid	Hexadecanoic acid, ethyl ester	Octadecanoic acid
Heterocyclic	**1**	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-			
Ketone	**1**	1,2-Cyclopentanedione			
Phenolic	**1**	1,2,3-Benzenetriol			

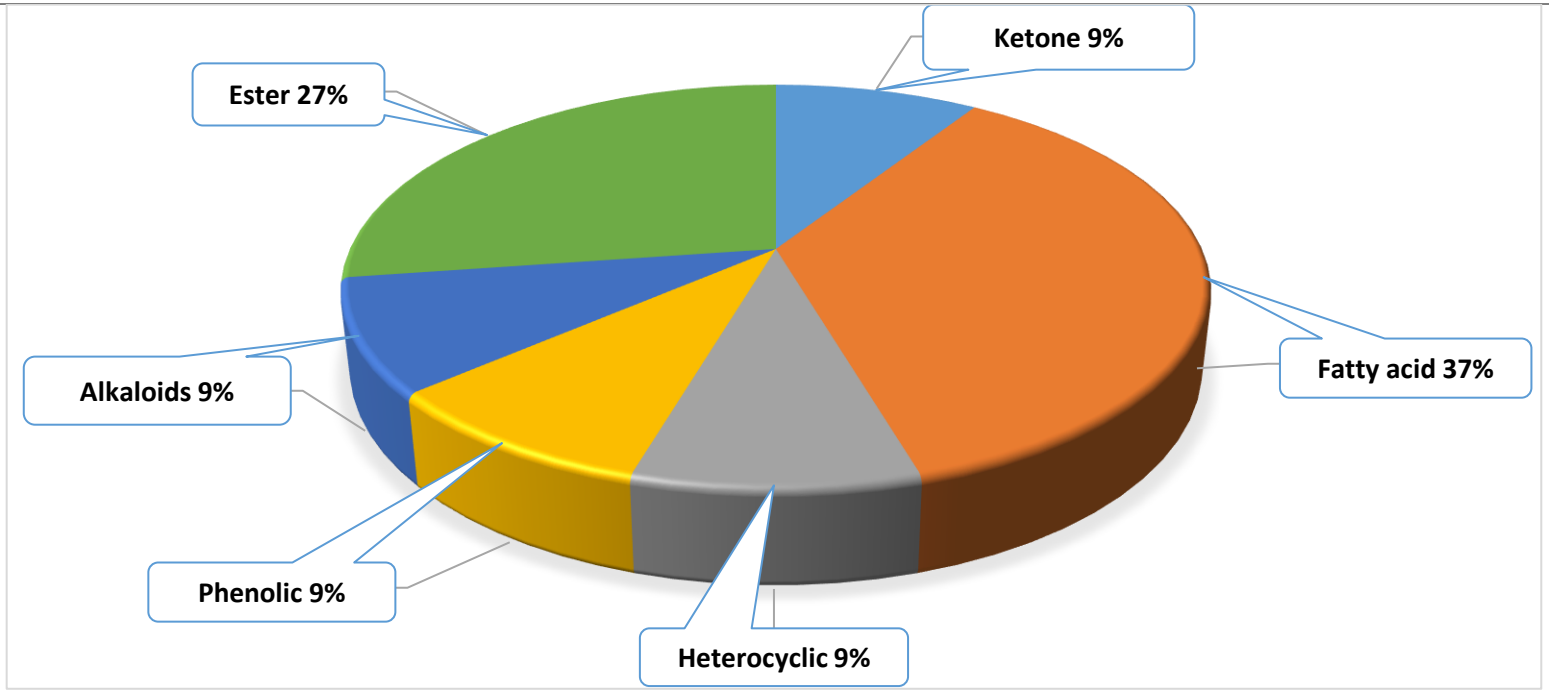


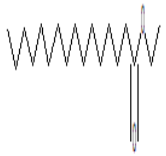
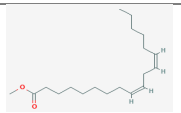
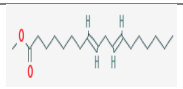
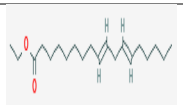
Figure (4.26): Relative content nature of compounds (%) from *Mangifera indica* (Seeds).

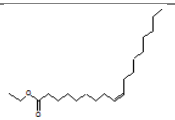
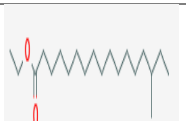
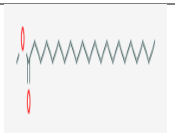
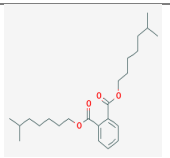
Gas chromatography and mass spectroscopy analysis of compounds was carried out in oil of the ethyl acetate sup-fraction (AK-2) from *M. indica* by column chromatographic technique, was presented in Table (4.23). Gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of 15 compounds, was shown in Figures (4.27 and 4.28). The first compound identified with less retention time (21.44 min) was Nonanoic acid, 9-oxo-, methyl ester, whereas Hexacosanoic acid, methyl ester was the last compound which took longest retention time (62.34 min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table (4.23). It was found to contain 8, 11-Octadecadienoic acid, methyl ester (29.49 %) was found as a major component followed by Hexadecanoic acid, methyl ester (24.52 %), Methyl 16-methyl-heptadecanoate (18.35 %), 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (16.92 %), Ethyl Oleate (1.97 %), Tetracosanoic acid, methyl ester (1.60 %), 1,2 Benzenedicarboxylic acid (1.22 %), Hexacosanoic acid, methyl ester (1.13 %) and Eicosanoic acid, methyl ester (1.01 %) as the major components, as shown in Figure (4.27).

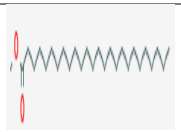
In the oil of the sup-fraction ethyl acetate (AK-2) from *M. indica* by column chromatographic technique, three types of compounds were determined, fatty acid compounds 13(87%), alkane 1(6%) and other compounds 1(7%), are shown as Figure (4.29).

The fatty acid (13) compounds including, 9,12-Octadecadienoic acid, ethyl ester (1), Hexadecanoic acid, methyl ester (2), Hexadecanoic acid, ethyl ester (3), Heptadecanoic acid, 15-methyl-, ethyl ester (4), Hexacosanoic acid, methyl ester (6), Eicosanoic acid, methyl ester (7), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (8), Nonionic acid, 9-oxo-, methyl ester (9), Tetracosanoic acid, methyl ester (10), 1,2-Benzenedicarboxylic acid, diisooctyl ester (11), Docosanoic acid, methyl ester (12) and Ethyl Oleate (13). Alkane (1) compound showed as Heptacosane (1), and other compound (1) showed as Methyl 16-methyl-heptadecanoate, are shown in Table (4.24). In addition, the oil was highly compounds as fatty acids 13(87 %).

Table (4.23): Major phytochemical compounds identified in sup-fraction (AK-2) of *Mangifera indica* by using GC-MS analysis:

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
1	Nonionic acid, 9-oxo-, methyl ester	21.44	0.18	$C_{10}H_{18}O_3$		Fatty acid		No activity reported.
2	**Hexadecanoic acid, methyl ester**	**40.95**	**24.52**	**$C_{17}H_{34}O_2$**	**270.5**	**Fatty acids**		Antibacterial and antifungal activities (Sudha *et al*., 2013).
3	Hexadecanoic acid, ethyl ester	45.41	0.93	$C_{18}H_{36}O_2$	284.5	Fatty acid		Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Anti-androgenic, Hemolytic and 5- Alpha reductase inhibitor activities (Sudha *et al*., 2013).
4	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**49.04**	**16.92**	**$C_{19}H_{34}O_2$**	**294.5**	**Fatty acid**		Anticancer activity (Lans and van Asseldonk, 2020).
5	**8,11-Octadecadienoic acid, methyl ester**	**49.3**	**29.49**	**$C_{19}H_{34}O_2$**	**294.5**	**Fatty acid**		No activity reported.
6	**Methyl 16-methyl-heptadecanoate**	**49.89**	**18.35**	**$C_{19}H_{38}O_2$**	**314.5**	**Other**		No activity reported.
7	9,12-Octadecadienoic acid, ethyl ester	50.64	0.54	$C_{20}H_{36}O_2$	308.5	Fatty acid		Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective Anti androgenic, Hypocholesterolemic Nematicide, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary Insectifuge, Antieczemic and Antiacne (Jananie *et al*., 2011).

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
8	**Ethyl Oleate**	**50.77**	**1.97**	**$C_{20}H_{38}O_2$**	**310.5**	**Fatty acid**		Anti-cancer, anti-proliferative (Win, 2005, Belakhdar *et al*., 2015, Asghar and Choudahry, 2011), antioxidant, anti-microbial (Abubakar and Majinda, 2016).
9	Heptadecanoic acid, 15-methyl-, ethyl ester	51.32	0.77	$C_{20}H_{40}O_2$	312.5	Fatty acid		No activity reported.
10	**Eicosanoic acid, methyl ester**	**53.76**	**1.01**	**$C_{21}H_{42}O_2$**	**326.6**	**Fatty acid**		Alpha-glucosidase inhibitors activity (Elaiyaraja and Chandramohan, 2016).
11	Docosanoic acid, methyl ester	56.65	0.67	$C_{23}H_{46}O_2$	354.6	Fatty acid		Therapeutic, Diagnostic activities (Elaiyaraja and Chandramohan, 2016).
12	**1,2-Benzenedicarboxylic acid, diisooctyl ester**	**56.98**	**1.22**	**$C_{24}H_{38}O_4$**	**390.6**	**Fatty acid**		Antimicrobial and antifouling activities (Salem *et al*., 2016).
13	Heptacosane	58.72	0.69	$C_{27}H_{56}$	280.7	Alkane		Antibacterial activity (Konovalova *et al*., 2013).
14	**Tetracosanoic acid, methyl ester**	**59.16**	**1.6**	**$C_{25}H_{50}O_2$**	**382.7**	**Fatty acids**		No activity reported.

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
15	**Hexacosanoic acid, methyl ester**	**62.34**	**1.13**	**$C_{27}H_{54}O_2$**	**410.7**	**Fatty acids**		Anti-oxidant activity (Basu *et al*., 2013)
	Total percentage		**99.9**					

Key: R. time= Retention time**, M. Weight**= Molecular Weight, **g/mol**= gram/molar. Main components (≥1%) marked in bold. **Activity Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases.

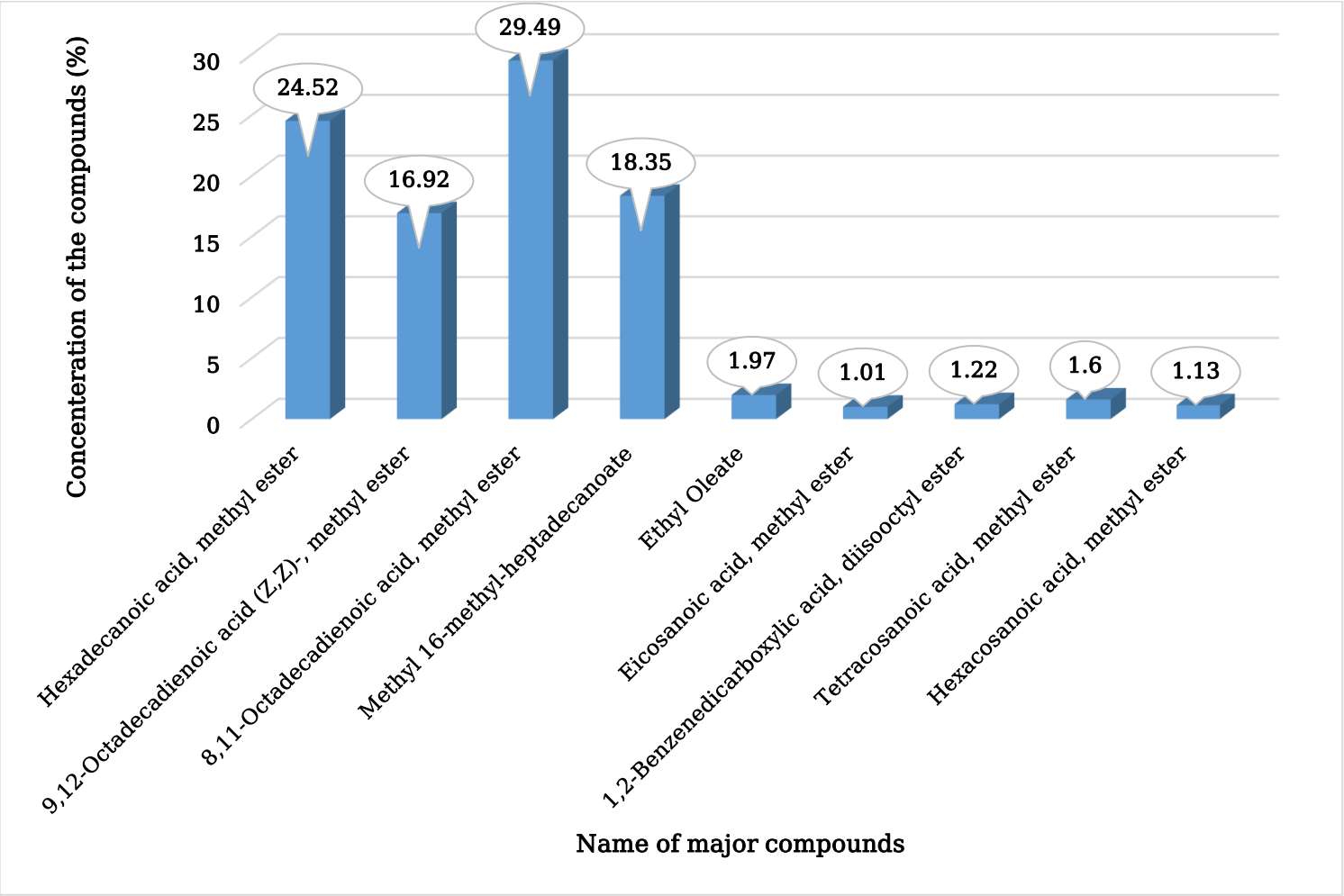


Figure (4.27): Nine major compounds of sup-fraction by column chromatography from *Mangifera indica* (seeds).

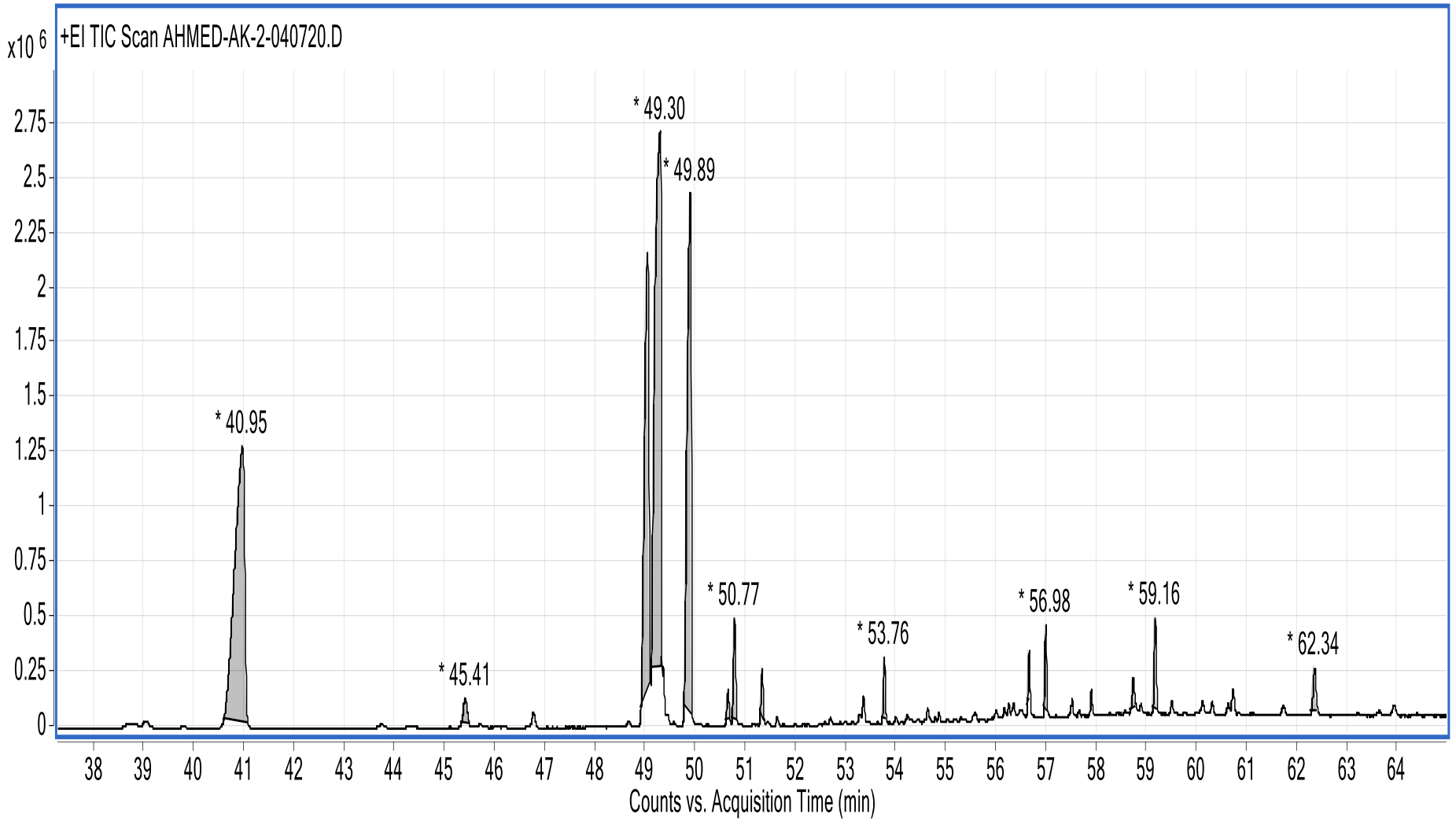


Figure (4.28): GC/MS analysis of sup-fraction (AK-2) of *Mangifera indica* (seeds).

Table (4.24): Nature of compounds, number and name of compounds in sup-fraction (AK-2) of *Mangifera indica* seeds:

Nature of compounds	Number of compounds	Name of compound	Name of compound	Name of compound
Fatty acid	**13**	9,12-Octadecadienoic acid, ethyl ester	Hexadecanoic acid, methyl ester	Hexadecanoic acid, ethyl ester
		Heptadecanoic acid, 15-methyl-, ethyl ester	Hexacosanoic acid, methyl ester	Eicosanoic acid, methyl ester
		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Nonionic acid, 9-oxo-, methyl ester	Tetracosanoic acid, methyl ester
		1,2-Benzenedicarboxylic acid, diisooctyl ester	Docosanoic acid, methyl ester	Ethyl Oleate
Alkane	**1**	Heptacosane		
Other	**1**	Methyl 16-methyl-heptadecanoate		

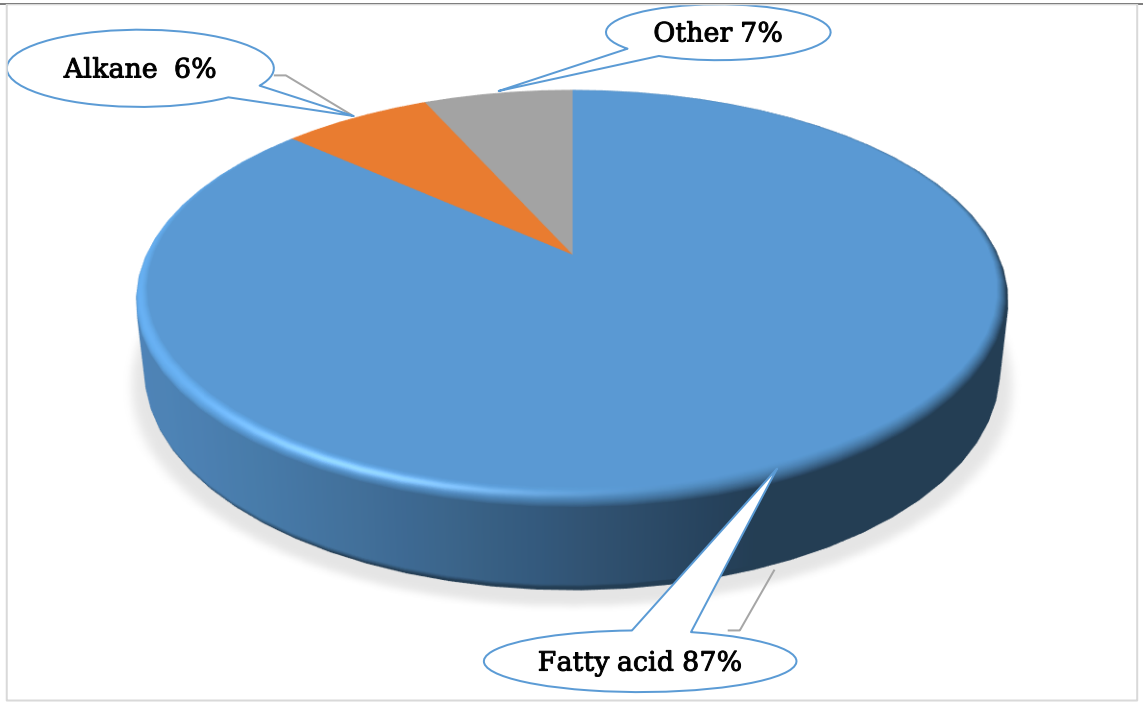


Figure (4.29): Relative content nature of compounds (%) of sup-fraction (AK-2) from *Mangifera indica* (Seeds).

The *R. officinalis* was subject to several phytochemical studies. In this study, Gas chromatography and mass spectroscopy (GC-MS) analysis of compounds was carried out in ethanolic extract of *R. officinalis*, was presented in Table (4.25). The GC-MS chromatogram of the twenty six (26) compounds representing 100% of the total amount was shown in Figures (4.30 and 4.31). The first compound identified with less retention time (4.739 min) was .gamma.-Terpinene, whereas 9(1H)-Phenanthrenone, 2, 3, 4, 4a, 10, 10 a-hexahydro-6-hydroxy-1, 1, 4 a-trimethyl-7-(1-methylethyl)-, (4aS-trans) was the last compound which took longest retention time (23.096 min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table (4.25). It was found to contain Eucalyptol (25.11 %) was found as a major components followed by (+)-2-Bornanone (19.34 %), Isocarnosol (8.74 %), endo-Borneol (7.62 %), Benz(a)acridine, 9,10,12-trimethyl (6.67 %), 9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-,(4aS-trans) (5.25 %), Ferruginol (4.72 %), n-Hexadecanoic acid (3.88 %), .alpha.-Terpineol (3.65 %), Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S) (2.97 %) and Andrographolide (2.50 %), 4H-Benzo[f]pyrrolo[1,2-a][1,4]diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro (1.95 %), Aromadendrene oxide- (1.39 %) and 2-Methoxy-4-vinylphenol (1.13 %) as the major components, as shown in Figure (4.30).

Studies showed that Eucalyptol is a promising compound for treating such conditions as it has been shown to have anti-inflammatory and antioxidant effects in various diseases, including respiratory disease, pancreatitis, colon damage, and cardiovascular and neurodegenerative diseases (Seol and Kim, 2016).

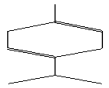
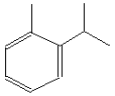
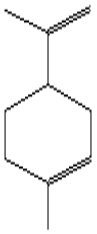
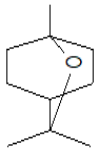
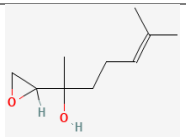
The comparison between the major components was presented in Figure (4.30). The above results are in accordance with that of Tomi *et al*. (2016), El-Ghorab (2003). Similar investigation showed that 1, 8-cineole and α-pinene were major constituents of the rosemary (Jiang *et al*., 2011). In another investigation, the most important constituents of the Iranian rosemary were shown to be 1, 8-cineole, α-pinene, berbonone, and camphor (Jalali-Heravi *et al*., 2011). Szumny *et al*. (2010) reported that the Polish fresh *Rosmarinus officinalis* essential oil analyzed and identified by GC/MS showed that α-pinene (33.3%), bornyl acetate (14.8%), camphene (13.8%), and 1, 8-cineole (12.3%) were the main compounds. These result similar to (Almeida *et al*., 2006)1, 8-cineol or eucalyptol was the most significant volatile compound of which concentrations ranged from (23 – 33 %).

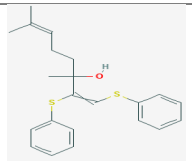
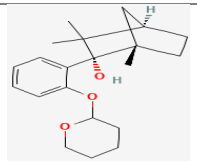
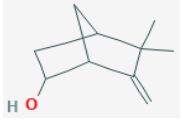
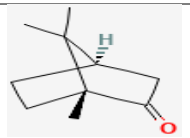
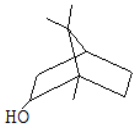
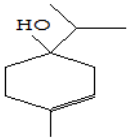
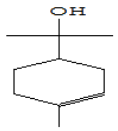
In the ethanolic extract of *R. officinalis*, eight nature of compounds were determined, Oxygenated monoterpenes 10(38%), Hydrocarbons monoterpenes 5(19%), Fatty acids and other compounds 3(12%), Diterpenoid 2(8%), phenol, Carbocyclic and Cyclic Hydrocarbons 1(4%), are shown as Figure (4.32).

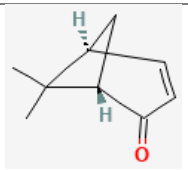
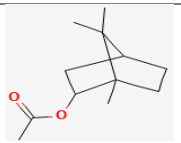
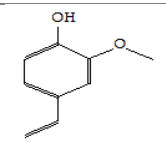
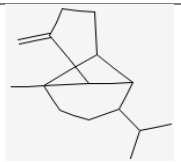
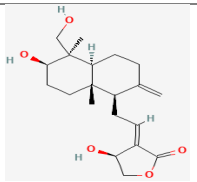
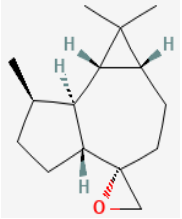
The Oxygenated monoterpenes (10) compounds including, Eucalyptol (1), 1,6-Octadien-3-ol, 3,7-dimethyl (2), Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl (3), Camphenol,6- (4), (+)-2-Bornanone (5), endo-Borneol (6), Terpinen-4-ol (7), .alpha.-Terpineol (8), Bornyl acetate (9) and Aromadendrene oxide- (10). Followed by Hydrocarbons monoterpenes (5) compounds including, .gamma. - Terpinene (1), O-Cymene (2), D-

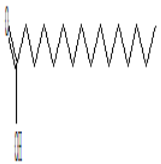
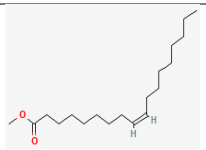
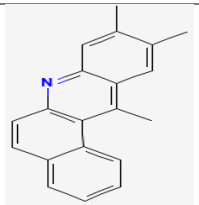
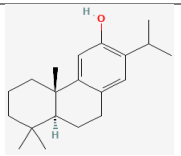
Limonene (3.), 3-Carene (4) and .beta.-ylangene (5). The Fatty acids (3) compounds including, .alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol (1), n-Hexadecanoic acid (2) and 9-Octadecenoic acid (Z)-, methyl ester (3). Diterpenoid (2) compounds including, Andrographolide (1) and Ferruginol (2). Carbocyclic (1) compound as Bicyclo [3.1.1] hept-3-en-2-one, 4,6,6-trimethyl-, (1S) (1). Phenol (1) compound as 2-Methoxy-4-vinylphenol (1). Cyclic Hydrocarbons (1) compound as Isocarnosol (1), and other (2) compounds including, Benz(a)acridine, 9,10,12-trimethyl (1), 4H-Benzo[f]pyrrolo[1,2-a][1,4]diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro (2) and 9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans), are shown in Table (4.26). In addition, the extract was highly compounds as Oxygenated monoterpenes 10(38%), and Hydrocarbons monoterpenes 5(19%).

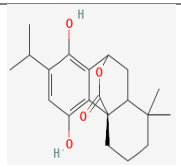
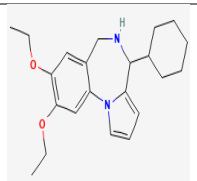
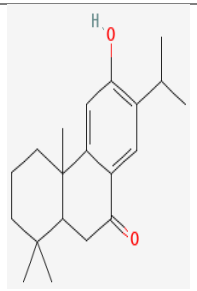
Table (4.25): Major phytochemical compounds identified in ethanolic extract of *Rosmarinus officinalis* by using GC-MS analysis:

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
1	gamma.- Terpinene	4.739	0.47	$C_{10}H_{16}$	136.23	Hydrocarbons monoterpenes		Anti-inflammatory activity (de Oliveira Ramalho *et al*., 2015).
2	O-Cymene	4.848	0.58	$C_{10}H_{14}$	134.22	Hydrocarbons monoterpenes		Antioxidant and antimicrobial activities (Mahmud *et al*., 2009).
3	D-Limonene	4.910	0.44	$C_{10}H_{16}$	136	Hydrocarbons monoterpenes		Antioxidant, antidiabetic, anticancer, anti-inflammatory, cardioprotective, gastroprotective, hepatoprotective, immunemodulatory, anti-fibrotic and anti-genotoxic (Anandakumar *et al*., 2021).
4	**Eucalyptol**	**4.963**	**25.11**	**$C_{10}H_{18}O$**	**154**	**Oxygenated monoterpenes**		Anti-inflammatory, gastroprotective, antitumorogenic, hepatoprotective, antimycotic and antibacterial activities (Bhowal and Gopal, 2015).
5	3-Carene	5.325	0.15	$C_{10}H_{16}$	136	Hydrocarbons monoterpenes		Antimicrobial and Anti-acetylcholinesterase Properties and Insecticidal activities (Znati *et al*., 2012, Langsi *et al*., 2020).
6	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	5.528	0.14	$C_{10}H_{18}O_2$	170	Fatty acids		No activity reported.

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
7	1,6-Octadien-3-ol, 3,7-dimethyl	5.885	0.30	$C_{10}H_{18}O$	154	Oxygenated monoterpenes		Anti-inflammatory and anti-cancer properties (Al-Marzoqi *et al*., 2015).
8	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	6.191	0.15	$C_{10}H_{18}O$	154	Oxygenated monoterpenes		Antimicrobial activity (Mujeeb *et al*., 2014).
9	Camphenol,6-	6.346	0.25	$C_{10}H_{16}O$	152	Oxygenated monoterpenes		No activity reported.
10	**(+)-2-Bornanone**	**6.670**	**19.34**	**$C_{10}H_{16}O$**	**152**	**Oxygenated monoterpenes**		No activity reported.
11	**endo-Borneol**	**6.989**	**7.62**	**$C_{10}H_{18}O$**	**154**	**Oxygenated monoterpenes**		No activity reported.
12	Terpinen-4-ol	7.128	0.50	$C_{10}H_{18}O$	154	Oxygenated monoterpenes		Antimicrobial and anti-inflammatory properties (Cox *et al*., 2001, Hart *et al*., 2000).
13	**.alpha.-Terpineol**	**7.322**	**3.65**	**$C_{10}H_{18}O$**	**154**	**Oxygenated monoterpenes**		Antimicrobial activity (Park *et al*., 2012).

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
14	**Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)**	**7.636**	**2.97**	**$C_{10}H_{14}O$**	**150**	**Carbocyclic**		No activity reported.
15	Bornyl acetate	8.692	0.99	$C_{12}H_{20}O_2$	196	Oxygenated monoterpenes		Antimicrobial activity (Rabib *et al.*, 2020).
16	**2-Methoxy-4-vinylphenol**	**9.162**	**1.13**	**$C_9H_{10}O_2$**	**150**	**phenol**		Anti-carcinogenic and anti-inflammatory activities (Lalthanpuii and Lalchhandama, 2019).
17	.beta.-ylangene	12.925	0.50	$C_{15}H_{24}$	204	Hydrocarbons monoterpenes		No activity reported.
18	**Andrographolide**	**13.751**	**2.50**	**$C_{20}H_{30}O_5$**	**350**	**Diterpenoid**		Anti-inflammatory, anti-infective and anti-hepatotoxic activities (Chao and Lin, 2010).
19	**Aromadendrene oxide-**	**13.909**	**1.39**	**$C_{15}H_{24}O$**	**220**	**Oxygenated monoterpenes**		No activity reported.

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
20	**n-Hexadecanoic acid**	**16.896**	**3.88**	**$C_{16}H_{32}O_2$**	**256**	**Fatty acids**		Antioxidant, Pesticide, Flavor, Hemolytic, Lubricant, and anti-cancer (Harada *et al*., 2002, Ponnamma and Manjunath, 2012). Anti-inflammatory, antiandrogenic and hypocholestrolemic (Aparna *et al*., 2012, Kumar *et al*., 2010).
21	9-Octadecenoic acid (Z)-, methyl ester	18.282	0.60	$C_{19}H_{36}O_2$	296	Fatty acids		Anti-inflammatory, antiandrogenic cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge (Krishnamoorthy and Subramaniam, 2014).
22	**Benz(a)acridine, 9,10,12-trimethyl**	**19.856**	**6.67**	**$C_{20}H_{17}N$**	**271**	**Other**		No activity reported.
23	**Ferruginol**	**20.741**	**4.72**	**$C_{20}H_{30}O$**	**286**	**Diterpenoid**		antibacterial, antifungal, antimicrobial, cardioprotective, anti-oxidative, anti-plasmodial, leishmanicidal, antiulcerogenic, anti-inflammatory and antitumor actions (Roa-Linares *et al*., 2016).

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
24	**Isocarnosol**	**21.444**	**8.74**	**$C_{20}H_{26}O_4$**	**330**	**Cyclic Hydrocarbons**		No activity reported.
25	**4H-Benzo[f]pyrrolo[1,2-a][1,4]diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro**	**21.774**	**1.95**	**$C_{22}H_{30}N_2O_2$**	**354**	**Other**		No activity reported.
26	**9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)**	**23.096**	**5.25**	**$C_{20}H_{28}O_2$**	**300**	**Other**		No activity reported.
	Total percentage		**100**					

Key: R. time= Retention time**, M. Weight**= Molecular Weight, **g/mol**= gram/molar. Main components (≥1%) marked in bold. **Activity Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases.

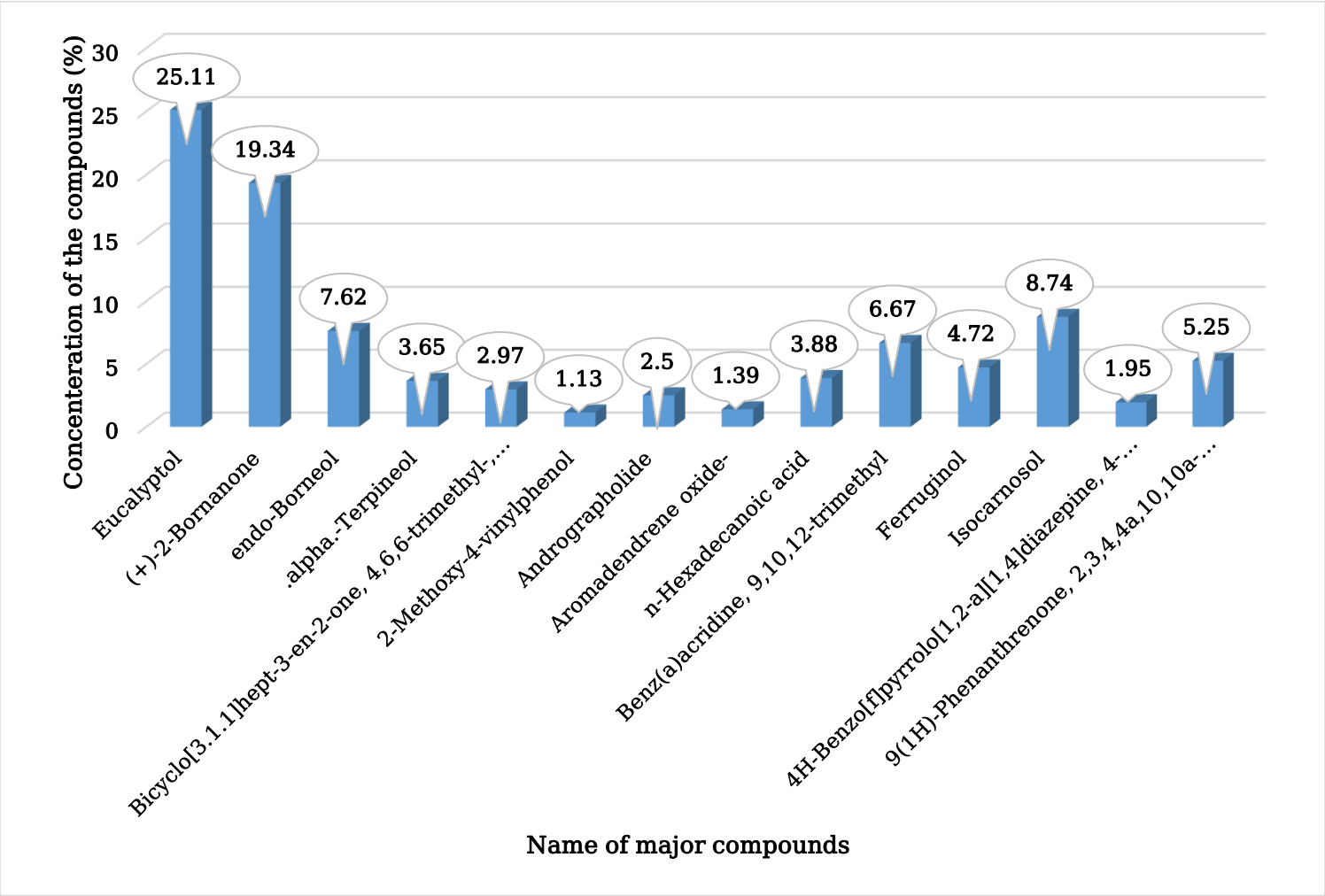


Figure (4.30): Fourteen major compounds of extract from *Rosmarinus officinalis* (pods).

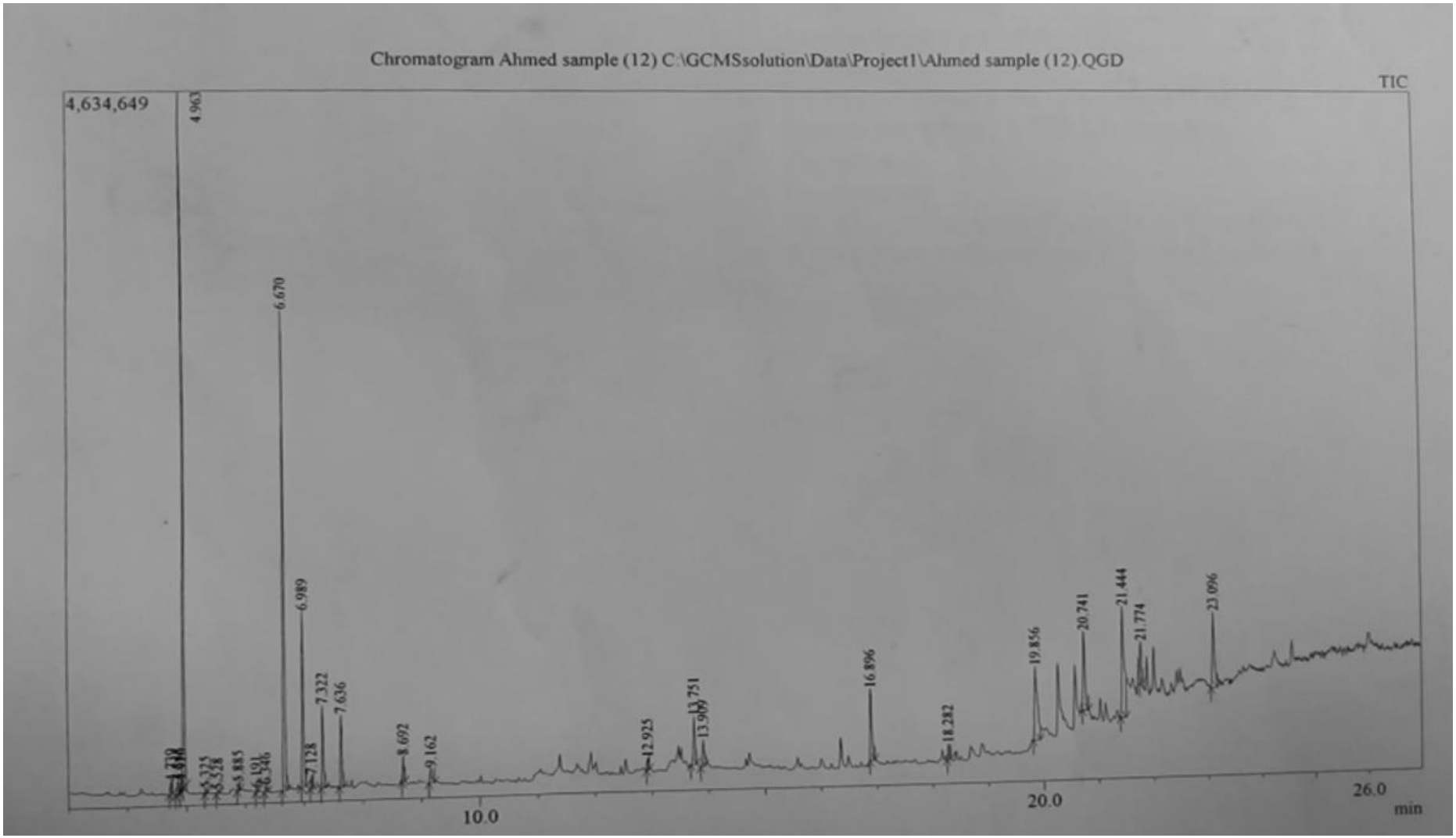


Figure (4.31): GC/MS analysis of ethanolic extract *Rosmarinus officinalis* (pods).

Table (4.26): Nature of compounds, number and name of compounds of *Rosmarinus officinalis* pods:

Nature of compounds	Number of compounds	Name of compound	Name of compound	Name of compound	Name of compound
Hydrocarbons monoterpenes	**5**	gamma.- Terpinene	O-Cymene	D-Limonene	3-Carene
		beta.-ylangene			
Oxygenated monoterpenes	**10**	Eucalyptol	1,6-Octadien-3-ol, 3,7-dimethyl	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	Camphenol,6-
		(+)-2-Bornanone	endo-Borneol	Terpinen-4-ol	.alpha.-Terpineol
		Bornyl acetate	Aromadendrene oxide-		
Fatty acids	**3**	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	n-Hexadecanoic acid	9-Octadecenoic acid (Z)-, methyl ester	
Carbocyclic	**1**	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)			
Phenol	**1**	2-Methoxy-4-vinylphenol			
Diterpenoid	**2**	Andrographolide	Ferruginol		
Cyclic Hydrocarbons	**1**	Isocarnosol			
Other	**3**	Benz(a)acridine, 9,10,12-trimethyl	4H-Benzo[f]pyrrolo[1,2-a][1,4]diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)	

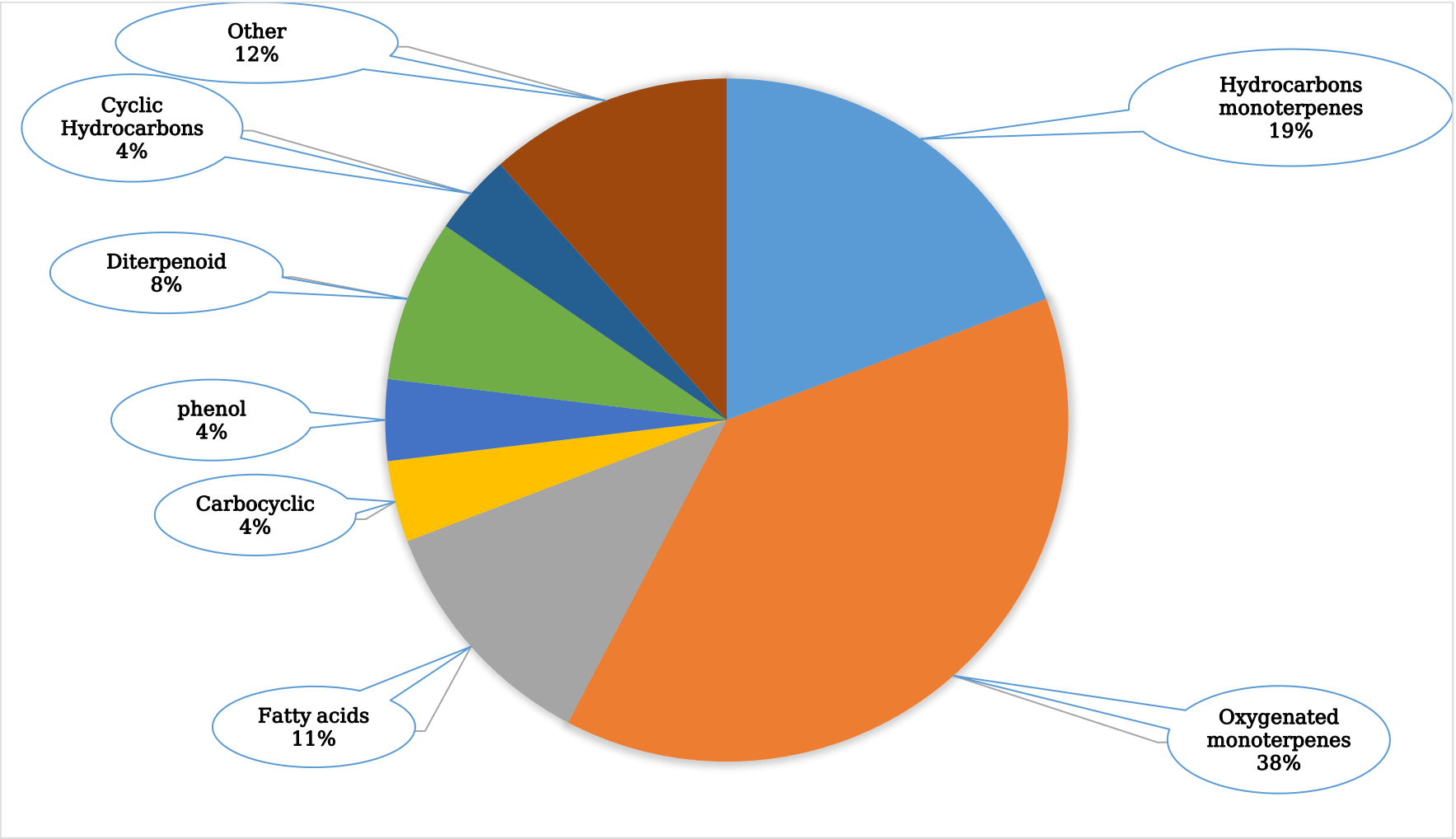


Figure (4.32): Relative content nature of compounds (%) of compounds from *Rosmarinus officinalis* pods.

There were no reports on the phytochemical investigations of these species hence the extract of *T. bakis* of the twenty two components were detected in the GC-MS profile. The components were found to have different pharmacological importance along with antioxidant and/or anticancer potential (Table 4.27) and Figures (4.33 and 4.34). The first compound identified with less retention time (3.730 min) was Glycerin, whereas Hyodeoxycholic acid was the last compound which took longest retention time (27.532 min) to identify. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table (4.27). 1H-3a, 7-Methanoazulene, octahydro-1, 9, 9-trimethyl-4-methylene (46.94 %) highest amount compared. Next, in relatively high concentrations were found: Androst-5, 15-dien-3ol acetate (8.18 %), Pregnan-20-one, 3-(acetyloxy)-5, 6-epoxy-, (3.beta. 5. alpha., 6.alpha.)- (7.95 %), 1H-2-Indenone, 2,4,5,6,7,7 a-hexahydro-3-(1-methylethyl)-7a-methyl (5.03 %), Hyodeoxycholic acid (4.48 %), Pregn-5-ene-3,20-dione (3.72 %) and Glycerin (3.69 %), Glaucine (2.88 %), 3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one (2.56 %), Octadec-9-enoic acid (2.16 %), n-Hexadecanoic acid (1.97 %), 5.beta.,14.beta.-Androstane-17.beta.-carboxylic acid, 3.beta.,14-dihydroxy-, .gamma.-lactone, acetate (1.92 %), (+)-(S)-Isocorydine (1.48 %), and Linoleic acid ethyl ester (1.09) represented the highest amount compared to the other components, as shown in Figure (4.33).

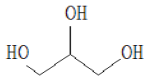
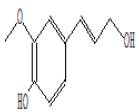
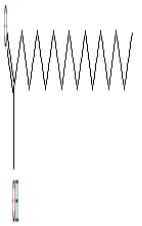
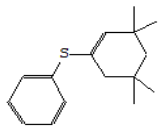
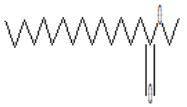
Some of the compounds present are anticancer in nature such as n-Hexadecanoic acid, Squalene and 9, 12-Octadecadienoic acid (Z, Z)-. This study explores the goodness of the whole plant of the *T. bakis* which has a commendable sense of purpose and can be advised as a plant of pharmaceutical importance.

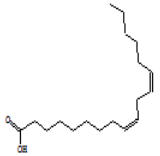
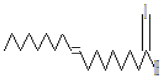
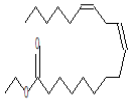
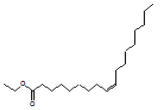
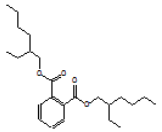
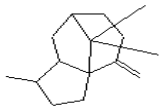
In the ethanolic extract of *T. bakis*, eleven nature of compounds were determined, Fatty acids 7(32%), Alkaloids 4(18%), other compounds 2(9%), Amino acid, Ester, Cyclic Hydrocarbons, Phenolic, Sesqueterpens hydrocarbon, Triterpene and Heterocyclic 1(5%), are shown as Figure (4.35). Therefore, 1H-3a, 7-Methanoazulene, octahydro-1, 9, 9-trimethyl-4-methylene has biological activity as anti-inflammatory, antiprotozoal, antibacterial and antimicrobial activities.

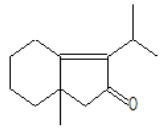
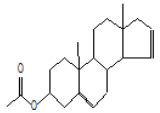
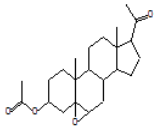
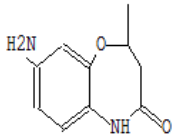
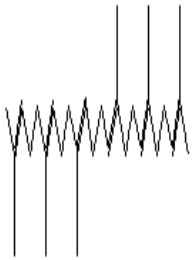
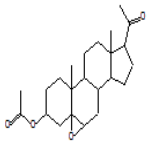
The Fatty acids (7) compounds including, 5.beta.,14 .beta.-Androstane-17.beta.-carboxylic acid, 3.beta.,14-dihydroxy-, .gamma.-lactone, acetate (1), Hyodeoxycholic acid (2), n-Hexadecanoic acid (3), Octadec-9-enoic acid (4), Hexadecanoic acid, ethyl ester (5), 9,12-Octadecadienoic acid (Z,Z)- (6) and Linoleic acid ethyl ester (7). Alkaloids (4) compounds including, 3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one (1), (+)-(S)-Isocorydine (2), Acridine, 9-butyl- (3) and Glaucine (4). The Carboxylic acid (2) compounds including, Ethyl Oleate (1) and Bis (2-ethylhexyl) phthalate (2). Amino acid (1) as Glycerin (1), Ester (1) as Pregnan-20-one, 3-(acetyloxy)-5, 6-epoxy-, (3. beta. 5. alpha., 6.alpha.)- (1), Cyclic Hydrocarbons (1) as Benzene, [(3,3,5,5-tetramethylcyclohex-1-en-1-yl)thio] (1), Phenolic (1) as (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol (1), Sesqueterpens hydrocarbon (1) as 1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene- (1), Triterpene (1) as Squalene (1), Heterocyclic (1) as 1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl (1) and other (2) compounds including, Androst-5,15-dien-3ol acetate (1) and Pregn-5-ene-3,20-dione (2), are

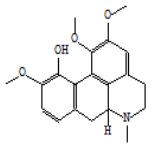
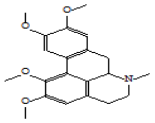
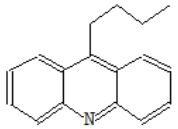
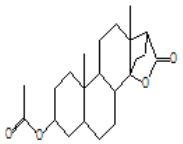
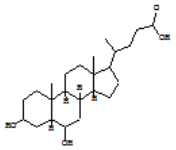
shown in Table (4.28). In addition, the extract contained higher number of compounds as fatty acids 7(32 %), and alkaloids 4(18 %). Although fatty acids have various therapeutic activities including anti-inflammatory and antioxidant activities (Kapoor and Huang, 2006, Galli and Calder, 2009, Poudel-Tandukar *et al*., 2009). The well-known alkaloids have antimicrobial (Gawali and Jadhav, 2011) and antidiabetic (Sharma, 2012) activities, and hence steroidal alkaloids are used as medicine.

Table (4.27): Major phytochemical compounds identified in ethanolic extract of *Tinospora bakis* by using GC-MS analysis:

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
1	**Glycerin**	**3.730**	**3.69**	**$C_3H_8O_3$**	**92**	**Amino acid**		No activity reported.
2	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	13.725	0.69	$C_{10}H_{12}O_3$	180	phenol		No activity reported.
3	**n-Hexadecanoic acid**	**15.892**	**1.97**	**$C_{16}H_{32}O_2$**	**256**	**Fatty acid**		Antioxidant, Pesticide, Flavor, Hemolytic, Lubricant, and anti-cancer (Harada *et al*., 2002, Ponnamma and Manjunath, 2012). Anti-inflammatory, antiandrogenic and hypocholestrolemic (Aparna et al., 2012, Kumar *et al*., 2010).
4	Benzene, [(3,3,5,5-tetramethylcyclohex-1-en-1-yl)thio]	15.996	0.82	$C_{16}H_{22}S$	246	Cyclic Hydrocarbons		No activity reported.
5	Hexadecanoic acid, ethyl ester	16.220	0.77	$C_{18}H_{36}O_2$	284	Fatty acid		Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic (Tyagi and Agarwal, 2017, Ponnamma and Manjunath, 2012, Sudha *et al*., 2013).

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
6	9,12-Octadecadienoic acid (Z,Z)-	17.548	0.85	$C_{18}H_{32}O_2$	280	Fatty acid		Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic,Antiacne (Sudha *et al*., 2013) Anticancer properties (Yu *et al*., 2005).
7	**Octadec-9-enoic acid**	**17.591**	**2.16**	**$C_{18}H_{34}O_2$**	**282**	**Fatty acid**		No activity reported.
8	**Linoleic acid ethyl ester**	**17.811**	**1.09**	**$C_{20}H_{36}O_2$**	**308**	**Fatty acids**		Antioxidant, anticancer and anti-inflammatory activities (Kim *et al*., 2020).
9	Ethyl Oleate	17.856	0.50	$C_{20}H_{38}O_2$	310	Carboxylic acid		Antioxidant, anti-inflammatory activities (Kim *et al*., 2020).
10	Bis(2-ethylhexyl) phthalate	21.080	0.56	$C_{24}H_{38}O_4$	390	Carboxylic Acids		No activity reported.
11	**1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-**	**21.855**	**46.94**	**$C_{15}H_{24}$**	**204**	**Sesqueterpens hydrocarbon**		Anti-inflammatory, antiprotozoal, antibacterial and antimicrobial activities.

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
12	**1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl**	**22.264**	**5.03**	**$C_{13}H_{20}O$**	**192**	**Heterocyclic**		No activity reported.
13	**Androst-5,15-dien-3ol acetate**	**22.450**	**8.16**	**$C_{21}H_{30}O_2$**	**314**	**other**		No activity reported.
14	**Pregnan-20-one, 3-(acetyloxy)-5, 6-epoxy-, (3. beta. 5. alpha., 6.alpha.)-**	**22.611**	**7.95**	**$C_{23}H_{34}O_4$**	**374**	**Ester**		No activity reported.
15	**3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one**	**22.800**	**2.56**	**$C_{10}H_{12}N_2O_2$**	**192**	**Alkaloids**		No activity reported.
16	Squalene	23.098	0.58	$C_{30}H_{50}$	410	Triterpene		Antiageing, Analgesic, Antidiabetic, Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary (Sudha *et al*., 2013, Spanova and Daum, 2011).
17	**Pregn-5-ene-3,20-dione**	**23.150**	**3.72**	**$C_{21}H_{30}O_2$**	**314**	**Other**		No activity reported.

No.	Name of compounds	R. Time	Area (%)	Formulas	M. Weight (g/mol)	Nature of compounds	Structures	Bioactivity of compounds
18	**(+)v-(S)-Isocorydine**	**23.651**	**1.84**	**$C_{20}H_{23}NO_4$**	**341**	**Alkaloids**		Anticancer activity (Zhong *et al*., 2014).
19	**Glaucine**	**24.083**	**2.88**	**$C_{21}H_{25}NO_4$**	**355**	**Alkaloids**		Antifungal activity (Thawabteh *et al*., 2019).
20	Acridine, 9-butyl-	24.788	0.80	$C_{17}H_{17}N$	335	Alkaloids		Antimalarial activity (Yu *et al*., 2012).
21	**.5.beta.,14.beta.-Androstane-17.beta.-carboxylic acid, 3.beta.,14-dihydroxy-, .gamma.-lactone, acetate**	**26.650**	**1.92**	**$C_{22}H_{32}O_4$**	**360**	**Fatty acids**		No activity reported.
22	**Hyodeoxycholic acid**	**27.532**	**4.48**	**$C_{24}H_{40}O_4$**	**392**	**Fatty acids**		No activity reported.
	Total percentage		**100**					

Key: R. time= Retention time**, M. Weight**= Molecular Weight, **g/mol**= gram/molar. Main components (≥1%) marked in bold. **Activity Source:** Dr. Duke's Phytochemical and Ethnobotanical Databases.

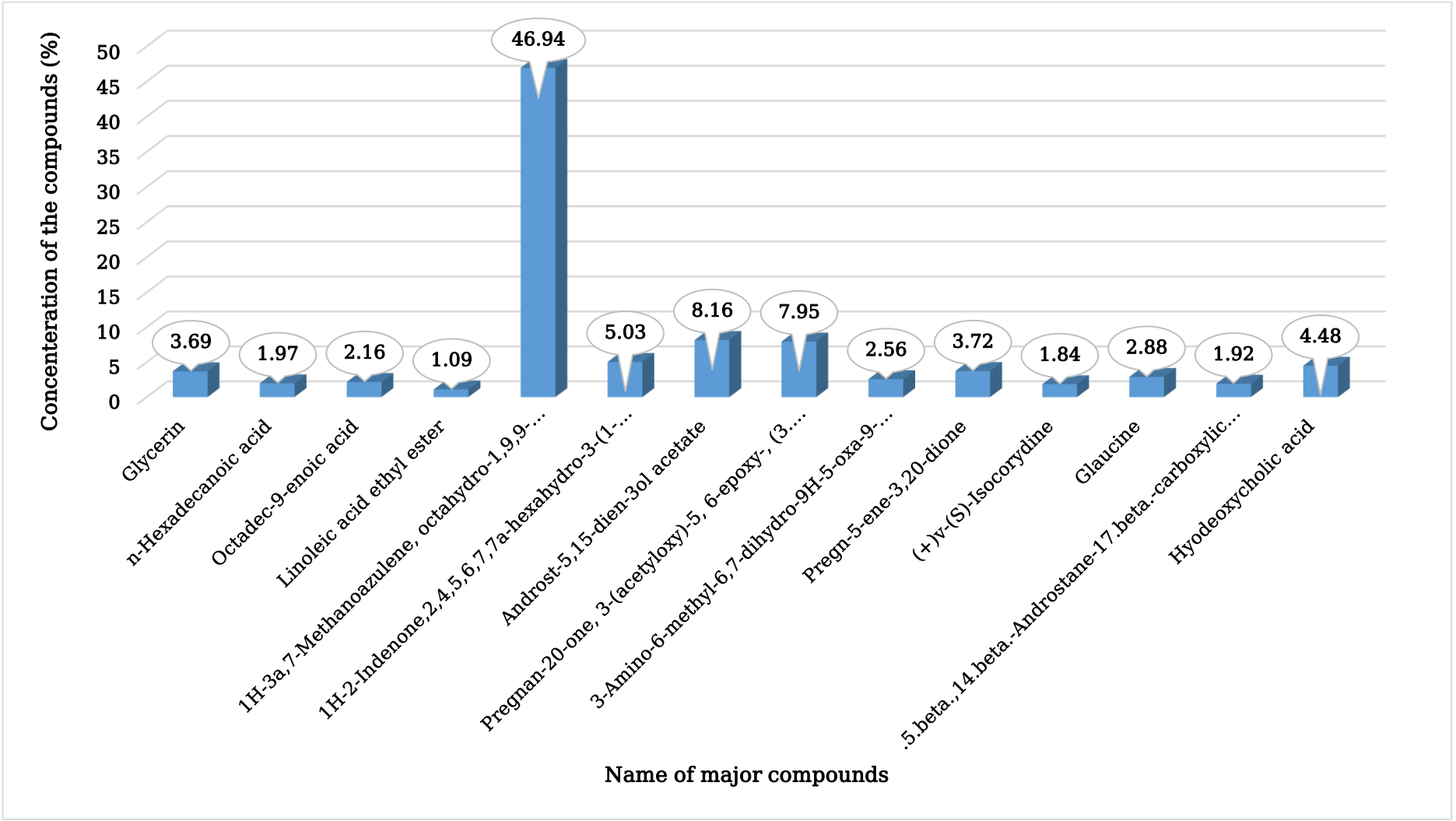


Figure (4.33): Fourteen major compounds of extract from *Tinospora bakis* (whole plant).

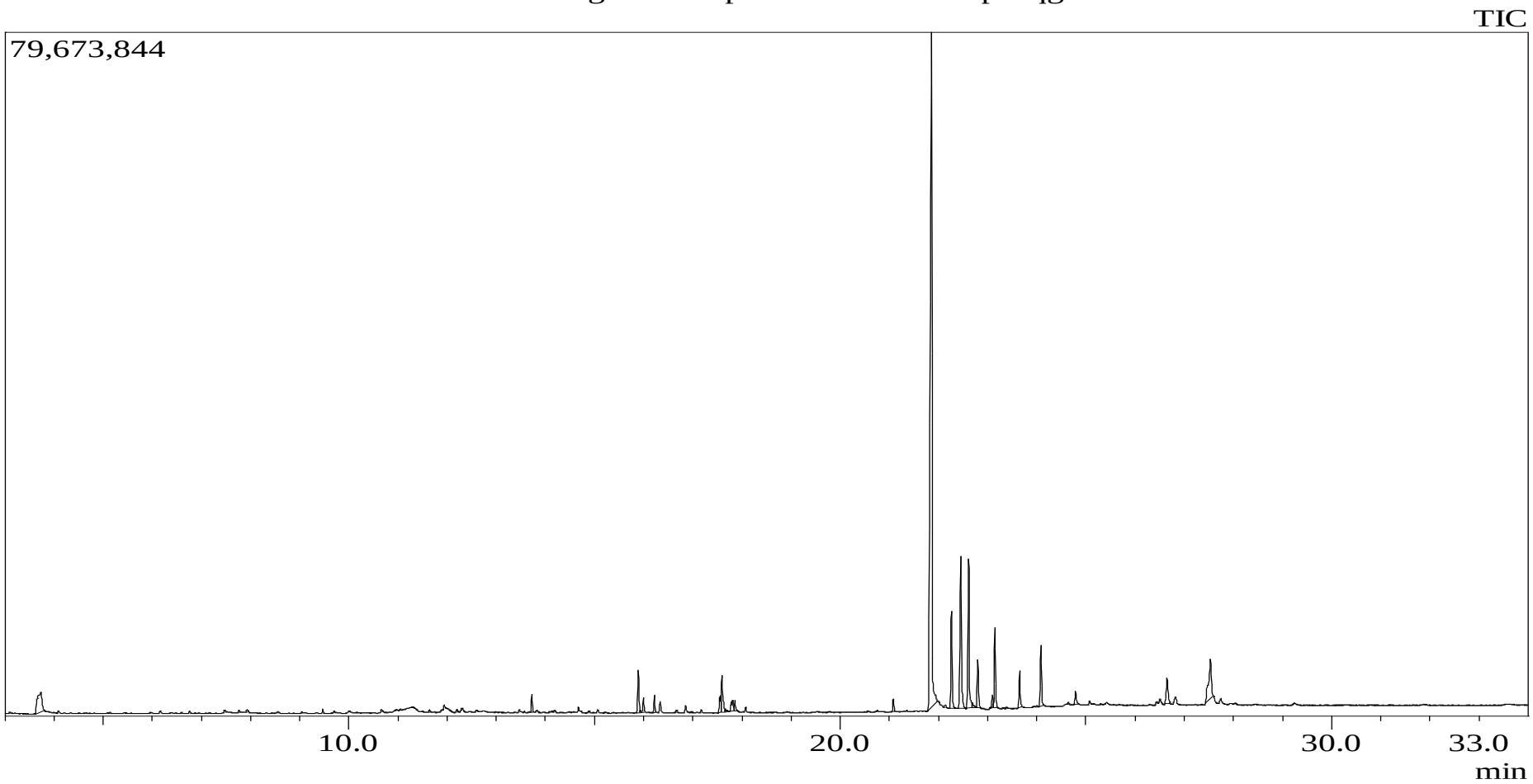


Figure (4.34): GC/MS analysis of ethanolic extract *Tinospora bakis* (whole plant).

Table (4.28): Nature of compounds, number and name of compounds of *Tinospora* whole plant:

Nature of compounds	Number of compounds	Name of compound	Name of compound	Name of compound	Name of compound
Amino acid	**1**	Glycerin			
Ester	**1**	Pregnan-20-one, 3-(acetyloxy)-5, 6-epoxy-, (3. beta. 5. alpha., 6.alpha.)-			
Fatty acids	**7**	5.beta.,14.beta.-Androstane-17.beta.-carboxylic acid, 3.beta.,14-dihydroxy-, .gamma.-lactone, acetate	Hyodeoxycholic acid	n-Hexadecanoic acid	Octadec-9-enoic acid
		Hexadecanoic acid, ethyl ester	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid ethyl ester	
Cyclic Hydrocarbons	**1**	Benzene, [(3,3,5,5-tetramethylcyclohex-1-en-1-yl)thio]			
Carboxylic acid	**2**	Ethyl Oleate	Bis(2-ethylhexyl) phthalate		
Phenolic	**1**	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol			
Sesqueterpens hydrocarbon	**1**	1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-			
Alkaloids	**4**	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	(+)-(S)-Isocorydine	Acridine, 9-butyl-	Glaucine
Triterpene	**1**	Squalene			
Heterocyclic	**1**	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl			
Other	**2**	Androst-5,15-dien-3ol acetate	Pregn-5-ene-3,20-dione		

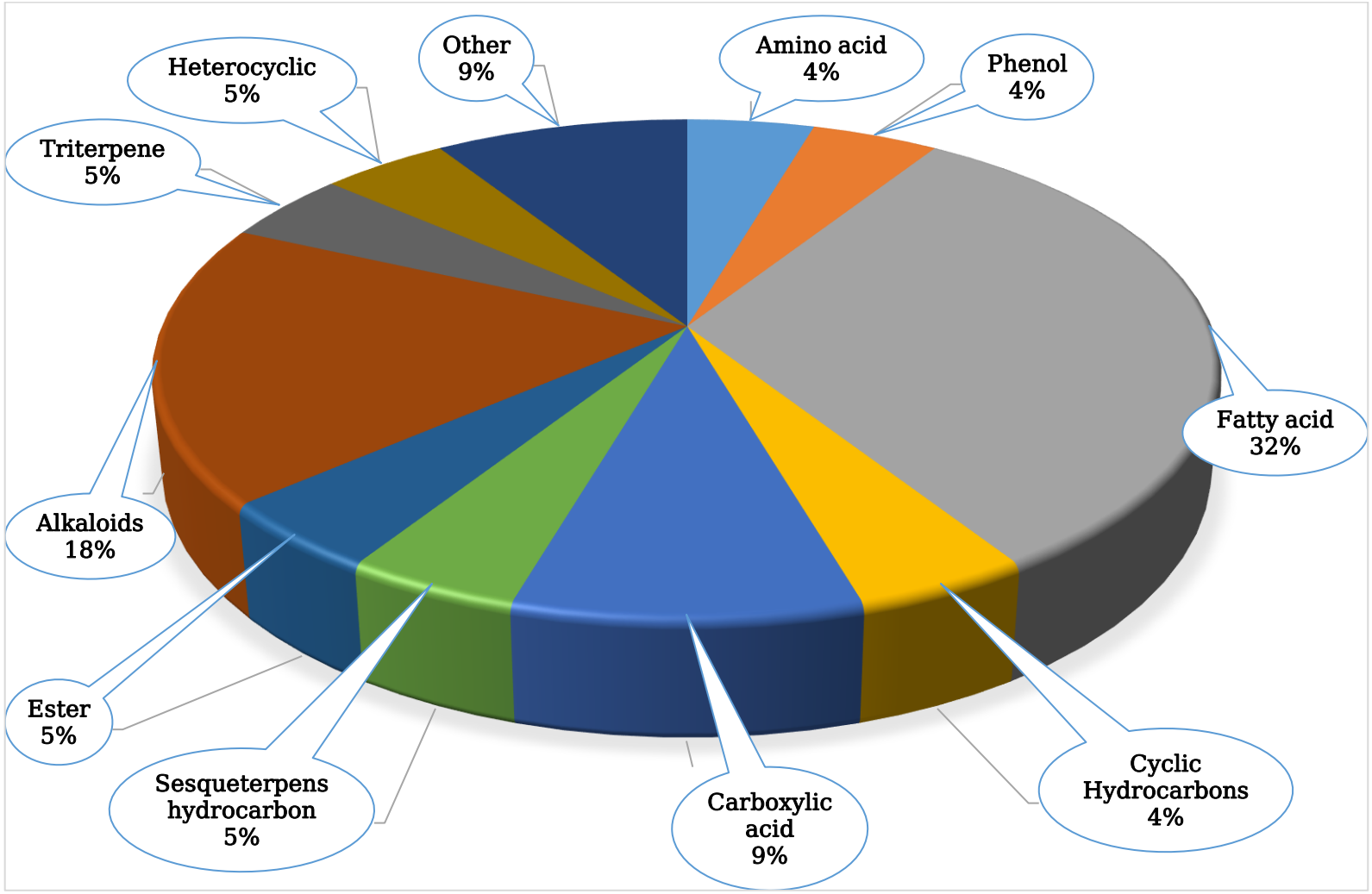


Figure (4.35): Relative content Nature of compounds (%) of compounds from *Tinospora bakis* (Whole plant).

The comparison between extracts and oil tested for GC-MS found some compounds were showed three and two crude extracts, are shown in Table (4.29) and Figure (4.36). Sixty six (66) compounds showed be checked in extracts and oil tested for GC-MS, and four (4) compounds were repeated twice and more of the tested extracts. The n-hexadecanoic acid was found in *M. indica* (4.36%), *R. officinalis* (3.88%) and *T. bakis* (1.97%). Studies showed that n-hexadecanoic acid was an antioxidant and its ester ascorbyl palmitate had a better lipid oxidation protecting effect than water-soluble ascorbic acid (Lan *et al*., 2017). In addition, the compound has also known to have anti-inflammatory (Arora and Pandey-Rai, 2014) and antihyperglacimic activity (Jiang *et al*., 2012). The Hexadecanoic acid, ethyl ester showed that *M. indica* (0.79%), AK-2 (0.93%) and *T. bakis* (0.77%). This compound has antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic, and alphareductase inhibitor (Sudha *et al*., 2013). The 9, 12-Octadecadienoic acid (Z, Z) was showed of *M. indica* (1.17%) and *T. bakis* (0.85%). It's the most abundant polyunsaturated fatty acid in human nutrition. In addition, it also play an important medicine for treatment of hyperlipoidemia and atherosclerosis (Yao *et al*., 2013, Aparna *et al*., 2012). And Ethyl Oleate was showed of AK-2 (1.97%) and *T. bakis* (0.5%). This compound was reported with antimicrobial activity (Adeoye-Isijola *et al*., 2018).

Table (4.29): The comparison of compounds between the different crude extracts using presence (+) and absence (-):

No.	Name of plants / Name of compounds	*Mangifera indica*	AK-2	*Rosmarinus officinalis*	*Tinospora bakis*
1.	(+)-(S)-Isocorydine	-	-	-	+
2.	(+)-2-Bornanone	-	-	+	-
3.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	-	-	-	+
4.	5.beta.,14.beta.-Androstane-17.beta.-carboxylic acid, 3.beta.,14-dihydroxy-, .gamma.-lactone, acetate	-	-	-	+
5.	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	-	-	+	-
6.	.alpha.-Terpineol	-	-	+	-
7.	.beta.-ylangene	-	-	+	-
8.	.gamma.- Terpinene	-	-	+	-
9.	1,2,3-Benzenetriol	+	-	-	-
10.	1,2-Benzenedicarboxylic acid, diisooctyl ester	-	+	-	-
11.	1,2-Cyclopentanedione	+	-	-	-
12.	1,6-Octadien-3-ol, 3,7-dimethyl	-	-	+	-
13.	1H-2-Indenone,2,4,5,6,7,7a-hexahydro-3-(1-methylethyl)-7a-methyl	-	-	-	+
14.	1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-	-	-	-	+
15.	2,5-Furandione, 3-methyl-	+	-	-	-
16.	2-Methoxy-4-vinylphenol	-	-	+	-
17.	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	-	-	-	+
18.	3-Carene	-	-	+	-
19.	4H-Benzo[f]pyrrolo[1,2-a][1,4]diazepine, 4-cyclohexyl-8,9-diethoxy-5,6-dihydro	-	-	+	-
20.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	+	-	-	-
21.	8,11-Octadecadienoic acid, methyl ester	-	+	-	-
22.	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)	-	-	+	-
23.	**9,12-Octadecadienoic acid (Z,Z)**	**+**	**-**	**-**	**+**
24.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-	+	-	-
25.	9,12-Octadecadienoic acid, ethyl ester	-	+	-	-
26.	9-Octadecenoic acid (Z)-, methyl ester	-	-	+	-

27.	9-Octadecenoic acid, (E)-	+	-	-	-
28.	Acridine, 9-butyl-	-	-	-	+
29.	Andrographolide	-	-	+	-
30.	Androst-5,15-dien-3ol acetate	-	-	-	+
31.	Aromadendrene oxide-	-	-	+	-
32.	Benz(a)acridine, 9,10,12-trimethyl	-	-	+	-
33.	Benzene, [(3,3,5,5-tetramethylcyclohex-1-en-1-yl)thio]	-	-	-	+
34.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl	-	-	+	-
35.	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)	-	-	+	-
36.	Bis(2-ethylhexyl) phthalate	-	-	-	+
37.	Bornyl acetate	-	-	+	-
36.	Camphenol,6-	-	-	+	-
37.	Diazinone	+	-	-	-
38.	D-Limonene	-	-	+	-
39.	Docosanoic acid, methyl ester	-	+	-	-
40.	Eicosanoic acid, methyl ester	-	+	-	-
41.	endo-Borneol	-	-	+	-
42.	**Ethyl Oleate**	**-**	**+**	**-**	**+**
43.	Eucalyptol	-	-	+	-
44.	Ferruginol	-	-	+	-
45.	Glaucine	-	-	-	+
46.	Glycerin	-	-	-	+
47.	Heptacosane	-	+	-	-
48.	Heptadecanoic acid, 15-methyl-, ethyl ester	-	+	-	-
49.	Hexacosanoic acid, methyl ester	-	+	-	-
50.	**Hexadecanoic acid, ethyl ester**	**+**	**+**	**-**	**+**
51.	Hexadecanoic acid, methyl ester	-	+	-	-
52.	Hyodeoxycholic acid	-	-	-	+
53.	Isocarnosol	-	-	+	-
54.	Linoleic acid ethyl ester	-	-	-	+
55.	Methyl 16-methyl-heptadecanoate	-	+	-	-
56.	**n-Hexadecanoic acid**	**+**	**-**	**+**	**+**

57.	Nonanoic acid, 9-oxo-, methyl ester	-	+	-	-
58.	Octadec-9-enoic acid	-	-	-	+
59.	Octadecanoic acid	+	-	-	-
60.	Octadecanoic acid, ethyl ester	+	-	-	-
61.	O-Cymene	-	-	+	-
62.	Pregn-5-ene-3,20-dione	-	-	-	+
63.	Pregnan-20-one, 3-(acetyloxy)-5, 6-epoxy-, (3. beta. 5. alpha., 6.alpha.)-	-	-	-	+
64.	Squalene	-	-	-	+
64.	Terpinen-4-ol	-	-	+	-
66.	Tetracosanoic acid, methyl ester	-	+	-	-

Key: AK-2: sup-fraction of *Mangifera indica*.

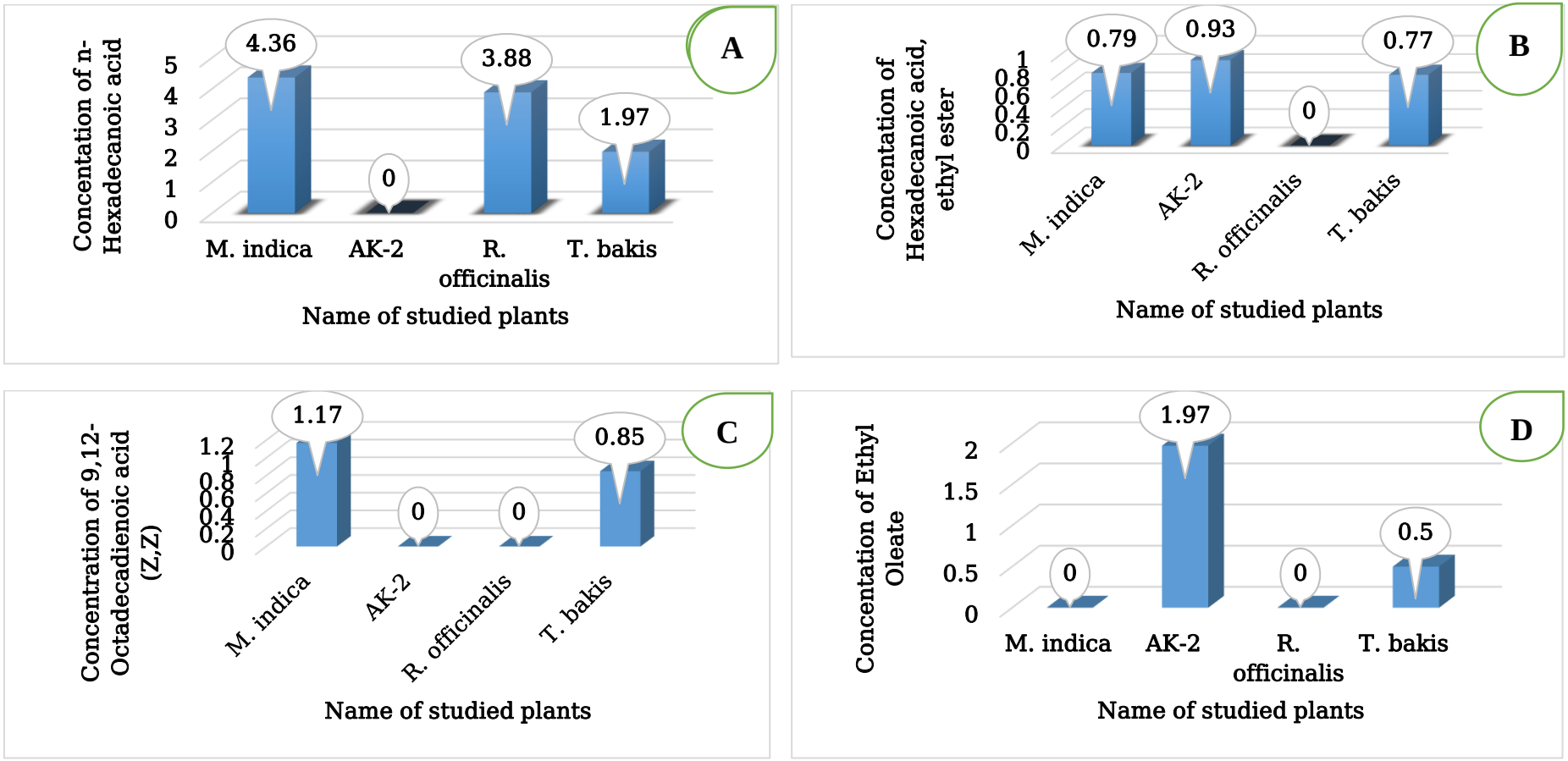


Figure (4.36): The percentage (%) composition and distribution of (A) n-Hexadecanoic acid, (B) Hexadecanoic acid, ethyl ester, (C) 9, 12-Octadecadienoic acid (Z, Z) and (D) Ethyl Oleate among the studied plants.

4.5 Identification and structural elucidation of isolated compounds

Plants are the main source of the natural compounds. Whenever a new active natural compound is isolated, the next major task is its structure elucidation. There are several techniques for structure elucidation; mass spectrometry (MS), X-ray crystallography, nuclear magnetic resonance (NMR), etc. In the present study 17 compounds were isolated from ethyl acetate fractions of *M. indica* (seeds) and *T. bakis* **(**whole plant**)** were selected for the isolation after an extensive screening process of three medicinally important plants of Sudan for their biological activities by utilizing: antimicrobial and anti-oxidant activities. These three plants were selected on the basis of information gathered from the local traditional healers. During this screening process that the extracts/fractions of *M. indica* and *T. bakis* showed the best results in multiple bioassays used in this study and also these plants had the oldest history of safe use in human. Characterization of the isolated compounds were carried out by using ESI-MS, 1D-NMR and 2D-NMR techniques at International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan. The new compounds isolated using all NMR spectra (^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, COSY and NOSY) of the compounds were recorded to propose the structure of the purified compound and MS spectrum was recorded to confirm the molecular weight (MW) of the compounds, and spectra for structure elucidation of each isolated compounds.

4.5.1 Isolated compounds from *M. indicia* seeds

Four compounds were isolated from ethyl acetate fraction of *M. indica* (seeds), and were screened for antimicrobial and antioxidant activities.

A. Methyl gallate

Methyl gallate or Methyl 3, 4, 5-trihydroxybenzoate (1) ($C_8H_8O_5$) (MW. 184) was obtained as white needle crystals powder (45 mg). The observed R_f value was 0.56 (dichloromethane: methanol 4:1). MS/ESI (−) m/z 183.0534 [M−H].^{1}H NMR (500 MHz, $CDCl_3$).: δ3.79 (3H, s, OCH3), δ 7.11 (2H, s, H-2, H-6); ^{13}C NMR (acetone-D6, 150.80 MHz) δ 51.0 (OCH3), δ 108.90 (C-2, C-6), δ 120.91 (C-1), δ 137.76 (C-4), δ 145.12 (C-3, C5), δ 166.27 (C=O). The molecular formula was determined from the MS and ^{13}C NMR. 8 Carbons and five protons attached to carbon were observed in the ^{13}C and ^{1}HNMR spectra, the number of hydroxyl groups, the NMR solvent was shifted to DMSO-d6 as hydroxyl were not seen with acetone-d6. ^{1}H-NMR (DMSO-d6, 600 MHz) clearly reveal the presence two hydroxyls at δ9.44 and one hydroxyl at δ9.11. Close examination of the ^{1}H and ^{13}C NMR spectrum showed a symmetrical molecule with two aromatic protons, δ 7.11 (2H, s, H-2, H-6), three hydroxyl, two hydroxyl at δ C 145.12 (C-3, C-5), and one hydroxyl at δ C 137.76 (C-4), a methyl δ3.79 (3H, s, OCH_3) and a ester carbonyl δ 166.27 (C=O), as shown in Figures (4.37). The result was consistent with Singh *et al*., (2007) and Zargari (1997) published NMR data hence, the compound structure revealed to be methyl 3, 4, 5-trihydroxybenzoate or methyl gallate**.**

B. β-sitosterol

β-sitosterol (2) ($C_{29}H_{50}O$) (MW. 414) was obtained as white-yellowish crystalline powder (85 mg). The observed R_f value was 0.56 (n-hexane/ethyl acetate 4:1). The compounds was identified using ^{1}H NMR spectra (400 MHz, $CDCl_3$) and confirmed using mass spectral data (m/z): 397 $[M\text{-}OH]^-$. Signals in the ^{1}H-NMR spectrum were observed mainly in the up field region. The spectra exhibited only two signals with high chemical shifts values; the first one resonated in the olefinic region and the other one was observed a little up field region. The olefinic signal at δ 5.3 (1H, br d, J = 4.8 Hz) appeared to be characteristic of the sterols, and it was assigned to H-6 proton in the β–sitosterol chemical skeleton. The ^{1}H-NMR spectra of compound 2 also exhibited a signal corresponding to the proton connected to C-3 hydroxyl group which appeared as a multiplet at δ 3.50 (1H, m). Six other proton signals were evident which include four secondary methyl groups (δH 0.91, 0.82, 0.81 and 0.79 all doublets with J = 6.6, 7.2, 6.4 and 6.4 Hz respectively) and two tertiary methyl groups (δH 0.66 and 0.99). The ^{13}C NMR spectra exhibited 29 carbon signals, characteristic of phytosterols. These data were in agreement with the structure of β-sitosterol. The NMR data of β-sitosterol, is in agreement with (Chaturvedula and Prakash, 2012). The observed molecular weight by ESI-MS was 414, and the chemical structure is shown in Figures (4.30).

C. Daucosterol

Daucosterol or β-sitosterol glucoside (3) ($C_{35}H_{60}O_6$) (MW. 576) was obtained as violet colour (40 mg). Isolated by sephadex column and gave an R_f value of 0.47 on thin layer chromatography. The ^{1}H-NMR spectrum of compound Daucosterol showed a chemical shift in the range δH 0.79-0.97 suggesting presence of methyl protons. A singlet observed at δH 0.75 was assigned to methyl protons. The proton attached to olefinic linkage was observed at δH 5.30. Methyl proton was observed at δH 0.91 with coupling constant J=6.4Hz. The proton of glucose was observed at δH 2.88-4.32 as a multiplet. The proton of CH-group of glucoside was observed at δH 4.40, as shown Figure (4.37). The signals observed at δH 0.79 and δH 0.91 were assigned to methyl groups of isopropenyl moiety (Peshin and Kar, 2017). Proton was observed at δH 2.88 the hydroxyl protons of the sugar moiety showed resonance at δH 3.56-3.63 (Tor-Anyiin *et al*., 2011).

D. Oleic acid

Oleic acid (4) ($C_{18}H_{34}O_2$) (MW. 282). Isolated by silica gel and gave an R_f value of 0.62 on thin layer chromatography. ^{1}H NMR ($CDCl_3$, 500 MHz) d 5.34 (2H, m), 2.35 (2H, t, J = 7.5 Hz), 2.01 (4H, m), 1.63 (2H, m), 1.2 – 1.4 (20H), 0.88 (3H, t, J = 7.0 Hz) and ESI-MS m/z 281 $[M–H]^–$. The ^{1}H-NMR (500 MHz) spectrum of the com-pound exhibited signals at δ 0.83 as a pair of trip-lets overlapped with each other indicating thepresence of two terminal methyl groups, at δ 1.29 as a broad singlet for a long chain of methyleneprotons, at δ 1.94 for methylene groups α to C=Cgroup, at δ 2.24 for methylene group α to carbo-nyl group, as showed in Figure (4.37). The multiplet signals appeared at δ 4.13 which may be attributed to oxymethylene groupand at δ 5.38 for an unsaturated proton. The ^{3}C-NMR spectrum showed twosignals in the unsaturated carbon region, at δ130.16 and δ 110.66. The signal at δ 155.34 ap-peared to be of an ester

carbonyl carbon atom.The signal at δ 60.39 could be assigned tooxymethylene carbon. The signals at δ 14.93 and13.97 might be due to methyl carbons. All theother signals at δ 19.05, 19.84, 20.43, 21.03,22.68, 23.07, 24.79, 27.22, 29.06, 29.82, 45.88and 56.17 were due to methylene carbons in along chain.

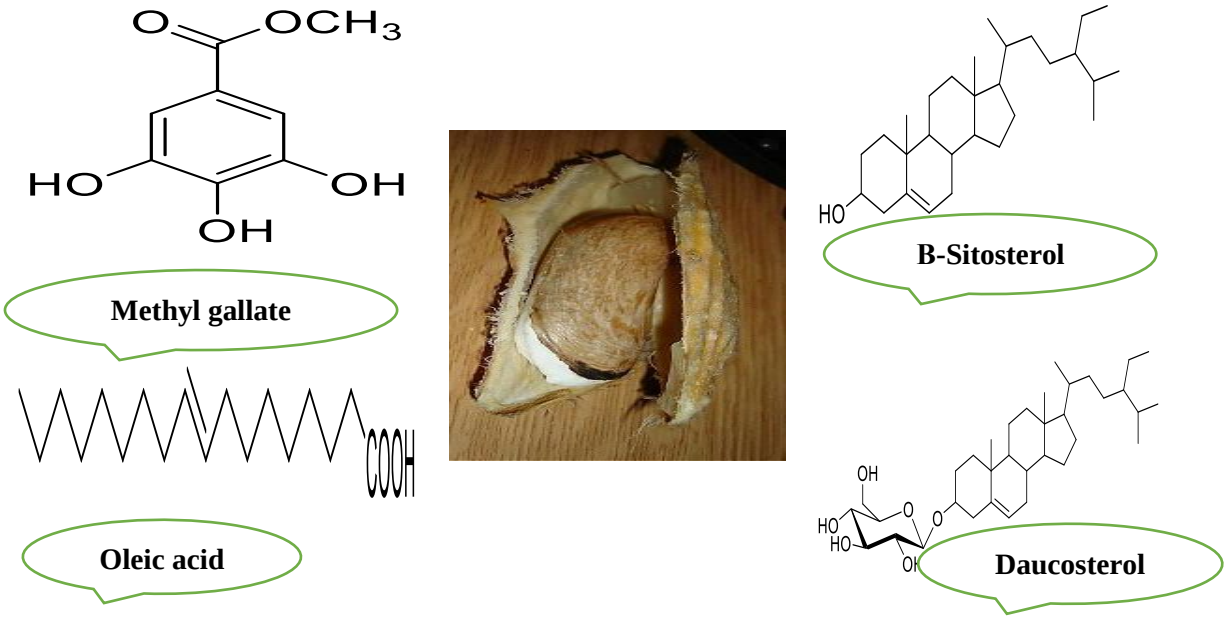


Figure (4.37): Chemical structure of isolated compounds (1). Methyl gallate, **(2) β-sitosterol**, **(3) B-sitosterol glucoside** and **(4) Oleic acid from *Mangifera indica* (seeds).**

4.5.2 Isolated compounds from *Tinospora bakis* (Whole plant)

The whole plant of *T. bakis* was separately extracted with ethanol (80 %) using cold maceration extraction protocol, and after drying, extracts further fractionated by liquid-liquid partitioning using solvents of increasing polarity (n-hexane, ethyl acetate, n-butanol and aqueous) using separation funnel. The most active fraction (ethyl acetate) was separated by chromatography over silica gel, sephadex LH-20, and C18 reverse-phased silica gel, as well as semi-preparative HPLC to afford four new diterpenoids and nine known ones, as shown in Figure (4.38). Characterization of the isolated compounds was carried out by using ESI-MS, 1D-NMR and 2D-NMR techniques. The new compounds isolated using all NMR spectra (^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, COSY and NOSY) of the compounds were recorded to propose the structure of the purified compound and MS spectrum was recorded to confirm the molecular weight of the compounds. In This present study 13 compounds were isolated from ethyl acetate fraction of *T. bakis* which were screened for antimicrobial and antioxidant activities.

A. **Tinocallone A**

Tinocallone A (5) ($C_{19}H_{22}O_4$) (MW. 314), was isolated as a white amorphous solid. Its molecular formula was established as $C_{19}H_{24}O_4$ by HR-ESI-MS showing a pseudo molecular ion [M + Na]+ peak at m/z 339.1568 (calcd for $C_{19}H_{24}O_4Na$, 339.1572). Signals of two methyls (dH 0.83 and 1.23), a furan ring (dH

6.42, 7.38, and 7.41), as well as two olefinic protons (dH 6.73 and 5.96) were observed in the ^{1}H NMR ($CDCl_3$, 500 MHz) of isolated compound, as shown in Figure (4.38).

B. Sagitone

Sagitone (6) ($C_{19}H_{22}O_5$) (MW. 330), was obtained as an amorphous powder and with positive reaction in the 10% H_2SO_4-EtOH test. The molecular formula was deduced from a pseudo-molecular ion $[M + H]^+$ at *m/z* 331.3816 in the HR-ESI-MS (calcd. for $C_{19}H_{23}O_5$, 331.3829). The ^{1}H-NMR (500 MHz) ($CDCl_3$) spectra of compound revealed 19 carbons, consisting of two methyls, four methylenes, seven methines, and six quaternary carbons. Taking into account the nine degrees of unsaturation, compound should include four rings. Careful analysis of its ^{1}H-NMR data strongly suggested that the compound exhibited typical clerodane diterpenoid signals. The characteristic ^{1}H-NMR signals of compound revealed the existence of a β-substituted furan ring (δ 6.42 s, 7.43 s and 7.45 s; δ 108.4, 143.8, 139.6, 125.0). The one proton double doublet at δ 5.42 (J = 12.4, 4.0 Hz) was assigned to the C-12 proton and two one-proton double doublets at δ 2.30 (J = 14.8, 4.0 Hz) and 1.73 (J = 14.8, 12.0 Hz) were attributed to the C-11eq and C-11ax protons, respectively. The presence of a carbinolic carbon was also evident from ^{13}C-NMR signal at δ 70.6 (C-12). The two methyl groups at C-9 and C-5 were observed as three proton singlets at δ 1.30 and 0.96, respectively. The signals at δ 2.30 and 2.20 were assigned to the protons at C-8 and C-10, and the C-6 and C-7 methylene protons resonating at δ 2.40 (m), 1.19 (dt, J = 14.0, 4.0 Hz) and 2.25 (m), 1.61 (m), respectively, as shown in Figure (4.38).

C. Fibrauretin H

Fibrauretin H (7), its molecular formula ($C_{19}H_{20}O_5$) (MW. 228), was isolated as optically yellow syrup, Compared ^{1}H-NMR ($CDCl_3$) spectral with 4, they indicated the structure of Fibrauretin F was similar to those of 5, but appeared an additional carbonyl group at C-1 position, which was further supported by the HMBC correlations of H-10 (d 2.69) and H-3 (d 6.85) with C-1 (d 199.0). Therefore, the structure of 6 was established as fibrauretin F, as shown in Figure (4.38).

D. Tinophyllol

Tinophyllol (8) ($C_{21}H_{26}O_6$) (MW. 374)- mp 229-231 °C (dec.), colorless prisms from MeOH, -19.3 (pyridine, c = 0.5). Anal. Calcd for C21E12606: C, 67.36; H, 7.00. Found: C, 67.37; H, 7.15. UV nm: 216. El-MS (m/z): 374 (Mt). IR vNujol cm°1 cm 1: 3470, 3130, 1730, 1718, 1495, 878, 815. 1H-NMR (400 MHz, DMSO-d6) : 7.73 (1H, d, J= 1.6 Hz, H-16), 7.68 (1H, dd, J=1.8, 1.6 Hz, H-15), 6.55 (1H, d, J= 1.8 Hz, H-14), 6.25 (1H, d, J=4.0 Hz, H-3), 5.49 (1H, dd, J= 12.0, 6.0 Hz, H-12), 4.96 (1H, br s, 2-OH), 4.34 (1H, ddd, J=8.0, 8.0, 4.0 Hz, H-2), 3.68 (3H, s, 4-OCH_3), 2.79 (1H, dd, J= 10.0, 3.0 Hz, H-8), 2.43 (1H, dd, J= 11.0, 3.0 Hz, H-6*f*À), 2.14 (1H, dd, J=14.0, 8.0 Hz, H-1*f*À), 2.06 (1H, dd, J= 14.0, 6.0 Hz, H-11*f*¿), 1.93 (1H, ddd, J= 14.0, 8.0, 6.0 Hz, H-1*f*¿), 1.87 (1H, dd, J= 14.0, 12.0 Hz, H-11*f*À), 1.80 (1H, d, J=6.0 Hz, H-10), 1.61 (1H, m, H-7a), 1.32 (3H, s, 5-CH_3), 1.18 (2H, m, H-6*f*¿ and H-7,6), 0.84 (3H, s, 9-CH_3), as shown Figure (4.38).

E. Tinospin C

Tinospin C (9) was isolated as colorless solid. The molecular formulas were established as $C_{21}H_{26}O_7$, by the HR-FAB-MS data. Compound was also cis-clerodane type diterpenoids, which were indicated by the typical C-12 furan ring downfield resonances in the ^{1}H-NMR spectrum. The superimposable ^{1}H- and ^{13}C-NMR spectroscopic data of compound, except for resonances of an additional β-glucopyranosyl moiety in compound suggested that compound was a β-glucopyranoside of compound. In the ^{1}H-NMR spectrum of compound, in addition to the 12-oxymethine proton resonance at δH 5.35, another oxymethine proton at δH 5.41 was also observed, which was assigned to be H-1 by the 1 H–1 H COSY correlation between δH 5.41 (H-1) and 2.08 (H-10). In the 1 H–1 H COSY spectrum, H-1 was showed to be correlated with a pair of coupled olefin proton resonances at δH 6.43 (H-2) and δH 6.53 (H-3), suggesting presence of an olefin moiety at C-2 and C-3. Another downfield olefin proton resonance at δH 7.27 was assigned.

F. β-sitosterol

β-sitosterol (10) ($C_{29}H_{50}O$) (MW. 414) was obtained as white-yellowish crystalline powder (85 mg). The observed R_f value was 0.56 (n-hexane/ethyl acetate 4:1). The compounds was identified using ^{1}H NMR spectra (500 MHz, $CDCl_3$) and confirmed using mass spectral data (m/z): 397 $[M\text{-}OH]^-$. Signals in the ^{1}H-NMR spectrum were observed mainly in the up field region. The spectra exhibited only two signals with high chemical shifts values; the first one resonated in the olefinic region and the other one was observed a little up field region. The olefinic signal at δ 5.3 (1H, br d, J = 4.8 Hz) appeared to be characteristic of the sterols, and it was assigned to H-6 proton in the β–sitosterol chemical skeleton. The ^{1}H-NMR spectra of compound 2 also exhibited a signal corresponding to the proton connected to C-3 hydroxyl group which appeared as a multiplet at δ 3.50 (1H, m). Six other proton signals were evident which include four secondary methyl groups (δH 0.91, 0.82, 0.81 and 0.79 all doublets with J = 6.6, 7.2, 6.4 and 6.4 Hz respectively) and two tertiary methyl groups (δH 0.66 and 0.99). The ^{13}C NMR spectra exhibited 29 carbon signals, characteristic of phytosterols. These data were in agreement with the structure of β-sitosterol. The NMR data of β-sitosterol, as shown in Figure (4.38), is in agreement with the published values (Chaturvedula and Prakash, 2012).

G. Daucosterol

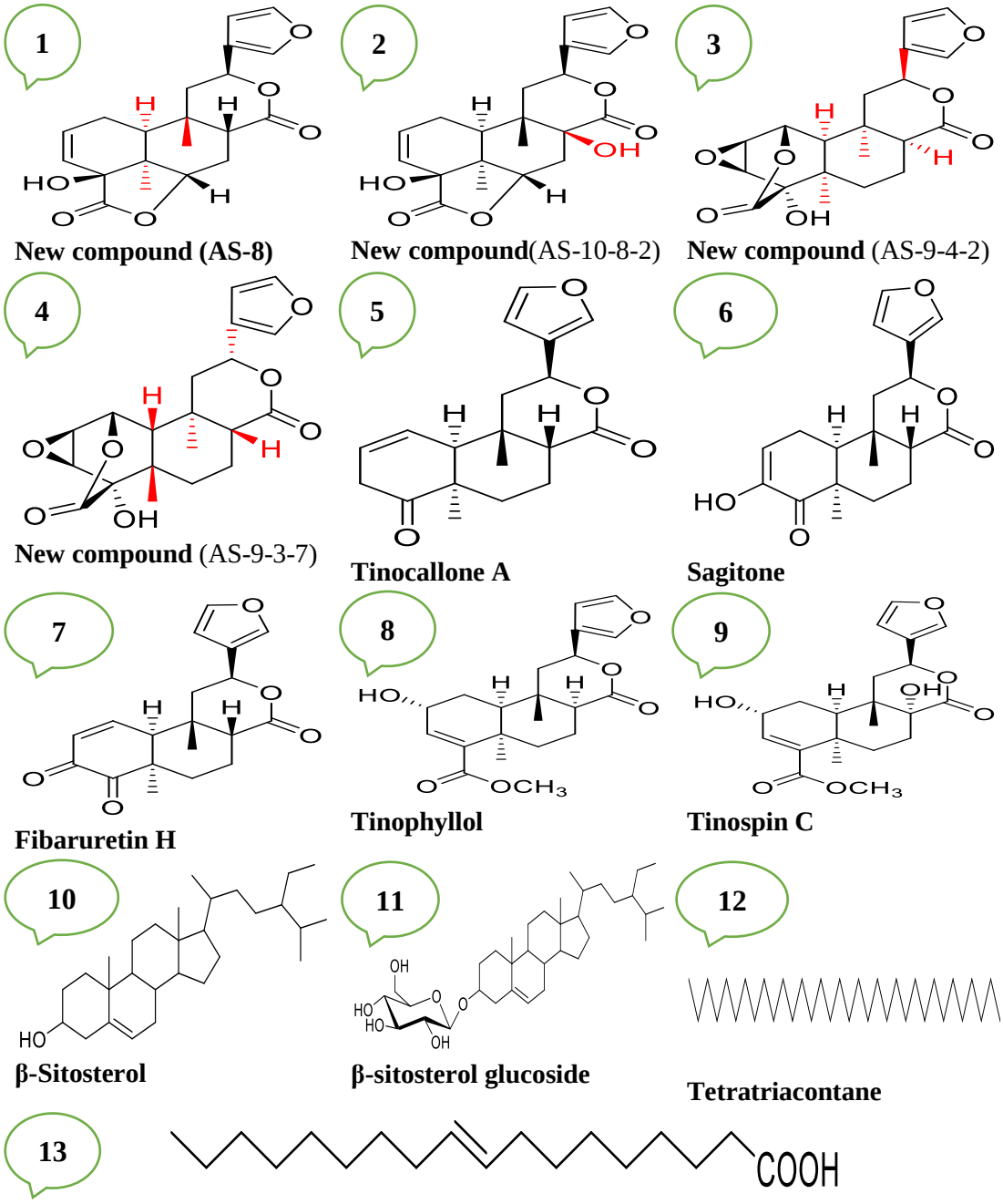
Daucosterol or β-sitosterol glucoside (11) ($C_{35}H_{60}O_6$) (MW. 576) was obtained as violet colour (40 mg). Isolated by sephadex column and gave an R_f value of 0.47 on thin layer chromatography. The ^{1}H-NMR spectrum of compound Daucosterol showed a chemical shift in the range δH 0.79-0.97 suggesting presence of methyl protons. A singlet observed at δH 0.75 was assigned to methyl protons. The proton attached to olefinic linkage was observed at δH 5.30. Methyl proton was observed at δH 0.91 with coupling constant J=6.4Hz. The proton of glucose was observed at δH 2.88-4.32 as a multiplet. The proton of CH-group of glucoside was observed at δH 4.40, as shown in Figure (4.38). The signals observed at δH 0.79 and δH 0.91 were assigned to methyl groups of isopropenyl moiety (Peshin and Kar, 2017). Proton was observed at δH 2.88 the hydroxyl protons of the sugar moiety showed resonance at δH 3.56-3.63 (Tor-Anyiin *et al*., 2011).

H. Tetratriacontane

Tetratriacontane (12) ($C_{34}H_{70}O$) (MW. 478). Isolated by silica gel. It was obtained as white powder from n-hexane fraction. 1H-NMR spectrum of Tetratriacontane presented signals of hydroxymethylene protons at δH 3.64 (2H, t, J = 7.0 Hz, H-1); two methylenes at δH 1.56 (4H, t, J=7.0 Hz, H2-2,3), sixty consecutive methylene protons at δH 1.25 – 1.26 (60H, s) and one methyl group (δH 0.88, 3H, t, J = 7.0 Hz, H-34). These data suggested that Tetratriacontane was a long-chain alcohol. The ESI-MS spectrum of Tetratriacontane in negative mode showed a pseudomolecular ion peak at m/z 493 [M-H]-, indicating the molecular formula of Tetratriacontane as $C_{34}H_{70}O$. Compound Tetratriacontane was determined to be n-tetratriacontanol, other name sapiol.

I. Oleic acid

Oleic acid (13) ($C_{18}H_{34}O_2$) (MW. 282). Isolated by silica gel and gave an R_f value of 0.62 on thin layer chromatography. ^{1}H NMR ($CDCl_3$, 500 MHz) d 5.34 (2H, m), 2.35 (2H, t, J = 7.5 Hz), 2.01 (4H, m), 1.63 (2H, m), 1.2 – 1.4 (20H), 0.88 (3H, t, J = 7.0 Hz) and ESI-MS m/z 281 $[M–H]^–$. The ^{1}H-NMR (500 MHz) spectrum of the com-pound exhibited signals at δ 0.83 as a pair of trip-lets overlapped with each other indicating thepresence of two terminal methyl groups, at δ 1.29 as a broad singlet for a long chain of methyleneprotons, at δ 1.94 for methylene groups α to C=Cgroup, at δ 2.24 for methylene group α to carbo-nyl group. The multiplet signals appeared at δ 4.13 which may be attributed to oxymethylene groupand at δ 5.38 for an unsaturated proton. The ^{3}C-NMR spectrum showed twosignals in the unsaturated carbon region, at δ130.16 and δ 110.66. The signal at δ 155.34 ap-peared to be of an ester carbonyl carbon atom.The signal at δ 60.39 could be assigned tooxymethylene carbon. The signals at δ 14.93 and13.97 might be due to methyl carbons. All the other signals at δ 19.05, 19.84, 20.43, 21.03,22.68, 23.07, 24.79, 27.22, 29.06, 29.82, 45.88and 56.17 were due to methylene carbons in along chain.



New compound (AS-8) **New compound**(AS-10-8-2) **New compound** (AS-9-4-2)

New compound (AS-9-3-7) **Tinocallone A** **Sagitone**

Fibaruretin H **Tinophyllol** **Tinospin C**

β-Sitosterol **β-sitosterol glucoside** **Tetratriacontane**

Oleic acid

Figure (4.38): Chemical structure of isolated compounds from *Tinospora bakis* (Whole plant).

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

CHABTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 General Conclusions

The main aim of the study was to evaluate the antimicrobial, antiprotozal, molluscicidal and antioxidant activities and cytotoxicity of the extracts from three medicinal plants selected were isolate and characterize the bioactive compounds. To attain this aim a number of objectives were formulated. The research work cited in this study contributes to justifying the traditional use of *Mangifera indica* L. (Famliy: Anacardiaceae), *Rosmarinus officinalis* L. (Famliy: Lamiaceae) and *Tinospora bakis* (A. Rich) Miers (Famliy: Menispermaceae) for the treatment of various health problems, collected from different geographical locations in Sudan.

In Sudan these plants may be used as a natural source for pharmaceutical purpose designed for antimicrobial, antiprotozoal, molluscicidal and antioxidant activities and may provide a new lead pharmacophore for more potent analogues, the present study can be concluded in two prospects on the basis of objectives as isolation of bioactive compound from the studied plants, which is responsible for antimicrobial, antioxidant activities and characterization and identification of antiprotozal, anti-schistosoma activities and cytotoxicity in studied plants.

5.1.1 Preliminary screening of extracts, fractions and compounds of plant species for biological activities

The powdered plant materials of selected medicinal plant were extracted with ethanol (80 %). The ethanol extract was fractionated using liquid-liquid fractionation to group the compounds based on polarity. Four fractions namely: hexane, ethyl actate, n-buotanol and aqueous were collected; tested for biological activities and cytotoxicity assay. The ethanolic extracts of *M. indica* and *T. bakis* were very active with an MIC value of ≤ 0.78 and 1.56 mg/ml, respectively. While, the ethyl actate fractions were with the highest activity against all microorganisms tested. In addition, the compounds tested showed the strongest antimicrobial activity. The extracts were active for antiprotozoal activity. However, the highest IC_{50} of antiprotozoal activity with 3 µg/ml against *Entamoeba histolytica* and *Giardia lamblia* after 72 h from *M. indica*. The extracts have activity for molluscicidal activity, while, the highest IC_{50} of molluscicidal activity with 0.3 µg/ml against *B. pfeiffer* after 48 h from *M. indica*. And extracts, fractions and compounds were showed highly activity and moderate for antioxidant activity. However, the highest IC_{50} of antioxidant activity with 0.00225 ± 0.001 and 0.0083 ± 0.0001 mg/ml from *R. officinalis* and *M. indica*, respectively. In addition, the Methyl gallate was strongest active IC_{50} with (0.03 ± 0.01 mg/ml) isolated from *M. indica* for antioxidant activity, while, comparison to the standard antioxidant (propyl gallate) IC_{50} (0.0141 ± 0.01 mg/ml). The cytotoxcity of crude extracts and fractions were screened on brine shrimp lethality assay and

normal cell line (BHK cell line) by MTT-assay, and the extracts and fractions as well as having a safe cytotoxicity profile.

Based on the results of flavonoids, alkaloids, saponins, tannins and phenols were present in larger amounts in all extracts for qualitative phytochemical analysis. The quantitative analysis of extracts were in highly amount as phenolic, flavonids and tannin. While, the *M. indica* was higher than *R. officinalis* for quantitative analysis. The results obtained from gas chromatography mass detector showed the presence of high number of many bioactive compounds that possess antioxidant, anti-inflammatory, anti-microbial and anticancer properties in all tested extracts.

In addition, in *M. indica* seeds 1,2,3-Benzenetriol (pyrogallol) ($C_6H_6O_3$) was found and is known as one of hydrolysable tannin (phenolic compound) and has been considered as the major compound of this plant that gave 68.61% in this study, with known biological properties. Its has many biological activities as antimalarial, antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide and anticancer. While, the *T. bakis* was found 1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene- ($C_{15}H_{24}$) has been found as the major compound of this plant that gave 46.94% Its has been maney biological properties. And in *R. officinalis* Eucalyptol ($C_{10}H_{18}O$) has been found as the major compound of this plant that gave 25.11%. Its has many biological properties vis; antibacterial, anti-inflammatory, gastroprotective, antitumorogenic, hepatoprotective and antimycotic activities.

Totally, seventeen purified compounds (1–17) were obtained from ethyl acetate fractions of *M. indica* and *T. bakis* by using standard techniques for isolations. Four compounds were isolated for the first time from *T. bakis*. Four isolated compounds (4) were isolated of ethyl acetate fraction from *M. indica* namely; Methyl gallate ($C_8H_8O_5$), β-sitosterol ($C_{29}H_{50}O$), Daucosterol or β-sitosterol glucoside ($C_{35}H_{60}O_6$) and Oleic acid ($C_{18}H_{34}O_2$). Its was highly activity for antimicrobial activity and modrate activity for antioxidant activity except Methyl gallate ($C_8H_8O_5$) has strong activity for antioxidant activity. On other hand, thirteen compounds (13) were isolated of ethyl acetate fraction from *T. bakis*. While, nine compounds (9) from diterpenoids classes and other compounds with different classes. In addition, the four new compounds for diterpenoids classes, (1), (2), (3) and (4), and five (5) known diterpenoids, Tinocallone A (5), Sagitone (6), Fibaruretin H (7), Tinophyllol (8) and Tinospin C (9). And other isolated compounds namely; β-sitosterol (10) ($C_{29}H_{50}O$), Daucosterol or β-sitosterol glucoside (11) ($C_{35}H_{60}O_6$), Tetratriacontane (12) ($C_{34}H_{70}$) and Oleic acid (13) ($C_{18}H_{34}O_2$). Its have highly for antimicrobial activity and modrate activity for antioxidant activity.

The isolated compounds have been shown for its antimicrobial and antioxidant activities and thus could also serve as useful precursor in the synthesis of drugs to treat diseases caused by some clinical pathogens which justifies the ethno medical claims of this plants uses for treatment of some diseases. Eventually, the investigated plants could be used as herbal remedies in the management of ailments caused and consequently to solve certain health problems. Since the isolated compounds have important medicinal role

in antibacterial, antifungal and antioxidant activities, so the isolation and characterization of bioactive phytocomponents can confirm/explain the medicinal importance of plants under study.

5.2 Recommendations:

The following highly recommended

1. Bio-assay guided fractionation and purification for to more isolation of active ingredients responsible activity.
2. Further, the crude extracts and compounds to be screened for other biological effects such as anti-inflammatory, antidiabetic, anti-viral, antileishmanial …..etc.
3. Various useful synthetic analogues of isolated compounds with enhanced therapeutic properties can be obtained from single lead compound by structural modification.
4. *In-vivo* studies should be carried out to assess their safety, therapeutic efficacy and potential it for commercial utilization as fundamental step for their application to human.
5. Studies regarding the mode of action for isolated compounds in the bacterial cells should be done.

References:

Abbasi, A. M., Liu, F., Guo, X., Fu, X., Li, T. & Liu, R. H. 2017. Phytochemical composition, cellular antioxidant capacity and antiproliferative activity in mango (*Mangifera indica* L.) pulp and peel. *International Journal of Food Science & Technology,* 52**,** 817-826.

Abd-Ulgadir, K. S., Suliman, S. I., Zakria, I. A. & Hassan, N. 2015. Antimicrobial potential of methanolic extracts of *Hibiscus sabdariffa* and *Ricinus communis*. *Advancement in Medicinal Plant Research,* 3**,** 18-22.

Abdalla, W. E. & Koko, W. S. 2018. Review of Sudanese Medicinal Plants Tested for their Antiparsitical Activity. *American Journal of Innovative Research and Applied Sciences*.

Abdallah, E. M. 2011. Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science,* 1**,** 16-20.

Abdallah, E. M. 2016a. Antibacterial efficiency of the Sudanese Roselle (*Hibiscus sabdariffa* L.), a famous beverage from Sudanese folk medicine. *Journal of intercultural ethnopharmacology,* 5**,** 186.

Abdallah, E. M. 2016b. Antibacterial properties of leaf extracts of *Moringa oleifera* Lam. growing in Sudan. *Journal of Advances in Medical and Pharmaceutical Sciences,* 5**,** 1-5.

Abdallah, E. M. 2017. Antibacterial Activity of Fruit Methanol Extract of *Ziziphus spina-christi* from Sudan. *Int. J. Curr. Microbiol. Appl. Sci,* 6**,** 38-44.

Abdallah, E. M., Hsouna, A. B. & Al-Khalifa, K. S. 2012. Antimicrobial, antioxidant and phytochemical investigation of *Balanites aegyptiaca* (L.) Del. edible fruit from Sudan. *African Journal of Biotechnology,* 11**,** 11535-11542.

Abdel-Aziz, A., Brain, K. & Bashir, A. K. 1990. Screening of sudanese plants for molluscicidal activity and identification of leaves of *Tacca leontopetaloides* (L.) O Ktze (Taccaceae) as a potential new exploitable resource. *Phytotherapy Research,* 4**,** 62-65.

Abdelkreem, M. I. M. & Ibrahim, D. A. 2016. Check list of Flora and vegetation of an archaeological habitat in North Sudan. *Pyrex Journal of Biodiversity and Conservation,* 1**,** 1-9.

Abdelmageed, E., Bushara, H. O. & Abdelgadir, M. 2018. Evidence of biological activity of *Pulicaria crispa* on *Biomphalaria pfeifferi* host snails of *Schistosoma mansoni*. *Bioscience Biotechnology Research Communications,* 11**,** 55-59.

Abdelrahman, M. M., Naguib, I. A., Elsayed, M. & Zaazaa, H. 2015. Three spectrophotometric methods for simultaneous determination of ampicillin and dicloxacillin in presence of their major impurity 6-aminopenicillanic acid. *Austin Journal of Analytical and Pharmaceutical Chemistry,* 2.

Abdelrahman, S. & Khalil, A. 2012. Activity and toxicity of *Tinospora bakis* in rats. *Asian Journal of Plant Sciences,* 11**,** 157-159.

Abdelsalam, I., Mohamed, Sayed Koko, W., Elamin Osman, E.-B., Mohamed Dahab, M., Ismail Garbi, M., Mohamed Alsadeg, A. & Saeed Kabbashi, A. 2016. Molluscicidal Activity of *Acacia seyal* (Dell) Bark Methanolic Extract Against *Biomphalaria pfeifferi* Snails. *International Biological and Biomedical Journal,* 2**,** 73-79.

Abdelwahid, M. A., Elsaman, T., Mohamed, M. S., Latif, S. A., Mukhtar, M. M. & Mohamed, M. A. 2019. Synthesis, Characterization, and Antileishmanial Activity of Certain Quinoline-4-carboxylic Acids. *Journal of Chemistry,* 2019.

Abdollaah, A. T., Barakat, T. A. M. & Abdalla, M. E. 2019. Medicinal Properties of Local Plants.

Abu-Al-Basal, M. A. 2010. Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. *Journal of ethnopharmacology,* 131**,** 443-450.

Abubakar, A. S. A. 2017. The Use of Traditional Medicine in Dairy Cattle in South Gezira Locality, Gezira State, Sudan. University of Gezira.

Abubakar, M. N. & Majinda, R. R. 2016. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and Pterocarpus angolensis (DC). *Medicines,* 3**,** 3.

Abudho, B. O., Ndombi, E. M., Guya, B., Carter, J. M., Riner, D. K., Kittur, N., Karanja, D. M., Secor, W. E. & Colley, D. G. 2018. Impact of four years of annual mass drug administration on prevalence and intensity of schistosomiasis among primary and high school children in Western Kenya: a repeated cross-sectional study. *The American journal of tropical medicine and hygiene,* 98**,** 1397-1402.

ACEVEDO-Rodríguez, P., STRONG, M. T. & STRONG, M. T. 2012. Catalogue of the Seed Plants of the West Indies. Smithsonian Contributions to Botany. Smithsonian Institution Scholarly Press Washington D. C.

Achimón, F., BRITO, V. D., PIZZOLITTO, R. P., SANCHEZ, A. R., Gómez, E. A. & ZYGADLO, J. A. 2021. Chemical composition and antifungal properties of commercial essential oils against the maize phytopathogenic fungus *Fusarium verticillioides*. *Revista Argentina de Microbiología*.

Adam, A. A. E. M. 2017. Isolation, Characterization and Biological Activity of Flavonoids from *CordiaSinensis* and *Acacia Orefota.* Sudan University of Science and Technology.

Adam, M., Elhassan, G. O., Yagi, S., Senol, F. S., Orhan, I. E., Ahmed, A. A. & Efferth, T. 2018. *In vitro* antioxidant and cytotoxic activities of 18 plants from the Erkowit Region, Eastern Sudan. *Natural products and bioprospecting,* 8**,** 97-105.

Adamu, S., Kela, S. & Suleiman, M. 2006. Antischistosomal Properties of Extracts of *Jatropha Curcas* (L) On *Schistosoma Mansoni* Infection In Mice. *African Journal of Traditional, Complementary and Alternative Medicines,* 3**,** 37-41.

Additives, E. P. O. F. & Food, N. S. A. T. 2014. Scientific Opinion on the re-evaluation of propyl gallate (E 310) as a food additive. *EFSA Journal,* 12**,** 3642.

Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I. & Kappo, A. P. 2015. Impact of human schistosomiasis in sub-Saharan Africa. *Brazilian Journal of Infectious Diseases,* 19**,** 196-205.

Adeoye-Isijola, M. O., Olajuyigbe, O. O., Jonathan, S. G. & Coopoosamy, R. M. 2018. Bioactive compounds in ethanol extract of *Lentinus squarrosulus* Mont-a Nigerian medicinal macrofungus. *African Journal of Traditional, Complementary and alternative medicines,* 15**,** 42-50.

Adhikari, P. P. & Paul, S. B. 2018. History of Indian traditional medicine: A medical inheritance. *History,* 11.

Ahmed, E., Babiker, O. & Abdalla, R. 2014. Molluscicidal activity of aqueous leave extract of *Solenostemma argel* (Del.) Hayne on *Biomphalaria pfeifferi* snails. *Journal of Basic and Applied Science Research,* 4**,** 179-184.

Ahmed, E., Bashir, A. & Khier, Y. 1985. Molluscicidal activity and chemical composition of *Gardenia lutea*. *Fitoterapia*.

Ahmed, N. F. M. 2016. Prevalence Rate of *Giardia lamblia*/*Helicobacter pylori* Co-infections in Khartoum State-Sudan. Sudan University of Science & Technology.

Ahmed, S. B. H., Sghaier, R., Guesmi, F., Kaabi, B., Mejri, M., Attia, H., Laouini, D. & Smaali, I. 2011. Evaluation of antileishmanial, cytotoxic and antioxidant activities of essential oils extracted from plants issued from the leishmaniasis-endemic region of Sned (Tunisia). *Natural product research,* 25**,** 1195-1201.

Ainsworth, E. A. & Gillespie, K. M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols,* 2**,** 875-877.

Ajila, C. & Rao, U. P. 2013. Mango peel dietary fibre: Composition and associated bound phenolics. *Journal of functional foods,* 5**,** 444-450.

Al-Marzoqi, A. H., Hameed, I. H. & Idan, S. A. 2015. Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). *African Journal of Biotechnology,* 14**,** 2812-2830.

Al-Sereiti, M., Abu-Amer, K. & Sena, P. 1999. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials.

Al Zuhairi, J. J. M. J., Kashi, F. J., Rahimi-Moghaddam, A. & Yazdani, M. 2020. Antioxidant, cytotoxic and antibacterial activity of *Rosmarinus officinalis* L. essential oil against bacteria isolated from urinary tract infection. *European Journal of Integrative Medicine,* 38**,** 101192.

Alabi, P., Oladejo, S. & Odaibo, A. 2018. Prevalence and intensity of urinary schistosomiasis in Ogun state, Southwest, Nigeria. *Journal of Public Health and Epidemiology,* 10**,** 413-417.

Alam, M. N., Bristi, N. J. & Rafiquzzaman, M. 2013. Review on *in-vivo* and *in-vitro* methods evaluation of antioxidant activity. *Saudi pharmaceutical journal,* 21**,** 143-152.

Alam, M. S. 2018. Analysis of Sequence Polymorphism of LINEs and SINEs in *Entamoeba histolytica*. *arXiv preprint arXiv:1809.03835*.

Alamin, M. A., Yagi, A. I. & Yagi, S. M. 2015. Evaluation of antidiabetic activity of plants used in Western Sudan. *Asian Pacific Journal of Tropical Biomedicine,* 5**,** 395-402.

Alebiosu, C. & Yusuf, A. 2015. Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of *Citrullus lanatus*. *Journal of Pharmaceutical, Chemical and Biological Sciences,* 3**,** 214-220.

Alexander, E. M., Aguiyi, J. C., Mdekera, I. W., Ogwu, O. S., Imoleayo, O. O., Ugokwe, C. V. & Pam, D. 2019. The climbing performance, neuromuscular transmitter (ACHE) activity, reproductive performance and survival of Drosophila melanogaster fed diet with *mangifera indica* cold aqueous leaf extract. *Journal of Applied Life Sciences International***,** 1-11.

Ali, H., König, G., Khalid, S., Wright, A. & Kaminsky, R. 2002. Evaluation of selected Sudanese medicinal plants for their *in vitro* activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory, and for cytotoxicity. *Journal of ethnopharmacology,* 83**,** 219-228.

Ali, R. K. M. 2016. Physical and chemical Characteristics of Three Mango Cultivars at Three Maturity Stages. Sudan University of Science and Technology.

Alilio, M. S., Bygbjerg, I. C. & Breman, J. G. 2004. Are multilateral malaria research and control programs the most successful? Lessons from the past 100 years in Africa. *The American journal of tropical medicine and hygiene,* 71**,** 268-278.

Almakhatreh, M., Hafez, E., Tousson, E. & Masoud, A. 2019. Biochemical and molecular studies on the role of rosemary (*Rosmarinus officinalis*) extract in reducing liver and kidney toxicity due to etoposide in male rats. *Asian journal of research in Medical and Pharmaceutical Sciences***,** 1-11.

Almeida, V., Goncalves, V., Galego, L., Miguel, G. & Costa, M. Volatile constituents of leaves and flowers of *Thymus mastichina* by Headspace Solid-Phase Microextraction. I International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation 723, 2006. 239-242.

Alnamer, R., Alaoui, K., Houcine Bouidida, E., Benjouad, A. & Cherrah, Y. 2011. Toxicity and psychotropic activity of essential oils of *Rosmarinus officinalis* and *Lavandula officinalis* from Morocco. *Journal of Biologically Active Products from Nature,* 1**,** 262-272.

Alsadeg, A. M., Koko, W. S., El-Badri, E. & Kabbashi, A. S. 2015. *In Vitro* Anthelminthic activity of the methanol stem bark extract of *Acacia senegal* against *Fasciola gigantica*. *International Invention Journal of Biochemistry and Bioinformatics,* 3**,** 18-22.

Aly, H. F. 2012. Challenges in Developing Vaccines in Parasitic Diseases with Special References to Schistosomiasis. *International Journal of Academic Research,* 4.

Amić, D., Davidović-Amić, D., Bešlo, D. & Trinajstić, N. 2003. Structure-radical scavenging activity relationships of flavonoids. *Croatica chemica acta,* 76**,** 55-61.

Amin, M. & Abubaker, H. 2017. Control of schistosomiasis in the Gezira irrigation scheme, Sudan. *Journal of biosocial science,* 49**,** 83.

Amin, M., Daffala, A. & El Moneim, O. 1972. Preliminary report on the molluscicidal properties of Habat El-Mollok, Jatropha sp. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 66**,** 805-806.

Anacarso, I., Sabia, C., De Niederhäusern, S., Iseppi, R., Condò, C., Bondi, M. & Messi, P. 2019. *In vitro* evaluation of the amoebicidal activity of rosemary (*Rosmarinus officinalis* L.) and cloves (*Syzygium aromaticum* L. Merr. & Perry) essential oils against *Acanthamoeba polyphaga* trophozoites. *Natural product research,* 33**,** 606-611.

Anandakumar, N. & Thajuddin, N. 2013. Diversity and biotechnological applications of microzooplankton from Gulf of Mannar, India.

Anandakumar, P., Kamaraj, S. & Vanitha, M. K. 2021. D-limonene: A multifunctional compound with potent therapeutic effects. *Journal of Food Biochemistry,* 45**,** e13566.

Ansari, J. & Inamdar, N. 2010. The promise of traditional medicines. *Int J Pharmacol,* 6**,** 808-812.

Antinozzi, D. I. 2020. Danse Macabre: The Black Death Through the Global Health Lens.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C. & Haridas, M. 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design,* 80**,** 434-439.

Aqyun, Q., Zein, A. & Meidianawaty, V. The comparison on antihyperglycemic activity between gedong gincu mango leaf (Mangifera indica var. gedong gincu) and metformin in streptozotocin-induced diabetic rats. Journal of Physics: Conference Series, 2019. IOP Publishing, 012010.

Arguelles-Arias, A., Ongena, M., Halimi, B., Lara, Y., Brans, A., Joris, B. & Fickers, P. 2009. *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microbial cell factories,* 8**,** 63.

Arguello-García, R., Cruz-Soto, M., Romero-Montoya, L. & Ortega-Pierres, G. 2004. Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitroimidazoles and benzimidazoles *in vitro*. *Journal of Antimicrobial Chemotherapy,* 54**,** 711-721.

Arogba, S. & Omede, A. 2012. Comparative antioxidant activity of processed mango (*Mangifera indica*) and bush mango (*Irvingia gabonensis*) kernels. *Nigerian Food Journal,* 30**,** 17-21.

Arora, N. & Pandey-Rai, S. 2014. GC–MS analysis of the essential oil of *Celastrus paniculatus* Willd. seeds and antioxidant, anti-inflammatory study of its various solvent extracts. *Industrial Crops and Products,* 61**,** 345-351.

Asghar, S. F. & Choudahry, M. 2011. Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of Iris germanica. *International Journal of Genetics and Molecular Biology,* 3**,** 95-100.

ASHBURN, T. T. & THOR, K. B. 2004. Drug repositioning: identifying and developing new uses for existing drugs. *Nature reviews Drug discovery,* 3**,** 673.

Ashok, V. G., Priya, S. B. & Pranita, A. G. 2014. Evaluation of antibacterial and phytochemical analysis of *Mangifera indica* bark extracts. *Int J Curr Microbiol App Sci,* 3**,** 567-580.

Ashworth, M. R. F. & Stahl, E. 2013. *Thin-layer chromatography: a laboratory handbook*, Springer Science & Business Media.

Astiti, N. P. A., Sudirga, S. K. & Ramona, Y. 2019. Analysis of Phenolic and Tannin Contents in the Methanol Extract of Sweet and Sour Star Fruit Plants (Averrhoa carambola L) Leaves Commonly

Used as Raw Materials of Lawar (A Balinese Traditional Food). *Advances in Tropical Biodiversity and Environmental Sciences,* 3**,** 5-7.

Augusto, O. & Miyamoto, S. 2011. Oxygen radicals and related species. *Principles of free radical biomedicine,* 1**,** 19-42.

Augustyniak, A., Bartosz, G., Čipak, A., Duburs, G., Horáková, L. U., Łuczaj, W., Majekova, M., Odysseos, A. D., Rackova, L. & Skrzydlewska, E. 2010. Natural and synthetic antioxidants: an updated overview. *Free radical research,* 44**,** 1216-1262.

Awadelkarim, S., Osama, A. & Fadul, E. 2017. Antioxidant Evaluation of Three Sudanese Medicinal Plants Used in Traditional Medicine. *World J Appl Chem,* 2**,** 77-9.

Ayala-Silva, T., Schnell, R. J., Meerow, A. W., Winterstein, M., Cervantes, C. & Brown, J. S. Determination of color and fruit traits of half-sib families of mango (*Mangifera indica* L.). Proc. Fla. State Hort. Soc, 2005. 253-257.

Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E. C. & Atangbayila, T. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research,* 7**,** 1019-1024.

Azeredo, C. M. O., Santos, T. G., De Noronha Sales, B. H. L. & Soares, M. J. 2014. *In vitro* biological evaluation of eight different essential oils against *Trypanosoma cruzi*, with emphasis on Cinnamomum verum essential oil. *BMC complementary and alternative medicine,* 14**,** 1-8.

Azim, M. K., Khan, I. A. & Zhang, Y. 2014. Characterization of mango (*Mangifera indica* L.) transcriptome and chloroplast genome. *Plant molecular biology,* 85**,** 193-208.

Azmir, J., Zaidul, I. S. M., Rahman, M., Sharif, K., Mohamed, A., Sahena, F., Jahurul, M., Ghafoor, K., Norulaini, N. & Omar, A. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of food engineering,* 117**,** 426-436.

Baba, S. A. & Malik, S. A. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science,* 9**,** 449-454.

Baer, H. A., Singer, M. & Susser, I. 2013. Medical Anthropology and the World System: Critical Perspectives*: Critical Perspectives*, Praeger.

Bahmani, M., Zargaran, A., Rafieian-Kopaei, M. & Saki, K. 2014. Ethnobotanical study of medicinal plants used in the management of *diabetes mellitus* in the Urmia, Northwest Iran. *Asian Pacific journal of tropical medicine,* 7**,** S348-S354.

Baj, T., Baryluk, A. & Sieniawska, E. 2018. Application of mixture design for optimum antioxidant activity of mixtures of essential oils from *Ocimum basilicum* L., *Origanum majorana* L. and *Rosmarinus officinalis* L. *Industrial Crops and Products,* 115**,** 52-61.

Bajalan, I., Rouzbahani, R., Pirbalouti, A. G. & Maggi, F. 2017. Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*. *Industrial crops and products,* 107**,** 305-311.

Bakheit, S. M. & Omer, A.-F. A. 2021. Antimicrobial Activity of *Boswellia papyrifera* Essential Oil against Clinical Bacterial Pathogens. *African Journal of Medical Sciences,* 6.

Balekar, N., Katkam, N. G., Nakpheng, T., Jehtae, K. & Srichana, T. 2012. Evaluation of the wound healing potential of *Wedelia trilobata* (L.) leaves. *Journal of Ethnopharmacology,* 141**,** 817-824.

Balouiri, M., Sadiki, M. & Ibnsouda, S. K. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis,* 6**,** 71-79.

Barbosa-Filho, J., Da-Cunha, E. V. L. & Gray, A. I. 2000. Alkaloids of the Menispermaceae.

Barbosa, E., Calzada, F. & Campos, R. 2007. *In vivo* antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. *Journal of Ethnopharmacology,* 109**,** 552-554.

Barry, A. L. 1976. *The antimicrobic susceptibility test: principles and practices*, Lippincott Williams & Wilkins.

Bartwal, A., Mall, R., Lohani, P., Guru, S. & Arora, S. 2013. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *Journal of plant growth regulation,* 32**,** 216-232.

Başgel, S. & Erdemoğlu, S. 2006. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Science of the total environment,* 359**,** 82-89.

Bashir, E. M. & El Shafie, H. A. 2013. Insecticidal and antifeedant efficacy of Jatropha oil extract against the desert locust, *Schistocerca gregaria* (Forskal)(Orthoptera: Acrididae). *Agricultural and Biological Journal of North America,* 4**,** 260-267.

Basu, S., Roychoudhury, U., Das, M. & Datta, G. 2013. Identification of bioactive components in ethanolic and aqueous extracts of *Amorphophallus campanulatus* tuber by GC-MS analysis. *International journal of Phytomedicine,* 5**,** 243.

Bbosa, G., Kyegombe, D., Ogwal-Okeng, J., Bukenya-Ziraba, R., Odyek, O. & Waako, P. 2007. Antibacterial activity of *Mangifera indica* (L.). *African Journal of Ecology,* 45**,** 13-16.

Begum, A., Sandhya, S., Ali, S. S., Vinod, K. R., Reddy, S. & Banji, D. 2013a. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta scientiarum polonorum. Technologia alimentaria,* 12.

Begum, A., Sandhya, S., Vinod, K. R., Reddy, S. & Banji, D. 2013b. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Scientiarum Polonorum Technologia Alimentaria,* 12.

Belakhdar, G., Benjouad, A. & Abdennebi, E. 2015. Determination of some bioactive chemical constituents from Thesium humile Vahl. *J Mater Environ Sci,* 6**,** 2778-2783.

Bendeddouche, M. S., Benhassaini, H., Hazem, Z. & Romane, A. 2011. Essential oil analysis and antibacterial activity of *Rosmarinus tournefortii* from Algeria. *Natural product communications,* 6**,** 1934578X1100601026.

Berdy, J. 2005. Bioactive microbial metabolites. *The Journal of antibiotics,* 58**,** 1.

Beretta, G., Artali, R., Facino, R. M. & Gelmini, F. 2011. An analytical and theoretical approach for the profiling of the antioxidant activity of essential oils: The case of *Rosmarinus officinalis* L. *Journal of pharmaceutical and biomedical analysis,* 55**,** 1255-1264.

Bernstein, N., Akram, M., Yaniv-Bachrach, Z. & Daniyal, M. 2020. Is it safe to consume traditional medicinal plants during pregnancy? *Phytotherapy Research*.

Beulah, G., Soris, P. T. & Mohan, V. 2018. GC-MS Determination of Bioactive Compounds of *Dendrophthoe falcata* (LF) Ettingsh: An Epiphytic Plant. *Int. J. Health Sci. Res,* 8**,** 261-269.

Bhatt, I. D., Rawat, S. & Rawal, R. S. 2013. Antioxidants in medicinal plants. *Biotechnology for medicinal plants.* Springer.

Bhattacharya, A., Tiwari, P., Sahu, P. K. & Kumar, S. 2018. A review of the phytochemical and pharmacological characteristics of *Moringa oleifera*. *Journal of pharmacy & bioallied sciences,* 10**,** 181.

Bhattacharya, S. 2015. Reactive oxygen species and cellular defense system. *Free radicals in human health and disease.* Springer.

Bhowal, M. & Gopal, M. 2015. Eucalyptol: Safety and pharmacological profile. *J. Pharm. Sci,* 5**,** 125-131.

Bills, G. F. & Gloer, J. B. 2017. Biologically active secondary metabolites from the fungi. *The Fungal Kingdom***,** 1087-1119.

Bladt, S. 2009. Plant Drug Analysis: A thin layer chromatography atlas, Springer Science & Business Media.

Bokhari, J., Khan, M. R., Shabbir, M., Rashid, U., Jan, S. & Zai, J. A. 2013. Evaluation of diverse antioxidant activities of *Galium aparine*. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 102**,** 24-29.

Bouaziz, A., Horchani, H., Salem, N. B., Chaari, A., Chaabouni, M., Gargouri, Y. & Sayari, A. 2010. Enzymatic propyl gallate synthesis in solvent-free system: optimization by response surface methodology. *Journal of Molecular Catalysis B: Enzymatic,* 67**,** 242-250.

Bouzekri, O. 2020. Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Rosmarinus Officinalis* L, cultivated in Fes-Meknes region. *RHAZES: Green and Applied Chemistry,* 8**,** 01-09.

Boy, H. I. A., Rutilla, A. J. H., Santos, K. A., Ty, A. M. T., Alicia, I. Y., Mahboob, T., Tangpoong, J. & Nissapatorn, V. 2018. Recommended medicinal plants as source of natural products: a review. *Digital Chinese Medicine,* 1**,** 131-142.

Bozin, B., Mimica-Dukic, N., Samojlik, I. & Jovin, E. 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. *Journal of agricultural and food chemistry,* 55**,** 7879-7885.

Brahmkshatriya, P. P. & Brahmkshatriya, P. S. 2013. Terpenes: Chemistry, biological role, and therapeutic applications. *Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes***,** 2665-2691.

Brand-Williams, W., Cuvelier, M.-E. & Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology,* 28**,** 25-30.

Burchfield, H. P. & Storrs, E. E. 1962. Biochemical applications of gas chromatography.

Cai, P., Weerakoon, K. G., Mu, Y., Olveda, D. U., Piao, X., Liu, S., Olveda, R. M., Chen, Q., Ross, A. G. & Mcmanus, D. P. 2017. A parallel comparison of antigen candidates for development of an optimized serological diagnosis of schistosomiasis japonica in the Philippines. *EBioMedicine,* 24**,** 237-246.

Caicedo, D., Díaz, O., Devesa, P. & Devesa, J. 2018. Growth hormone (GH) and cardiovascular system. *International journal of molecular sciences,* 19**,** 290.

Carocho, M., Barreiro, M. F., Morales, P. & Ferreira, I. C. 2014. Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive reviews in food science and food safety,* 13**,** 377-399.

Carrillo-Muñoz, A. J., Tur-Tur, C., Giusiano, G., Marcos-Arias, C., Eraso, E., Jauregizar, N. & Quindós, G. 2013. Sertaconazole: an antifungal agent for the topical treatment of superficial candidiasis. *Expert review of anti-infective therapy,* 11**,** 347-358.

Čechovská, L., CEJPEK, K., Konečný, M. & Velíšek, J. 2011. On the role of 2, 3-dihydro-3, 5-dihydroxy-6-methyl-(4 H)-pyran-4-one in antioxidant capacity of prunes. *European Food Research and Technology,* 233**,** 367-376.

Cedillo-Rivera, R., Chávez, B., González-Robles, A., Tapia, A. & Yepez-Mulia, L. 2002. *In vitro* effect of nitazoxanide against *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* trophozoites. *Journal of Eukaryotic Microbiology,* 49**,** 201-208.

Centeno, S., Calvo, M., Adelantado, C. & Figueroa, S. 2010. Antifungal activity of extracts of *Rosmarinus officinalis* and *Thymus vulgaris* against *Aspergillus flavus* and A. ochraceus. *Pakistan journal of biological sciences: PJBS,* 13**,** 452-455.

Ceylan, O., Uğur, A., Sarac, N., Ozcan, F. & Baygar, T. 2014. The *in vitro* antibiofilm activity of *Rosmarinus officinalis* L. essential oil against multiple antibiotic resistant Pseudomonas sp. and Staphylococcus sp. *J. Food. Agric. Environ,* 12**,** 82-86.

Chang, S.-T. & Wasser, S. P. 2012. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International journal of medicinal mushrooms,* 14.

Chang, S. K., Alasalvar, C. & Shahidi, F. 2019. Superfruits: Phytochemicals, antioxidant efficacies, and health effects–A comprehensive review. *Critical reviews in food science and nutrition,* 59**,** 1580-1604.

Chao, J., Dai, Y., Verpoorte, R., Lam, W., Cheng, Y.-C., Pao, L.-H., Zhang, W. & Chen, S. 2017. Major achievements of evidence-based traditional Chinese medicine in treating major diseases. *Biochemical pharmacology,* 139**,** 94-104.

Chao, W.-W. & Lin, B.-F. 2010. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chinese medicine,* 5**,** 1-15.

Chattopadhyay, D., Ojha, D., Mondal, S. & Goswami, D. 2015. Validation of antiviral potential of herbal ethnomedicine. *Evidence-Based Validation of Herbal Medicine.* Elsevier.

Chaturvedula, V. S. P. & Prakash, I. 2012. Isolation of Stigmasterol and?-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal,* 1**,** 239-242.

Chavan, S. S., Jadhav, R. S., Khemnar, K. S. & Tambe, V. B. 2015. Evaluation of antibacterial activity and phytochemical screening of *Medicago sativa* leaves. *Int J Curr Res Acad Rev,* 3**,** 308-13.

Chi, S., She, G., Han, D., Wang, W., Liu, Z. & Liu, B. 2016. Genus Tinospora: ethnopharmacology, phytochemistry, and pharmacology. *Evidence-Based Complementary and Alternative Medicine,* 2016.

Chinou, I. 2008. Primary and secondary metabolites and their biological activity. *Thin layer chromatography in phytochemistry.* CRC Press.

Chintamunnee, V. & Mahomoodally, M. F. 2012. Herbal medicine commonly used against non-communicable diseases in the tropical island of Mauritius. *Journal of Herbal Medicine,* 2**,** 113-125.

Choi, I. H. & Moore, P. A., Jr. 2008. Effects of liquid aluminum chloride additions to poultry litter on broiler performance, ammonia emissions, soluble phosphorus, total volatile Fatty acids, and nitrogen contents of litter. *Poult Sci,* 87**,** 1955-63.

Chokshi, A., Sifri, Z., Cennimo, D. & Horng, H. 2019. Global contributors to antibiotic resistance. *Journal of global infectious diseases,* 11**,** 36.

Chung, J. H., Choi, S. Y., Kim, J. Y., Kim, D. H., Lee, J. W., Choi, J. S. & Chung, H. Y. 2007. 3-Methyl-1, 2-cyclopentanedione down-regulates age-related NF-κB signaling cascade. *Journal of agricultural and food chemistry,* 55**,** 6787-6792.

Coelho, E. M., De Souza, M. E. A. O., Correa, L. C., Viana, A. C., De Azevedo, L. C. & Dos Santos Lima, M. 2019. Bioactive compounds and antioxidant activity of mango peel liqueurs (*Mangifera indica* L.) produced by different methods of maceration. *Antioxidants,* 8**,** 102.

Cömert, E. D. & Gökmen, V. 2017. Antioxidants bound to an insoluble food matrix: Their analysis, regeneration behavior, and physiological importance. *Comprehensive Reviews in Food Science and Food Safety,* 16**,** 382-399.

Cong, Y. 1998. Biosystématique des mollusques d'eau douce d'intérêt médical et vétérinaire de Cuba. Paris, EPHE.

Cooper, R. & Deakin, J. J. 2016. *Botanical miracles: chemistry of plants that changed the world*, CRC Press.

Cordell, G. A. & Colvard, M. D. 2012. Natural products and traditional medicine: turning on a paradigm. *Journal of natural products,* 75**,** 514-525.

Corradini, E., Foglia, P., Giansanti, P., Gubbiotti, R., Samperi, R. & Lagana, A. 2011. Flavonoids: chemical properties and analytical methodologies of identification and quantitation in foods and plants. *Natural product research,* 25**,** 469-495.

Cos, P., Vlietinck, A. J., Berghe, D. V. & Maes, L. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *Journal of ethnopharmacology,* 106**,** 290-302.

Costain, A. H., Macdonald, A. S. & Smits, H. H. 2018. Schistosome egg migration: mechanisms, pathogenesis and host immune responses. *Frontiers in Immunology,* 9**,** 3042.

Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical microbiology reviews,* 12**,** 564-582.

Cox, S., Mann, C. & Markham, J. 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. *Journal of applied microbiology,* 91**,** 492-497.

Cragg, G. M. & Newman, D. J. 2013. Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects,* 1830**,** 3670-3695.

Cruickshank, R. 1975. Medical Microbiology: Volume 2: The Practice of Medical Microbiology, Churchill Livingstone.

Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A. & Brielmann, H. L. 2016. *Natural products from plants,* CRC press.

Cushnie, T. T. & Lamb, A. J. 2005. Antimicrobial activity of flavonoids. *International journal of antimicrobial agents,* 26**,** 343-356.

Da Paixão Siqueira, L., Fontes, D. A. F., Aguilera, C. S. B., Timóteo, T. R. R., Ângelos, M. A., Silva, L. C. P. B. B., De Melo, C. G., Rolim, L. A., Da Silva, R. M. F. & Neto, P. J. R. 2017. Schistosomiasis: Drugs used and treatment strategies. *Acta tropica,* 176**,** 179-187.

Da Silva Bomfim, N., Kohiyama, C. Y., Nakasugi, L. P., Nerilo, S. B., Mossini, S. A. G., Romoli, J. C. Z., Graton Mikcha, J. M., Abreu Filho, B. A. D. & Machinski Jr, M. 2020. Antifungal and antiaflatoxigenic activity of rosemary essential oil (*Rosmarinus officinalis* L.) against *Aspergillus flavus*. *Food Additives & Contaminants: Part A,* 37**,** 153-161.

Dagil, R., O'shea, C., Nykjær, A., Bonvin, A. M. & Kragelund, B. B. 2013. Gentamicin binds to the megalin receptor as a competitive inhibitor using the common ligand binding motif of complement type repeats: insight from the nmr structure of the 10^{th} complement type repeat domain alone and in complex with gentamicin. *Journal of Biological Chemistry,* 288**,** 4424-4435.

Dahab, A. 2015. Effect of *Syzygium aromaticum* and *Rosmarinus officinalis* extracts on *Giardia lamblia* and *Streptococcus agalactiae*. *Egyptian Veterinary Medical Society of Parasitology Journal.,* 11**,** 67-75.

Dahab, M., Koko, W. & Osman, E. 2011. *In vitro* antitrichomonal activity of *Xanthium brasilicum* vell and Argemone mexicana L different extracts. *Journal of medicinal plants research,* 5**,** 151-155.

Dahab, M. M., Koko, W. S. & Osman, E. A. 2010. *In vitro* Antitrichomonal Activity of *Acacia nilotica* L different extracts. *International journal of natural product and pharmaceutical sciences,* 1**,** 10-14.

Dai, J. & Mumper, R. J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,* 15**,** 7313-7352.

Daniel, I. E. & Ibok, E. N. 2019. Toxicological Evaluation of Different Extracts of *Andrographis paniculata*. *South Asian Research Journal of Natural Products***,** 1-10.

Dar, M. S., Oak, P., Chidley, H., Deshpande, A., Giri, A. & Gupta, V. 2016. Nutrient and flavor content of mango (*Mangifera indica* L.) cultivars: an appurtenance to the list of staple foods. *Nutritional composition of fruit cultivars.* Elsevier.

Das, K., Tiwari, R. & Shrivastava, D. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of medicinal plants research,* 4**,** 104-111.

Dasrao, P., Pradeep, H. & Anil, B. 2014. *In-vitro* anthelminetic activity of methanoilic extract of *Mangifera indica* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS),* 3**,** 771-776.

David, B., Wolfender, J.-L. & Dias, D. A. 2015. The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews,* 14**,** 299-315.

Davies, P. J. 2010. The plant hormones: their nature, occurrence, and functions. *Plant hormones.* Springer.

De Beer, J. & Whitlock, E. 2009. Indigenous knowledge in the Life Sciences classroom: put on your De Bono hats! *The American Biology Teacher,* 71**,** 209-217.

De Macedo, L. M., Santos, É. M. D., Militão, L., Tundisi, L. L., Ataide, J. A., Souto, E. B. & Mazzola, P. G. 2020. Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) and its topical applications: a review. *Plants,* 9**,** 651.

De Moraes, J., Nascimento, C., Lopes, P. O., Nakano, E., Yamaguchi, L. F., Kato, M. J. & Kawano, T. 2011. *Schistosoma mansoni*: *in vitro* schistosomicidal activity of piplartine. *Experimental parasitology,* 127**,** 357-364.

De Oliveira Ramalho, T. R., De Oliveira, M. T. P., De Araujo Lima, A. L., Bezerra-Santos, C. R. & Piuvezam, M. R. 2015. Gamma-terpinene modulates acute inflammatory response in mice. *Planta Medica,* 81**,** 1248-1254.

De Oliveira, W. F., Dos Santos Silva, P. M., Breitenbach Barroso Coelho, L. C. & Dos Santos Correia, M. T. 2018. Plant Antioxidants and Mechanisms of Action. *Letters in Drug Design & Discovery,* 15**,** 1103-1115.

De, P. K. & Pal, A. 2014. Effects of aqueous young leaves extract of Mangifera indica on Gram negative microorganisms causing gastro-intestinal disorders. *Asian J of Plant Sci Res,* 4**,** 23-7.

De Vijlder, T., Valkenborg, D., Lemiere, F., Romijn, E. P., Laukens, K. & Cuyckens, F. 2018. A tutorial in small molecule identification via electrospray ionization-mass spectrometry: The practical art of structural elucidation. *Mass spectrometry reviews,* 37**,** 607-629.

De Waele, J. J., Akova, M., Antonelli, M., Canton, R., Carlet, J., De Backer, D., Dimopoulos, G., Garnacho-Montero, J., Kesecioglu, J. & Lipman, J. 2018. Antimicrobial resistance and antibiotic stewardship programs in the ICU: insistence and persistence in the fight against resistance. A position statement from ESICM/ESCMID/WAAAR round table on multi-drug resistance. *Intensive care medicine,* 44**,** 189-196.

De Wet, H., Van Heerden, F. R. & Van Wyk, B.-E. 2004. Alkaloids of *Antizoma angustifolia* (Menispermaceae). *Biochemical systematics and ecology,* 32**,** 1145-1152.

De Wet, H., Van Heerden, F. R. & Van Wyk, B.-E. 2005. Alkaloids of *Antizoma miersiana* (Menispermaceae). *Biochemical systematics and ecology,* 33**,** 799-807.

De Wet, H. & Van Wyk, B.-E. 2008. An ethnobotanical survey of southern African Menispermaceae. *South African Journal of Botany,* 74**,** 2-9.

Degirmen, N. 2010. From Traditional To Modern; Thyme Process In Medical History. *Full Text Proceedings Book***,** 9.

Deressa, T., Lemessa, F. & Wakjira, M. 2015. Antifungal activity of some invasive alien plant leaf extracts against mango (Mangifera indica) anthracnose caused by Colletotrichum gloeosporioides. *International Journal of Pest Management,* 61**,** 99-105.

Dhama, K., Sachan, S., Khandia, R., Munjal, A., Mn Iqbal, H., K Latheef, S., Karthik, K., A Samad, H., Tiwari, R. & Dadar, M. 2016. Medicinal and beneficial health applications of *Tinospora cordifolia*

(Guduchi): a miraculous herb countering various diseases/disorders and its Immunomodulatory effects. *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery,* 10**,** 96-111.

Dhanavade, M. J., Jalkute, C. B., Ghosh, J. S. & Sonawane, K. D. 2011. Study antimicrobial activity of lemon (*Citrus lemon* L.) peel extract. *British Journal of pharmacology and Toxicology,* 2**,** 119-122.

Diánez, F., Santos, M., Parra, C., Navarro, M., Blanco, R. & Gea, F. 2018. Screening of antifungal activity of 12 essential oils against eight pathogenic fungi of vegetables and mushroom. *Letters in applied microbiology,* 67**,** 400-410.

Divya, K. 2015. Ethno-Medicinal Plants used in East Godavari District, Andhra Pradesh, India. *IJPR,* 5**,** 293.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. & Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food chemistry,* 97**,** 654-660.

Domis, M. & Oyen, L. 2008. Piper umbellatum L.[Internet] Record from Protabase. Schmelzer, GH & Gurib-Fakim, A.(Editors). PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. *database prota. org/recherche. htm*.

Dorta, E., González, M., Lobo, M. & Laich, F. 2016. Antifungal activity of mango peel and seed extracts against clinically pathogenic and food spoilage yeasts. *Natural product research,* 30**,** 2598-2604.

Dorta, E., González, M., Lobo, M. G., Sánchez-Moreno, C. & De Ancos, B. 2014. Screening of phenolic compounds in by-product extracts from mangoes (*Mangifera indica* L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. *Food Research International,* 57**,** 51-60.

Doughari, J. H. 2012. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. *Phytochemicals-A global perspective of their role in nutrition and health,* 3**,** 1-27.

Drews, J. 2000. Drug discovery: a historical perspective. *Science,* 287**,** 1960-1964.

Dudley, N., Bhagwat, S., Higgins-Zogib, L., Lassen, B., Verschuuren, B. & Wild, R. 2010. Conservation of biodiversity in sacred natural sites in Asia and Africa: a review of the scientific literature. *Sacred natural sites: Conserving nature and culture***,** 19-32.

Duke, J. & Bogenschutz, M. J. 1994. *Dr. Duke's phytochemical and ethnobotanical databases,* USDA, Agricultural Research Service.

Dwivedi, M. K. & Singh, P. K. 2020. Anti-Malarial Drug Resistance: Need for Novel Natural Products. *Advanced Pharmacological Uses of Medicinal Plants and Natural Products.* IGI Global.

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M.-L. & Jouad, H. 2002. Ethnopharmacological survey of medicinal plants used for the treatment of *diabetes mellitus*, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). *Journal of ethnopharmacology,* 82**,** 97-103.

Ediriweera, M. K., Tennekoon, K. H. & Samarakoon, S. R. 2017. A review on ethnopharmacological applications, pharmacological activities, and bioactive compounds of *Mangifera indica* (Mango). *Evidence-Based Complementary and Alternative Medicine,* 2017.

Eisa, A. I., Elfaki, T. E. M., Abdellah, M. A. & Mohammed, A. 2019. Molluscicidal Activity of *Khaya Grandifoliola* Extracts Against Freshwater Snails in Khartoum State-Sudan. *apex,* 3**,** 29-31.

EL-GHORAB, A. H. 2003. Supercritical fluid extraction of the Egyptian rosemary (Rosmarinus officinalis) leaves and *Nigella sativa* L. seeds volatile oils and their antioxidant activities. *Journal of Essential Oil Bearing Plants,* 6**,** 67-77.

El-Gied, A. A. A., Joseph, M. R., Mahmoud, I. M., Abdelkareem, A. M., Al Hakami, A. M. & Hamid, M. E. 2012. Antimicrobial activities of seed extracts of mango (*Mangifera indica* L.).

El-Kamali, H. H. & El-Amir, M. Y. 2010. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. *Current Research Journal of Biological Sciences,* 2**,** 143-146.

El-Kamali, H. H., El-Nour, R. O. & Khalid, S. A. 2010. Molluscicidal activity of the essential oils of *Cymbopogon nervatus* leaves and *Boswellia papyrifera* resins. *Current Research Journal of Biological Sciences,* 2**,** 139-142.

El-Mahmood, A., Doughari, J. & Ladan, N. 2008. Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology,* 2**,** 089-094.

El-Sherbini, G. T. & Osman, S. M. 2013. Anthelmintic activity of unripe *Mangifera indica* L.(Mango) against *Strongyloides stercoralis*. *International Journal of Current Microbiology and Applied Sciences,* 2**,** 401-409.

El-Tahir, A., Satti, G. M. & Khalid, S. A. 1999. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Acacia nilotica*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,* 13**,** 474-478.

El Ghazali, G., Abdalla, W., Khalid, H., Khalafalla, M. & Hamad, A. 2003. Medicinal plants of the Sudan part V. Medicinal plants of Ingassana area. *Ministry of Science and Technology National Centre for Research Medicinal and Aromatic Plants Research Institute*.

El Kheir, Y. & El Tohami, M. 1979. Investigation of molluscicidal activity of certain Sudanese plants used in folk-medicine. II. Molluscicidal activity of the different morphological organs of *Gnidia kraussiana* Meisn" abu gotna" family Thymelaeaceae. *The Journal of Tropical Medicine and Hygiene,* 82**,** 242-247.

Elaiyaraja, A. & Chandramohan, G. 2016. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. *Journal of pharmacognosy and phytochemistry,* 5**,** 158.

Elbadr, A., Raouf, U., Abo-Dahab, N. & Badr, S. 2013a. Antiprotozoal activity of *Rosmarinus officinalis* and *Ocimum basilicum* against *Giardia lamblia*. *Egyptian Veterinary Medical Society of Parasitology Journal.,* 9**,** 21-29.

Elbadr, A., Raouf, U. A., Abo-Dahab, N. & Badr, S. G. 2013b. Antiprotozoal activity of *Rosmarinus officinalis* and *Ocimum basilicum* against *Giardia lamblia*. *EVMSPJ,* 9**,** 21-29.

Elbashir, I. B. E. 2016. *Phytochemical Study and Biological Activity Evaluation of Fifteen Plants Indigenous to Sudan.* University of Khartoum.

Elegami, A. A., Almagboul, A. Z., Omer, M. E. A. & El Tohami, M. S. 2001. Sudanese plants used in folkloric medicine: screening for antibacterial activity. Part X. *Fitoterapia,* 72**,** 810-817.

Elhadi, I. M., Koko, W. S., Dahab, M. M., El Imam, Y. M. & El Mageed, M. A. E. A. 2013. Antigiardial activity of some Cucurbita species and *Lagenaria siceraria*. *laboratory animals,* 3**,** 8.

Ellison, S. 2009. The role of phenoxymethylpenicillin, amoxicillin, metronidazole and clindamycin in the management of acute dentoalveolar abscesses–a review. *British dental journal,* 206**,** 357.

Elnour, M., Elegamy, A., Koko, W., Khalid, A. & Fadul, E. 2013. Antioxidant Activity and Cytotoxicity of some Sudanese Medicinal Plants. *Inter J Adv Indust Eng,* 1**,** 20-3.

Elnour, M., Penech, F. & Mesaik, M. 2017. Four selected Sudanese medicinal plants induce anticancer and cytotoxic effects in prostate cancer cell line. *Clinic. Med. Biochem,* 3**,** 2471-2663.1000134.

Elnour, M. A., Khalid, A. & Penech, F. 2018. Selected Sudanese Medicinal Plants Induce Anticancer and Cytotoxic Effects in Cervical Cancer Cell Line. *Significance Bioeng. Biosci,* 2**,** 147-151.

Eloff, J., Masoko, P. & Picard, J. 2007. Resistance of animal fungal pathogens to solvents used in bioassays. *South African Journal of Botany,* 73**,** 667-669.

Elrofaei, N., Elsharif, K., Elshikh, A., Bashir, M. & Ahmed, I. 2018. Studies on Antibacterial Activity of some Medicinal Plants against Selected Bacterial Strain. *J Antimicrob Agents,* 4**,** 2472-1212.1000170.

Eltayeb, A. & Ibrahim, K. 2012. Potential antileishmanial effect of three medicinal plants. *Indian journal of pharmaceutical sciences,* 74**,** 171.

Emam, M. A. 2012. Comparative evaluation of antidiabetic activity of *Rosmarinus officinalis* L. and *Chamomile recutita* in streptozotocin induced diabetic rats. *Agric. Biol. JN Am,* 3**,** 247-252.

Engwa, G. A. 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. *Phytochemicals: source of antioxidants and role in disease prevention***,** 49-73.

Esteghamati, A., Khanaliha, K., Bokharaei-Salim, F., Sayyahfar, S. & Ghaderipour, M. 2019. Prevalence of intestinal parasitic infection in cancer, organ transplant and primary immunodeficiency patients in Tehran, Iran. *Asian Pacific journal of cancer prevention: APJCP,* 20**,** 495.

Etuh, M. A., Aguiyi, J. C., Ochala, S. O., Simeon, O., Oyeniran, O. I., Oshibanjo, O. D. & Pam, D. 2019. The In vivo antioxidant protective activity of *Mangifera indica* cold aqueous leaf extract in Drosophila melanogaster.

Evans, W. C. 2009a. Trease and evans' pharmacognosy E-book, Elsevier Health Sciences.

Evans, W. C. 2009b. Trease and Evans Pharmacognosy, *International Edition E-Book*, Elsevier Health Sciences.

Eyong, C. T. 2007. Indigenous knowledge and sustainable development in Africa: Case study on Central Africa. *Indigenous knowledge systems and development: Relevance for Africa***,** 121-139.

Farah, H. M., El Hussein, A. R. M., El Amin, T. H. & Khalid, H. E. S. 2013. *In vitro* effect of aqueous extract of *Tinospora bakis* roots on *Theileria lestoquardi*. *Top-class Journal of Herbal Medicine,* 2**,** 1-6.

Farah, H. M., Khalid, H. E., El Hussein, A. M. & Osman, H. M. 2018. Toxic Activity of *Tinospora bakis* (Irg al-hagar) Roots in Wistar Albino Rats. *European Journal of Medicinal Plants***,** 1-9.

Faria, C. P., Zanini, G. M., Dias, G. S., Da Silva, S., De Freitas, M. B., Almendra, R., Santana, P. & Do Ceu Sousa, M. 2017. Geospatial distribution of intestinal parasitic infections in Rio de Janeiro (Brazil) and its association with social determinants. *PLoS neglected tropical diseases,* 11**,** e0005445.

Fatemeh, D., Mitra, M., Homa, A. & Somayyeh, K.-M. 2021. Antioxidant and anticholinergic effects of rosemary extract: a double-blind randomized controlled trial. *Advances in Integrative Medicine*.

Fawole, O., Amoo, S., Ndhlala, A., Light, M., Finnie, J. & Van Staden, J. 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. *Journal of Ethnopharmacology,* 127**,** 235-241.

Felsociova, S., Kacaniova, M., Horská, E., Vukovic, N., Hleba, L., Petrová, J., Rovná, K., Stricik, M. & Hajduová, Z. 2015. Antifungal activity of essential oils against selected terverticillate penicillia. *Annals of agricultural and Environmental Medicine,* 22.

Fernández-Ponce, M. T., Casas, L., Mantell, C., Rodríguez, M. & De La Ossa, E. M. 2012. Extraction of antioxidant compounds from different varieties of *Mangifera indica* leaves using green technologies. *The Journal of Supercritical Fluids,* 72**,** 168-175.

Fiedor, J. & Burda, K. 2014. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients,* 6**,** 466-488.

Finkelstein, J. L., Schleinitz, M. D., Carabin, H. & Mcgarvey, S. T. 2008. Decision-model estimation of the age-specific disability weight for schistosomiasis japonica: a systematic review of the literature. *PLoS neglected tropical diseases,* 2**,** e158.

Fitriasih, F., Komariyah, S. M., Sandra, F., Pratiwi, N. & Hidayati, D. N. 2019. *Mangifera indica* L. Leaves Extract Induced Intrinsic Apoptotic Pathway in MCF-7 Cells by Decreasing Bcl-2 Expression and Inducing Bax Expression. *Indonesian Journal of Cancer Chemoprevention,* 10**,** 1-6.

Fletcher, S. M., Stark, D., Harkness, J. & Ellis, J. 2012. Enteric protozoa in the developed world: a public health perspective. *Clinical microbiology reviews,* 25**,** 420-449.

Folashade, O., Omoregie, H. & Ochogu, P. 2012. Standardization of herbal medicines-A review. *International Journal of Biodiversity and Conservation,* 4**,** 101-112.

Fontenelle, R. O. D. S., Sobrinho, A. C. N., Soares, B. V., Aguiar, F. L. L. D., Brito, E. H. S. D., Cavalcante, C. S. D. P., Rocha, M. F. G. & Morais, S. M. D. 2017. Efeito dos óleos essenciais de variedades de "*Mangifera indica*" L. na susceptibilidade antifúngica de cepas de Candida spp. isoladas de cães. *Revista Brasileira de Saúde e Produção Animal,* 18**,** 337-346.

Foote, M. J. 2008. *Recovery of Vegetation and Fuel Load Following a 1990 Fire in the Kanuti National Wildlife Refuge, Alaska: A Survey, 1991-1999,* Kanuti National Wildlife Refuge.

Fowler, P., Smith, K., Young, J., Jeffrey, L., Kirkland, D., Pfuhler, S. & Carmichael, P. 2012. Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. I. Choice of cell type. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 742**,** 11-25.

Friedrich, M. J. 2017. Depression is the leading cause of disability around the world. *Jama,* 317**,** 1517-1517.

Fuhrman, B., Volkova, N., Rosenblat, M. & Aviram, M. 2000. Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. *Antioxidants and Redox Signaling,* 2**,** 491-506.

Fuller, D. Q. 2011. Pathways to Asian civilizations: tracing the origins and spread of rice and rice cultures. *Rice,* 4**,** 78-92.

Gadir, S. A. 2012. Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *J Chem Pharm Res,* 4**,** 5145-4148.

Galli, C. & Calder, P. C. 2009. Effects of fat and fatty acid intake on inflammatory and immune responses. *Annals of nutrition & metabolism,* 55**,** 123-139.

Gami, B. & Parabia, M. 2010. Pharmacognostic evaluation of bark and seeds of Mimusops elengi L. *Int J Pharm Pharm Sci,* 2**,** 110-3.

Ganesan, A. 2008. The impact of natural products upon modern drug discovery. *Current opinion in chemical biology,* 12**,** 306-317.

Ganogpichayagrai, A., Palanuvej, C. & Ruangrungsi, N. 2017. Antidiabetic and anticancer activities of *Mangifera indica* cv. Okrong leaves. *Journal of advanced pharmaceutical technology & research,* 8**,** 19.

Garbi, M., Kabbashi, A. & El-Badri Elamin, O. 2018. *In Vitro* gardicidal and amoebicidal activity of Anogeissus leicarpus leaves extracts. *Horticult Int J,* 2**,** 232-235.

Garbi, M. I., Osman, E. E., Dahab, M. M., Koko, W. S., Kabbashi, A. S., Elegami, A. A., Hamed, S. Y. J. M. R. J. O. B. & Bioinformatics 2014. Antigiardial, Amoebicidal and Cytotoxic activity of the plant *Prosopis juliflora* leave extracts. 2**,** 1-7.

Gargouri, M., Hamed, H., Akrouti, A., Christian, M., Ksouri, R. & El Feki, A. 2017. Immunomodulatory and antioxidant protective effect of Sarcocornia perennis L.(swampfire) in lead intoxicated rat. *Toxicology mechanisms and methods,* 27**,** 697-706.

Gaspard, J., Usey, M. M., Fredericks-James, M., Sanchez-Martin, M. J., Atkins, L., Campbell Jr, C. H., Corstjens, P. L., Van Dam, G. J., Colley, D. G. & Secor, W. E. 2020. Survey of schistosomiasis in Saint Lucia: evidence for interruption of transmission. *The American Journal of Tropical Medicine and Hygiene,* 102**,** 827-831.

Gawali, P. & Jadhav, B. 2011. Antioxidant activity and antioxidant phytochemical analysis of mangrove species *Sonneratia alba* and *Bruguiera cylindrica*. *Asian J. Microbiol. Biotechnol. Environ. Sci,* 13**,** 257-261.

Geetha, S., Ram, M. S., Mongia, S., Singh, V., Ilavazhagan, G. & Sawhney, R. 2003. Evaluation of antioxidant activity of leaf extract of Seabuckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. *Journal of Ethnopharmacology,* 87**,** 247-251.

Gershenzon, J. & Dudareva, N. 2007. The function of terpene natural products in the natural world. *Nature chemical biology,* 3**,** 408.

Geta, K. 2019. Factors, impacts and possible solutions of antibiotic resistance. *World Scientific News,* 138**,** 225-247.

Gillen, A. L. & Augusta, M. 2018. The Coliform Kind: *E. coli* and Its "Cousins" The Good, the Bad, and the Deadly.

Ginwala, R., Bhavsar, R., Chigbu, D. G. I., Jain, P. & Khan, Z. K. 2019. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants,* 8**,** 35.

Giovanelli, F., Mattellini, M., Fichi, G., Flamini, G. & Perrucci, S. 2018. *In vitro* anthelmintic activity of four plant-derived compounds against sheep gastrointestinal nematodes. *Veterinary sciences,* 5**,** 78.

Göksungur, Y., GUVENC, U. J. G. T. J. O. F. & 6, C. S. 2002. Cell Immobilization in Calcium Alginate and Biotechnological Applications (Turkish with English Abstract).

González-MINERO, F. J., BRAVO-Díaz, L. & AYALA-Gómez, A. 2020. *Rosmarinus officinalis* L.(Rosemary): An Ancient Plant with Uses in Personal Healthcare and Cosmetics. *Cosmetics,* 7**,** 77.

Goodman, L., Gilman, A. & Gilman, A. 1980. Antiproliferative agents and immunosuppressive drugs. The Pharmacological basis of therapeutics. Macmillan Publishing Co., Inc., USA.

Goswami, H. K. & Ram, H. K. 2017. Ancient food habits dictate that food can be medicine but medicine cannot be "food"!! *Medicines,* 4**,** 82.

Goudie, A. S. 2018. *Human impact on the natural environment*, John Wiley & Sons.

Gouveia, M. J., Brindley, P. J., Gärtner, F., Costa, J. M. & Vale, N. 2018. Drug repurposing for schistosomiasis: combinations of drugs or biomolecules. *Pharmaceuticals,* 11**,** 15.

Guanghou, S. 2004. Characterization of effective antioxidant components of tropical fruit and vegetable species.

Gulcin, İ., Huyut, Z., Elmastaş, M. & Aboul-Enein, H. Y. 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry,* 3**,** 43-53.

Gulfraz, M., Sadiq, A., Tariq, H., Imran, M., Qureshi, R. & Zeenat, A. 2011. Phytochemical analysis and antibacterial activity of *Eruca sativa* seed. *Pak. J. Bot,* 43**,** 1351-1359.

Habtemariam, S. 2019. Natural products in Alzheimer's disease therapy: would old therapeutic approaches fix the broken promise of modern medicines? *Molecules,* 24**,** 1519.

Hagr, T. E., Adam, I. A., Almain, A. A. & Mohammed, M. M. 2019. Phytochemical Screening, GC-MS Analysis, Antibacterial and Antioxidant Activity of Seeds Oil of *Annona Squmosa* L. Sudanese Medicinal Plant. *Journal of Pharmacy and Pharmacology,* 7**,** 1-6.

Hahn-Deinstrop, E. 2007. Applied thin-layer chromatography: best practice and avoidance of mistakes, John Wiley & Sons.

Halliwell, B. 2011. Free radicals and antioxidants–quo vadis? *Trends in pharmacological sciences,* 32**,** 125-130.

Halliwell, B. & Whiteman, M. 2004. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *British journal of pharmacology,* 142**,** 231-255.

Hamadnalla, H. M. 2020. Anti-Microbial and Anti-Oxidant Activities of *Ziziphus Spina Christi*, Sudanese Medicinal Plant. *Journal of Biotechnology & Bioinformatics Research. SRC/JBBR-108***,** 3.

Hamadnalla, H. M. & Jack, M. 2019. Phytochemical Screening and Antibacterial Activity of *Solenostemma argel*: A Medicinal Plant. *Acta Scientific Agriculture,* 3**,** 2-4.

Hamidi, M., Jovanova, B. & Panovska, T. 2014. Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. *Maced pharm bull,* 60**,** 9-18.

Han, Y. H., Moon, H. J., You, B. R., Kim, S. Z., Kim, S. H. & Park, W. H. 2010. Propyl gallate inhibits the growth of HeLa cells via caspase-dependent apoptosis as well as a G1 phase arrest of the cell cycle. *Oncology reports,* 23**,** 1153-1158.

Handa, S. 2008. An overview of extraction techniques for medicinal and aromatic plants. *Extraction technologies for medicinal and aromatic plants,* 1.

Harada, H., Yamashita, U., Kurihara, H., Fukushi, E., Kawabata, J. & Kamei, Y. 2002. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer research,* 22**,** 2587-2590.

Harbone, B. 1984. Phytochemical methods. 2nd. *New York, Champan Hall,* 4**,** 4-7.

Harborne, A. 1998. *Phytochemical methods a guide to modern techniques of plant analysis*, springer science & business media.

Harborne, J. 1984. Methods of plant analysis. *Phytochemical methods.* Springer.

Hart, P., Brand, C., Carson, C., Riley, T., Prager, R. & Finlay-Jones, J. 2000. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research,* 49**,** 619-626.

Hassan, A., Abutalib, A., Almagboul, A. & Kabbashi, A. 2017. Antimicrobial activity of the rhizome essential oil of Zingiber officinale Roscoe. *Advancement in Medicinal Plant Research,* 5**,** 5-10.

Hassan, L. E. A., Ahamed, M. B. K., Majid, A. S. A., Baharetha, H. M., Muslim, N. S., Nassar, Z. D. & Majid, A. M. A. 2014. Correlation of antiangiogenic, antioxidant and cytotoxic activities of some Sudanese medicinal plants with phenolic and flavonoid contents. *BMC complementary and alternative medicine,* 14**,** 1-14.

Hassan, L. E. A., Koko, W. S., Osman, E.-B. E., Dahab, M. M. & Sirat, H. M. 2011. *In vitro* antigiardial activity of *Citrullus lanatus* var. citroides extracts and cucurbitacins isolated compounds. *Journal of Medicinal Plants Research,* 5**,** 3338-3346.

Hastings, J., De Matos, P., Dekker, A., Ennis, M., Harsha, B., Kale, N., Muthukrishnan, V., Owen, G., Turner, S. & Williams, M. 2012. The ChEBI reference database and ontology for biologically relevant chemistry: enhancements for 2013. *Nucleic acids research,* 41**,** D456-D463.

Heinrich, M., Williamson, E. M., Gibbons, S., Barnes, J. & Prieto-Garcia, J. 2017. Fundamentals of Pharmacognosy and Phytotherapy E-Book, Elsevier Health Sciences.

Hemmateenejad, B., Nekoeinia, M., Absalan, G. & Ansari, M. 2014. Photodegradation study of nystatin by UV-Vis spectrophotometry and chemometrics modeling. *Journal of AOAC International,* 97**,** 1206-1212.

Hemraj, V., Diksha, S. & Avneet, G. 2013. A review on commonly used biochemical test for bacteria. *Innovare J Life Sci,* 1**,** 1-7.

Hernando-Amado, S., Blanco, P., Alcalde-Rico, M., Corona, F., Reales-Calderón, J. A., Sánchez, M. B. & Martínez, J. L. 2016. Multidrug efflux pumps as main players in intrinsic and acquired resistance to antimicrobials. *Drug Resistance Updates,* 28**,** 13-27.

Hijazi, A., Al Masri, D. S., Farhan, H., Nasser, M., Rammal, H. & Annan, H. 2015. Effect of different ethanol concentrations, using different extraction techniques, on the antioxidant capacity of Lebanese *Eryngium creticum*. *Journal of pharmaceutical, chemical and biological sciences,* 3**,** 262-271.

Holmes, K. K., Bertozzi, S., Bloom, B. R., Jha, P., Gelband, H., Demaria, L. M. & Horton, S. 2017. Major infectious diseases: key messages from disease control priorities.

Hossain, M., Khan, M., Anisuzzaman, A., Ahmed, M., Amran, M. & Islam, A. 2010. Antidiabetic and glycogenesis effects of different fractions of ethanolic extract of leaves of *Mangifera indica* (Linn.) in normal and alloxan induced diabetic rats. *Journal of Medical Sciences (Pakistan),* 10**,** 80-86.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. & Armand, R. 2015. The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. *International Journal of Clinical Medicine,* 6**,** 635.

Hostettmann, K., Marston, A., Ndjoko, K. & Wolfender, J.-L. 2000. The potential of African plants as a source of drugs. *Current Organic Chemistry,* 4**,** 973-1010.

Hotez, P. J. & Kamath, A. 2009. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS neglected tropical diseases,* 3**,** e412.

House, S. A. 2012. The Role of Flagella and the Lateral Crest in Giardialamblia Attachment to *In Vitro* Substrates, University of California, Davis.

Howes, M. J. R., Quave, C. L., Collemare, J., Tatsis, E. C., Twilley, D., Lulekal, E., Farlow, A., Li, L., Cazar, M. E. & Leaman, D. J. 2020. Molecules from nature: Reconciling biodiversity conservation and global healthcare imperatives for sustainable use of medicinal plants and fungi. *Plants, People, Planet,* 2**,** 463-481.

Hullatti, K. & Murthy, U. D. 2010. Activity guided isolation of cytotoxic compounds from Indian medicinal plants using BSL bioassay. *Journal of Current Pharmaceutical Research,* 1**,** 16-18.

Hussain, A., Abbasi, M., Hussain, N. & Majid, S. 2013. A survey of important indigenous medicinal plants of district Bhimber Azad Jammu Kashmir, Pakistan. *Int J Adv Res,* 1**,** 635-44.

Hussain, M. S., Fareed, S., Saba Ansari, M., Rahman, A., Ahmad, I. Z. & Saeed, M. 2012. Current approaches toward production of secondary plant metabolites. *Journal of pharmacy & bioallied sciences,* 4**,** 10.

Hussain, S. M., Syeda, A. F., Alshammari, M., Alnasser, S., Alenzi, N., Alanazi, S. & Krishnadas, N. 2020. Cognition Enhancing Effect of Rosemary Plant (*Rosmarinus officinalis*, L) in Preclinical Studies: A Systematic Review and Meta-Analysis.

Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. & Shimotohno, K. 2000. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,* 14**,** 510-516.

Hussein, M. B. & Hamad, M. N. M. 2021. Phytochemical Screening, Antimicrobial and Antioxidant Activity of *Ziziphus Spina-Christi* (L.)(Rhamnaceae) Leaves and Bark extracts.

Ibrahim, D. & Osman, H. 1995. Antimicrobial activity of *Cassia alata* from Malaysia. *Journal of ethnopharmacology,* 45**,** 151-156.

IBRAHIM, F. Y. & EL-KHATEEB, A. 2013. Effect of herbal beverages of Foeniculum vulgare and Cymbopogon proximus on inhibition of calcium oxalate renal crystals formation in rats. *Annals of Agricultural Sciences,* 58**,** 221-229.

Ibrahim, M. K., Mattar, Z. A., Abdel-Khalek, H. H. & Azzam, Y. M. 2017. Evaluation of antibacterial efficacy of anise wastes against some multidrug resistant bacterial isolates. *Journal of Radiation Research and Applied Sciences,* 10**,** 34-43.

Idris, Y. M. A., Mariod, A. A. & Hamad, S. I. 2011. Physicochemical properties, phenolic contents and antioxidant activity of Sudanese honey. *International Journal of Food Properties,* 14**,** 450-458.

Ifeoma, O. & Oluwakanyinsola, S. 2013. Screening of herbal medicines for potential toxicities. *New insights into toxicity and drug testing,* 244**,** 63-88.

Iliffe, J. 2017. *Africans: the history of a continent*, Cambridge University Press.

Iman, S., Saleem, U. & Ahmad, B. 2020. Preclinical assessment of antiurolithiatic activity of Mangifera indica seeds on ethylene glycol induced urolithiasis rat model. *Pak Vet J*, 10.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P. & Khelurkar, V. C. 2017. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry,* 6**,** 32-36.

Irondi, E. A., Oboh, G. & Akindahunsi, A. A. 2016. Antidiabetic effects of *Mangifera indica* Kernel Flour-supplemented diet in streptozotocin-induced type 2 diabetes in rats. *Food science & nutrition,* 4**,** 828-839.

Islam, M., Mannan, M., Kabir, M., Islam, A. & Olival, K. 2010. Analgesic, anti-inflammatory and antimicrobial effects of ethanol extracts of mango leaves. *Journal of the Bangladesh Agricultural University,* 8.

Jacques, F. & Bertolino, P. 2008. Molecular and morphological phylogeny of Menispermaceae (Ranunculales). *Plant Systematics and Evolution,* 274**,** 83-97.

Jahan, R., Khatun, A., Nahar, N., Jahan, F. I., Chowdhury, A. R., Nahar, A., Seraj, S., Mahal, M. J., Khatun, Z. & Rahmatullah, M. 2010. Use of Menispermaceae family plants in folk medicine of Bangladesh. *Advances in Natural and Applied Sciences,* 4**,** 1-10.

Jahurul, M., Zaidul, I., Ghafoor, K., Al-Juhaimi, F. Y., Nyam, K.-L., Norulaini, N., Sahena, F. & Omar, A. M. 2015. Mango (*Mangifera indica* L.) by-products and their valuable components: A review. *Food chemistry,* 183**,** 173-180.

Jain, C., Khatana, S. & Vijayvergia, R. 2019. Bioactivity of secondary metabolites of various plants: a review. *International Journal of Pharmaceutical Sciences and Research,* 10**,** 494-498.

Jain, P. & Nafis, G. 2011. Antifungal activity of crude aqueous and methanolic amchur (*Mangifera indica*) extracts against Candida species. *Int J Pharm Sci Rev Res,* 9**,** 85-87.

Jalali-Heravi, M., Moazeni, R. S. & Sereshti, H. 2011. Analysis of Iranian rosemary essential oil: Application of gas chromatography–mass spectrometry combined with chemometrics. *Journal of chromatography A,* 1218**,** 2569-2576.

Jamshidi-Kia, F., Lorigooini, Z. & Amini-Khoei, H. 2018. Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology,* 7.

Jananie, R., Priya, V. & Vijayalakshmi, K. 2011. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. *NY Sci J,* 4**,** 1-5.

Jarrar, N., Abu-Hijleh, A. & Adwan, K. 2010. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin–resistant *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Medicine,* 3**,** 121-123.

Jeyasakthy, S., Jeyadevan, P., Thavaranjit, A., Manoranjan, T., Srikaran, R. & Krishnapillai, N. 2013. Antifungal activity and qualitative phytochemical analysis of extracts obtained by sequential extraction of some medicinal plants in *Jaffna peninsula*. *Archiv. of Appli. Sci. Res,* 5**,** 214-221.

Jiang, S., Lai, P., Li, J. & Wang, G. 2012. Antioxidant activities and essential oil composition of Herba *Artemisiae Scopariae* from China. *Journal of oleo science,* 61**,** 291-295.

Jiang, Y., Wu, N., Fu, Y.-J., Wang, W., Luo, M., Zhao, C.-J., Zu, Y.-G. & Liu, X.-L. 2011. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental toxicology and pharmacology,* 32**,** 63-68.

Jøraholmen, M. W. B. 2012. Development of chitosan-coated liposomes for improved therapy of vaginal infections: clotrimazole as model drug. Universitetet i Tromsø.

Jordán, M. J., LAX, V., ROTA, M. C., Lorán, S. & SOTOMAYOR, J. A. 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of Rosmarinus officinalis L. *Food Control,* 30**,** 463-468.

Jorgensen, J. H. & Turnidge, J. D. 2015. Susceptibility test methods: dilution and disk diffusion methods. *Manual of Clinical Microbiology, Eleventh Edition.* American Society of Microbiology.

Jouda, M. M., Elbashiti, T., Masad, A. & Albayoumi, M. 2015. The Antibacterial Effect of Some Medicinal Plant Extracts and Their Synergistic Effect With Antibiotics. *World Journal of Pharmacy and Pharmaceutical Sciences,* 5**,** 23-33.

Junsongduang, A., Kasemwan, W., Lumjoomjung, S., Sabprachai, W., Tanming, W. & Balslev, H. 2020. Ethnomedicinal Knowledge of Traditional Healers in Roi Et, Thailand. *Plants,* 9**,** 1177.

Kaab, S. B., Rebey, I. B., Hanafi, M., Berhal, C., Fauconnier, M.-L., De Clerck, C., Ksouri, R. & Jijakli, H. 2019. Rosmarinus officinalis essential oil as an effective antifungal and herbicidal agent. *Spanish journal of agricultural research,* 17**,** 1006.

Kabbashi, A. S., Almagboul, A. Z., Garbi, M. I., Osman, E.-B. E., Koko, W. S., Hassan, A. M., Dahab, M. M. & Abuzeid, N. 2016. Antigiaridial activity and cytotoxicity of ethanolic bark extract of *Acacia nilotica* (L.). *Mediterranean Journal of Biosciences,* 1**,** 138-146.

Kabbashi, A. S., Garbi, M. I. & Osman, E. E. 2015a. Antigiardial, Antioxidant and Cytotoxicity of Ethanolic Leaves Extract of *Acacia nilotica* (L). *Journal of Forest Products & Industries,* 3**,** 100-106.

Kabbashi, A. S., Garbi, M. I. & Osman, E. E. J. A. M. P. R. 2015b. Antigiardial, antioxidant activities and cytotoxicity of ethanolic extract of leaves of *Acacia nilotica* (L). 3**,** 33-38.

Kabbashi, A. S., Koko, W. S., Mohammed, S. E. A., Musa, N., Osman, E. E., Dahab, M. M., Allah, E. F. F. & Mohammed, A. K. 2014. *In vitro* amoebicidal, antimicrobial and antioxidant activities of the plants *Adansonia digitata* and *Cucurbit maxima*. *Advancement in Medicinal Plant Research,* 2**,** 50-57.

Kabbashi, A. S., Osman, E.-B. E., Garbi, M. I., Ahmed, I. F. A., Saleh, M. S., Mohamed, A., Badri, A. A. E. & Abuzeid, N. 2017. *In vitro* Antiprotozoal activities and cytotoxicity of selected Sudanese medicinal plants. *Int J Biomed Eng Clin Sci,* 3.

Kabera, J. N., Semana, E., Mussa, A. R. & He, X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J Pharm Pharmacol,* 2**,** 377-392.

Kahraman, C., Arituluk, Z. C. & Cankaya, I. I. T. 2020. The Clinical Importance of Herb-Drug Interactions and Toxicological Risks of Plants and Herbal Products. *Medical Toxicology.* IntechOpen.

Kala, C. P., Dhyani, P. P. & Sajwan, B. S. 2006. Developing the medicinal plants sector in northern India: challenges and opportunities. *Journal of Ethnobiology and Ethnomedicine,* 2**,** 1-15.

Kalita, P. 2021. An overview on mangifera indica: importance and its various pharmacological action. *Pharma News*.

Kamath, K. & Ramakrishna, A. 2017. Comparison of antibacterial activity of leaves extracts of *Tectona grandis*, *Mangifera indica*, and *Anacardium occidentale*. *Int J Curr Pharm Res,* 9**,** 36-9.

Kang, K. B., Ernst, M., Van Der Hooft, J. J., Da Silva, R. R., Park, J., Medema, M. H., Sung, S. H. & Dorrestein, P. C. 2019. Comprehensive mass spectrometry-guided phenotyping of plant specialized metabolites reveals metabolic diversity in the cosmopolitan plant family Rhamnaceae. *The Plant Journal,* 98**,** 1134-1144.

Kanwal, Q., Hussain, I., Latif Siddiqui, H. & Javaid, A. 2010. Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. *Natural Product Research,* 24**,** 1907-1914.

Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R. & Singh, J. 2019. Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene,* 19**,** 100182.

Kapoor, R. & Huang, Y.-S. 2006. Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. *Current pharmaceutical biotechnology,* 7**,** 531-534.

Karak, P. 2019. Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research,* 10**,** 1567-1574.

Karar, M. G. E. & Kuhnert, N. 2017. Herbal drugs from Sudan: Traditional uses and phytoconstituents. *Pharmacognosy reviews,* 11**,** 83.

Karpiński, T. M. 2020. Essential oils of Lamiaceae family plants as antifungals. *Biomolecules,* 10**,** 103.

Kasote, D. M., Katyare, S. S., Hegde, M. V. & Bae, H. 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International journal of biological sciences,* 11**,** 982.

Kasparavičienė, G., Ramanauskienė, K., Savickas, A., Velžienė, S., Kalvėnienė, Z., Kazlauskienė, D., Ragažinskienė, O. & Ivanauskas, K. 2013. Evaluation of total phenolic content and antioxidant activity of different Rosmarinus officinalis L. ethanolic extracts. *Biologija,* 59.

Kaur, J., Rathinam, X., Kasi, M., Leng, K. M., Ayyalu, R., Kathiresan, S. & Subramaniam, S. 2010. Preliminary investigation on the antibacterial activity of mango (Mangifera indica L: Anacardiaceae) seed kernel. *Asian Pacific Journal of Tropical Medicine,* 3**,** 707-710.

Kavanagh, D. 1972. *Political culture*, Macmillan International Higher Education.

Kayuni, S., Lampiao, F., Makaula, P., Juziwelo, L., Lacourse, E. J., Reinhard-Rupp, J., Leutscher, P. D. & Stothard, J. R. 2019. A systematic review with epidemiological update of male genital schistosomiasis (MGS): a call for integrated case management across the health system in sub-Saharan Africa. *Parasite epidemiology and control,* 4**,** e00077.

Khabar, K. S., Al-Zoghaibi, F., Dzimiri, M., Taha, M., Al-Tuwaijri, A. & Al-Ahdal, M. N. 1996. MTS interferon assay: a simplified cellular dehydrogenase assay for interferon activity using a water-soluble tetrazolium salt. *Journal of interferon & cytokine research,* 16**,** 31-33.

Khabnadideh, S., Rezaei, Z., Motazedian, M. & Eskandari, M. 2007. Synthesis of metronidazole derivatives as antigiardiasis agents. *DARU Journal of Pharmaceutical Sciences,* 15**,** 17-20.

Khalil, O. A., Ramadan, K. S., Danial, E. N., Alnahdi, H. S. & Ayaz, N. O. 2012. Antidiabetic activity of Rosmarinus officinalis and its relationship with the antioxidant property. *African Journal of Pharmacy and Pharmacology,* 6**,** 1031-1036.

Khan, M. & Khan, M. 1989. Alkyl gallates of flowers of Mangifera Indica. *Fitoterapia,* 60**,** 284.

Khan, M. A. A. & Islam, M. T. 2012. Analgesic and cytotoxic activity of *Acorus calamus* L., *Kigelia pinnata* L., *Mangifera indica* L. and *Tabernaemontana divaricata* L. *Journal of pharmacy & bioallied sciences,* 4**,** 149.

Khan, N., Khushtar, M., Ahmad, N., Hasan, N., Khan, Z., Idris, S., Ahmad, A. & Zishan, M. 2017. Nutritional importance and pharmacological activity of Mangifera indica. *World Journal of Pharmacy and Pharmaceutical Sciences,* 6**,** 258-73.

Khanbabaee, K. & Van Ree, T. 2001. Tannins: classification and definition. *Natural product reports,* 18**,** 641-649.

Khider, T. O. & Hubbe, M. A. 2018. Towards Rational Utilization of Indigenous Plant Resources. *BioResources,* 13**,** 7172-7174.

Khiralla, A., Mohamed, I., Thomas, J., Mignard, B., Spina, R., Yagi, S. & Laurain-Mattar, D. 2015. A pilot study of antioxidant potential of endophytic fungi from some Sudanese medicinal plants. *Asian Pacific Journal of Tropical Medicine,* 8**,** 701-704.

Khoo, H. E., Azlan, A., Kong, K. W. & Ismail, A. 2016. Phytochemicals and medicinal properties of indigenous tropical fruits with potential for commercial development. *Evidence-Based Complementary and Alternative Medicine,* 2016.

Kim, B.-R., Kim, H. M., Jin, C. H., Kang, S.-Y., Kim, J.-B., Jeon, Y. G., Park, K. Y., Lee, I.-S. & Han, A.-R. 2020. Composition and antioxidant activities of volatile organic compounds in radiation-bred Coreopsis cultivars. *Plants,* 9**,** 717.

Kim, D.-O., Jeong, S. W. & Lee, C. Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry,* 81**,** 321-326.

Kim, H., Moon, J. Y., Kim, H., Lee, D.-S., Cho, M., Choi, H.-K., Kim, Y. S., Mosaddik, A. & Cho, S. K. 2010. Antioxidant and antiproliferative activities of mango (Mangifera indica L.) flesh and peel. *Food Chemistry,* 121**,** 429-436.

King, C. H., Muchiri, E. M. & Ouma, J. H. 2000. Evidence against rapid emergence of praziquantel resistance in *Schistosoma haematobium*, Kenya. *Emerging infectious diseases,* 6**,** 585.

Kiran, S. & Prakash, B. 2015. Toxicity and biochemical efficacy of chemically characterized *Rosmarinus officinalis* essential oil against *Sitophilus oryzae* and *Oryzaephilus surinamensis*. *Industrial Crops and Products,* 74**,** 817-823.

Klimenko, L. Y., Shkarlat, G., Shovkova, Z. & Kolisnyk, O. 2019. Development and validation of HPLC/UV-procedures for quantification of metronidazole in blood and urine.

Kodedová, M. & Sychrová, H. 2015. Changes in the sterol composition of the plasma membrane affect membrane potential, salt tolerance and the activity of multidrug resistance pumps in Saccharomyces cerevisiae. *PLoS One,* 10**,** e0139306.

Koko, W., Abdalla, H., Galal, M. & Khalid, H. J. F. 2005. Evaluation of oral therapy on Mansonial Schistosomiasis using single dose of *Balanites aegyptiaca* fruits and praziquantel. 76**,** 30-34.

Koko, W., Galal, M. & Khalid, H. J. J. O. E. 2000. Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole. 71**,** 247-252.

Koko, W., Mesaik, M. A., Yousaf, S., Galal, M. & Choudhary, M. I. 2008a. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. *Journal of ethnopharmacology,* 118**,** 26-34.

Koko, W., Mesaik, M. A., Yousaf, S., Galal, M. & Choudhary, M. I. J. J. O. E. 2008b. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. 118**,** 26-34.

Kompelly, A., Kompelly, S., Vasudha, B. & Narender, B. 2019. *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. *Journal of Drug Delivery and Therapeutics,* 9**,** 323-330.

Kong, J.-M., Chia, L.-S., Goh, N.-K., Chia, T.-F. & Brouillard, R. 2003. Analysis and biological activities of anthocyanins. *Phytochemistry,* 64**,** 923-933.

Konovalova, O., Gergel, E. & Herhel, V. 2013. GC-MS Analysis of bioactive components of *Shepherdia argentea* (Pursh.) Nutt. from Ukrainian Flora. *The Pharma Innovation,* 2**,** 7.

Kpobi, L. N., Swartz, L. & Omenyo, C. N. 2019. Traditional herbalists' methods of treating mental disorders in Ghana. *Transcultural Psychiatry,* 56**,** 250-266.

Krishnamoorthy, K. & Subramaniam, P. 2014. Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *International scholarly research notices,* 2014.

Krull, I., Strong, R., Sosic, Z., Cho, B.-Y., Beale, S., Wang, C.-C. & Cohen, S. 1997. Labeling reactions applicable to chromatography and electrophoresis of minute amounts of proteins. *Journal of Chromatography B: Biomedical Sciences and Applications,* 699**,** 173-208.

Ksouri, S., Djebir, S., Bentorki, A., Gouri, A., Hadef, Y. & Benakhla, A. 2017. Antifungal activity of essential oils extract from *Origanum floribundum* Munby, *Rosmarinus officinalis* L. and *Thymus ciliatus* Desf. against *Candida albicans* isolated from bovine clinical mastitis. *Journal de mycologie medicale,* 27**,** 245-249.

Kumar, P. P., Kumaravel, S. & Lalitha, C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research,* 4**,** 191-195.

Kumar, S. & Pandey, A. K. 2015. Free radicals: health implications and their mitigation by herbals. *Journal of Advances in Medicine and Medical Research***,** 438-457.

Kuo, C. K. & Ma, P. X. 2001. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. *Biomaterials,* 22**,** 511-521.

Kusari, S., Hertweck, C. & Spiteller, M. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry & biology,* 19**,** 792-798.

La Hoz, R. M., Morris, M. I. & Practice, A. I. D. C. O. 2019. Intestinal parasites including *cryptosporidium, cyclospora,* giardia, and microsporidia, *entamoeba histolytica,* strongyloides, schistosomiasis, and echinococcus: guidelines from the American society of transplantation infectious diseases community of practice. *Clinical transplantation,* 33**,** e13618.

Ladan Moghadam, A. R. 2015. Antioxidant activity and chemical composition of *Rosmarinus officinalis* L. essential oil from Iran. *Journal of Essential Oil Bearing Plants,* 18**,** 1490-1494.

Lahlou, M. 2007. Screening of natural products for drug discovery. *Expert opinion on drug discovery,* 2**,** 697-705.

Lahlou, M. 2013. The success of natural products in drug discovery. *Pharmacol Pharm,* 4**,** 17-31.

Lahlou, Y., El Amraoui, B. & Bamhaoud, T. 2019. The Antibacterial Activity Screening of the Extracts of Some Moroccan Medicinal Plants. *International Journal of New Technology and Research (IJNTR),* 5**,** 31-38.

Lail, L. I. A. 2006. An atomic force microscopy study of bacterial adhesion to natural organic matter-coated surfaces in the environment. Citeseer.

Lajayer, B. A., Ghorbanpour, M. & Nikabadi, S. 2017. Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicology and Environmental Safety,* 145**,** 377-390.

Lalisan Jeda, A., Nuñeza Olga, M. & Uy Mylene, M. 2014. Brine shrimp (Artemia salina) bioassay of the medicinal plant Pseudelephantopus spicatus from Iligan City, Philippines.

Lalthanpuii, P. & Lalchhandama, K. 2019. Chemical profiling, antibacterial and antiparasitic studies of Imperata cylindrica. *J. Appl. Pharm. Sci,* 9**,** 117-121.

Lam, K. S. 2007. New aspects of natural products in drug discovery. *Trends in microbiology,* 15**,** 279-289.

Lan, W., Lin, S., Li, X., Zhang, Q. & Qin, W. Chemical composition of the leaf and stem essential oil of *Adenophorae Radix*. AIP Conference Proceedings, 2017. AIP Publishing LLC, 030001.

Langsi, J. D., Nukenine, E. N., Oumarou, K. M., Moktar, H., Fokunang, C. N. & Mbata, G. N. 2020. Evaluation of the insecticidal activities of α-Pinene and 3-Carene on Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae). *Insects,* 11**,** 540.

Lans, C. & Van Asseldonk, T. 2020. Dr. Duke's Phytochemical and Ethnobotanical Databases, a Cornerstone in the Validation of Ethnoveterinary Medicinal Plants, as Demonstrated by Data on Pets in British Columbia. *Medicinal and Aromatic Plants of North America.* Springer.

Latha, M., Latha, K., Vagdevi, H., Vaidya, V., Virupaxappa, S. & Swetha, G. 2010. Anthelmintic activity of the crude extracts of *Mangifera indica* L. *Biomed,* 5**,** 125-131.

Lauwers, G. Y., Mino-Kenudson, M. & Kradin, R. L. 2018. Infections of the gastrointestinal tract. *Diagnostic pathology of infectious disease***,** 232.

Lawi, Y., Saria, J. & Kidukuli, A. 2018. Brine shrimp cytotoxicity, phytochemical screening and larvicidal activities of *Plectranthus barbatus* extracts. *Res Rev Insights,* 2**,** 2-4.

Lewies, A., Du Plessis, L. H. & Wentzel, J. F. 2019. Antimicrobial peptides: the Achilles' heel of antibiotic resistance? *Probiotics and antimicrobial proteins,* 11**,** 370-381.

Lewis, W. H. & Elvin-Lewis, M. P. 2003. Medical botany: plants affecting human health, John Wiley & Sons.

Lidani, K. C. F., Andrade, F. A., Bavia, L., Damasceno, F. S., Beltrame, M. H., Lucas Sandri, T. & De Messias-Reason, I. 2019. Chagas disease: from discovery to a worldwide health problem. *Frontiers in public health,* 7**,** 166.

Lin, J.-J., Huang, C.-C., Su, Y.-L., Luo, H.-L., Lee, N.-L., Sung, M.-T. & Wu, Y.-J. 2019. Proteomics Analysis of Tangeretin-Induced Apoptosis through Mitochondrial Dysfunction in Bladder Cancer Cells. *International Journal of Molecular Sciences,* 20**,** 1017.

Lin, J.-Y. & Tang, C.-Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chemistry,* 101**,** 140-147.

Lobo, V., Patil, A., Phatak, A. & Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews,* 4**,** 118.

López-Ráez, J. A., SHIRASU, K. & FOO, E. 2017. Strigolactones in plant interactions with beneficial and detrimental organisms: the Yin and Yang. *Trends in Plant Science,* 22**,** 527-537.

Löscher, W., Klitgaard, H., Twyman, R. E. & Schmidt, D. 2013. New avenues for anti-epileptic drug discovery and development. *Nature reviews drug discovery,* 12**,** 757.

Lovkova, M. Y., Buzuk, G., Sokolova, S. & Kliment'eva, N. 2001. Chemical features of medicinal plants. *Applied Biochemistry and Microbiology,* 37**,** 229-237.

Lu, L., Zhang, L., Wai, M. S. M., Yew, D. T. W. & Xu, J. 2012. Exocytosis of MTT formazan could exacerbate cell injury. *Toxicology in Vitro,* 26**,** 636-644.

Luckner, M. 2013. *Secondary metabolism in microorganisms, plants and animals,* Springer Science & Business Media.

Machado, D. G., Cunha, M. P., Neis, V. B., Balen, G. O., Colla, A., Bettio, L. E., Oliveira, Á., Pazini, F. L., Dalmarco, J. B. & Simionatto, E. L. 2013. Antidepressant-like effects of fractions, essential oil, carnosol and betulinic acid isolated from *Rosmarinus officinalis* L. *Food Chemistry,* 136**,** 999-1005.

Mäder, P., RENNAR, G. A., VENTURA, A. M. P., GREVELDING, C. G. & SCHLITZER, M. 2018. Chemotherapy for fighting schistosomiasis: past, present and future. *ChemMedChem,* 13**,** 2374-2389.

Mahmoud, A. B., Mäser, P., Kaiser, M., Hamburger, M. & Khalid, S. 2020. Mining Sudanese Medicinal Plants for Antiprotozoal Agents. *Frontiers in Pharmacology,* 11**,** 865.

Mahmud, S., Saleem, M., Siddique, S., Ahmed, R., Khanum, R. & Perveen, Z. 2009. Volatile components, antioxidant and antimicrobial activity of *Citrus acida* var. sour lime peel oil. *Journal of Saudi Chemical Society,* 13**,** 195-198.

Makaula, P., Sadalaki, J. R., Muula, A. S., Kayuni, S., Jemu, S. & Bloch, P. 2014. Schistosomiasis in Malawi: a systematic review. *Parasites & vectors,* 7**,** 570.

Makhloufi, A., Moussaoui, A., Lazouni, H., Hasnat, N. & Abdelouahid, D. 2011. Antifungal activity of *Rosmarinus officinalis* L. and its impact on the conservation of a local variety of dates. *J Med Plants,* 3**,** 1-6.

Malann, Y., Matur, B. & Mailafia, S. 2013. Evaluation of the antimalarial activity of aqueous leaf extracts of *Casuarina equistifolia* and *Mangifera indica* against *Plasmodium berghei* in mice. *Journal of Pharmacy & Bioresources,* 10**,** 8-16.

Mantawy, M. M., Ali, H. F. & Rizk, M. Z. 2011. Therapeutic effects of *Allium sativum* and *Allium cepa* in *Schistosoma mansoni* experimental infection. *Revista do instituto de medicina tropical de são paulo,* 53**,** 155-163.

Manzur, A. G., Sm Junior, V., Morais-Costa, F., Mariano, E. G., Careli, R. T., Da Silva, L. M., Coelho, S. G., De Almeida, A. C. & Duarte, E. R. 2020. Extract of *Mangifera indica* L. leaves may reduce biofilms of Staphylococcus spp. in stainless steel and teatcup rubbers. *Food Science and Technology International,* 26**,** 11-20.

Mari, L., Gatto, M., Ciddio, M., Dia, E. D., Sokolow, S. H., De Leo, G. A. & Casagrandi, R. 2017. Big-data-driven modeling unveils country-wide drivers of endemic schistosomiasis. *Scientific reports,* 7**,** 1-11.

Martin, G. J. 2010. *Ethnobotany: a methods manual*, Routledge.

Masoko, P., Mmushi, T., Mogashoa, M., Mokgotho, M., Mampuru, L. & Howard, R. 2008. *In vitro* evaluation of the antifungal activity of *Sclerocarya birrea* extracts against pathogenic yeasts. *African Journal of Biotechnology,* 7.

Masoko, P., Picard, J. & Eloff, J. 2005. Antifungal activities of six south African Terminalia species (Combretaceae). *Journal of Ethnopharmacology,* 99**,** 301-308.

Matsuzaki, Y., Tsujisawa, T., Nishihara, T., Nakamura, M. & Kakinoki, Y. 2013. Antifungal activity of chemotype essential oils from rosemary against *Candida albicans*.

Mayer, F. L., Wilson, D. & Hube, B. 2013. *Candida albicans* pathogenicity mechanisms. *Virulence,* 4**,** 119-128.

Mcgaw, L., Jäger, A. & Van Staden, J. 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *Journal of ethnopharmacology,* 72**,** 247-263.

Mclaughlin, J. L., Rogers, L. L. & Anderson, J. E. 1998. The use of biological assays to evaluate botanicals. *Drug information journal,* 32**,** 513-524.

Medi, M. & Jasprica, I. 2008. 16 Application of TLC. *Thin Layer Chromatography in Phytochemistry***,** 405.

Mehraj, V., Hatcher, J., Akhtar, S., Rafique, G. & Beg, M. A. 2008. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PloS one,* 3**,** e3680.

Mekonnen, A., Yitayew, B., Tesema, A. & Taddese, S. 2016. *In vitro* antimicrobial activity of essential oil of *Thymus schimperi*, *Matricaria chamomilla*, *Eucalyptus globulus*, and *Rosmarinus officinalis*. *International journal of microbiology,* 2016.

Meneses, M. A., Caputo, G., Scognamiglio, M., Reverchon, E. & Adami, R. 2015. Antioxidant phenolic compounds recovery from *Mangifera indica* L. by-products by supercritical antisolvent extraction. *Journal of Food Engineering,* 163**,** 45-53.

Menkir, S. & Ayenew, M. 2013. Prevalence and Major Risk Factors of Intestinal Parasitic Infections among Under-five Aged Children Visiting the Health Center at Debre Birhan Town, Central Ethiopia. Haramaya University.

Mensah, M., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K. & Dickson, R. A. 2019. Toxicity and safety implications of herbal medicines used in Africa. *Herbal medicine,* 63**,** 1992-0849.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. & Mclaughlin, J. L. 1982a. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta med,* 45**,** 31-34.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. J. & Mclaughlin, J. L. 1982b. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica,* 45**,** 31-34.

Michael, A., Thompson, C. & Abramovitz, M. 1956. Artemia salina as a test organism for bioassay. *Science,* 123**,** 464-464.

Middleton, E., Kandaswami, C. & Theoharides, T. C. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews,* 52**,** 673-751.

Miles, A. A., Misra, S. & Irwin, J. 1938. The estimation of the bactericidal power of the blood. *Epidemiology & Infection,* 38**,** 732-749.

Moglad, E. H., Alhassan, M. S., Koko, W. & Saadabi, A. 2012. *In vitro* antimicrobial activity of Sudanese medicinal plants. *J. Med. Sci,* 12**,** 219-223.

Moglad, E. H., Hamad, A. M., Fatima, F., Seshadri, V. D. & Naz, M. 2020. Antimicrobial and wound healing activities of certain Sudanese medicinal plants. *Saudi journal of biological sciences,* 27**,** 1766-1772.

Mohamed, I. E. T., Nur, E. & Abdelrahman, M. E. N. 2010. The antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. *EurAsian Journal of BioSciences,* 4.

Mohamed, I. T. A., Mohamed, A. H. & Ayoub, S. M. H. 2016. Antioxidant activity and phytochemical screening of Sudanese *Solanum dubium* seeds in combination with Sudanese honey. *Am. J. Res. Comm,* 4**,** 192-199.

Mohammed, G. J., Mohammed, J. A. & Hameed, I. H. 2016a. Anti-bacterial, Antifungal Activity and Chemical Analysis of *Punica grantanum* (Pomegranate peel) Using GC-MS and FTIR Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research* 8**,** 480 - 494.

Mohammed, N. N., Pandey, P., Khan, N. S., Elokely, K. M., Liu, H., Doerksen, R. J. & Repka, M. A. 2016b. Clotrimazole–cyclodextrin based approach for the management and treatment of Candidiasis–A formulation and chemistry-based evaluation. *Pharmaceutical development and technology,* 21**,** 619-629.

Mohammed, S. E. A., Kabashi, A. S., Koko, W. S. & Azim, M. K. 2015. Antigiardial activity of glycoproteins and glycopeptides from Ziziphus honey. *Natural product research,* 29**,** 2100-2102.

Mohammed, Z. M. A. 2014. Morphological and Genetical Diversity of Mango (*Mangifera Indica* L.) Cultivars In Shendi Area. Shendi University.

Mohan, C., Deepak, M., Viswanatha, G., Savinay, G., Hanumantharaju, V., Rajendra, C. & Halemani, P. D. 2013. Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. *Asian Pacific journal of tropical medicine,* 6**,** 311-314.

Moon, J.-K. & Shibamoto, T. 2009. Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry,* 57**,** 1655-1666.

Morens, D. M., Folkers, G. K. & Fauci, A. S. 2004. The challenge of emerging and re-emerging infectious diseases. *Nature,* 430**,** 242.

Mosa, E., Justin, D., Hamam, S., Mohamed, E. & Saad, M. 2014. Evaluation of phytochemical and antimicrobial activities of some sudanese medicinal plants. World. *J Pharm Pharm Sci,* 3**,** 1769-76.

Moshi, M. J., Mbwambo, Z. H., Kapingu, M. C., Mhozya, V. H. & Marwa, C. 2006. Antimicrobial and brine shrimp lethality of extracts of *Terminalia mollis* Laws. *African Journal of Traditional, Complementary and Alternative Medicines,* 3**,** 59-69.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods,* 65**,** 55-63.

Mounanga, M. B., Mewono, L. & Angone, S. A. 2015. Toxicity studies of medicinal plants used in sub-Saharan Africa. *Journal of ethnopharmacology,* 174**,** 618-627.

Moundipa, P. F., Flore, K. G. M., Bilong, C. F. & Bruchhaus, I. 2005. *In vitro* amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). *African Journal of Traditional, Complementary and Alternative Medicines,* 2**,** 113–121-113–121.

Mugnaini, L., Nardoni, S., Pinto, L., Pistelli, L., Leonardi, M., Pisseri, F. & Mancianti, F. 2012. *In vitro* and *in vivo* antifungal activity of some essential oils against feline isolates of *Microsporum canis*. *Journal de mycologie medicale,* 22**,** 179-184.

Muhammadi, M., Shafiq, S. & Hussain, F. 2020. Phytochemical Screening of Leaves from *Mangifera Indica* and *Morus Alba* For Bioactive Compounds. *Journal of Plant and Environment,* 1.

Mujeeb, F., Bajpai, P. & Pathak, N. 2014. Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos*. *BioMed research international,* 2014.

Musher, D. M., Logan, N., Mehendiratta, V., Melgarejo, N. A., Garud, S. & Hamill, R. J. 2007. Clostridium difficile colitis that fails conventional metronidazole therapy: response to nitazoxanide. *Journal of antimicrobial chemotherapy,* 59**,** 705-710.

Mushore, J. & Matuvhunye, M. 2013. Antibacterial properties of *Mangifera indica* on *Staphylococcus aureus*. *African journal of clinical and experimental microbiology,* 14**,** 62-74.

Mustafa, G., Arif, R., Atta, A., Sharif, S. & Jamil, A. 2017. Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan.

Mwonga, K. B. 2014. Evaluationof Molluscicidal and Antischistosomal Activities of Selected Medicinal Plants Traditionally Used In Makueni County-Kenya. Kenyatta University.

Naiel, M. A., Ismael, N. E. & Shehata, S. A. 2019. Ameliorative effect of diets supplemented with rosemary (*Rosmarinus officinalis*) on aflatoxin B1 toxicity in terms of the performance, liver histopathology, immunity and antioxidant activity of Nile Tilapia (Oreochromis niloticus). *Aquaculture,* 511**,** 734264.

Najumudin, K., Ayubu, J. & Elnazeer, A. M. 2018. Antigiardial activity of some plant extracts used in traditional medicine in Sudan in comparison with metronidazole.

Nakamura, C. V., Ueda-Nakamura, T., Bando, E., Melo, A. F. N., Cortez, D. A. G. & Dias Filho, B. P. 1999. Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Memórias do Instituto Oswaldo Cruz,* 94**,** 675-678.

Namivandi-Zangeneh, R., Sadrearhami, Z., Dutta, D., Willcox, M., Wong, E. H. & Boyer, C. 2019. Synergy between Synthetic Antimicrobial Polymer and Antibiotics: A Promising Platform To Combat Multidrug-Resistant Bacteria. *ACS infectious diseases,* 5**,** 1357-1365.

Nascimento, G. G., Locatelli, J., Freitas, P. C. & Silva, G. L. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology,* 31**,** 247-256.

National, C., For Clinical, Laboratory, Standards 1999. Methods for determining bactericidal activity of antimicrobial agents: approved guideline, National Committee for Clinical Laboratory Standards Wayne, PA.

Ncube, N., Afolayan, A. & Okoh, A. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African journal of biotechnology,* 7.

Nemudzivhadi, V. & Masoko, P. 2014. *In vitro* assessment of cytotoxicity, antioxidant, and anti-inflammatory activities of Ricinus communis (Euphorbiaceae) leaf extracts. *Evidence-Based Complementary and Alternative Medicine,* 2014.

Newman, D. J. & Cragg, G. M. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of natural products,* 75**,** 311-335.

Nguyen, V. H., Le, M. N., Nguyen, H. B., Ha, K. O., Pham, T. H. V., Nguyen, T. H., Dao, N. S. H., Nguyen, V. G., Nguyen, D. L. & Trinh, N. T. 2021. Propyl Gallate. *Molbank,* 2021**,** M1201.

Nimse, S. B. & Pal, D. 2015. Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances,* 5**,** 27986-28006.

Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E. & Capaccioli, S. 2009. Natural compounds for cancer treatment and prevention. *Pharmacological research,* 59**,** 365-378.

Nour, A. A., Khalid, K. S. & Osman, G. A. 2011. Suitability of some Sudanese mango varieties for jam making. *Am. J. Ind. Res,* 2**,** 17-23.

Nour, A. M., Khalid, S. A., Kaiser, M., Brun, R., Wai’ l, E. A. & Schmidt, T. J. 2010. The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides* L. *Journal of ethnopharmacology,* 129**,** 127-130.

Nowruzi, B., Sarvari, G. & Blanco, S. 2020. The cosmetic application of cyanobacterial secondary metabolites. *Algal Research,* 49**,** 101959.

Nwoke, K., Nneli, R. & Konyefom, N. 2016. Evaluating the Anti-Pyretic and Anti-Inflammatory Properties of the Ethanolic Extract of *Mangifera Indica* (Mango) Bark in Albino Wistar Rats.

Nwonu, C., Ilesanmi, O., Agbedahunsi, J. & Nwonu, P. 2019. Natural products as veritable source of novel drugs and medicines: A review. *International Journal of Herbal Medicine,* 7**,** 50-54.

Obiri, D. D., Osafo, N., Ayande, P. G. & Antwi, A. O. 2014. *Xylopia aethiopica* (Annonaceae) fruit extract suppresses Freund′ s adjuvant-induced arthritis in Sprague-Dawley rats. *Journal of ethnopharmacology,* 152**,** 522-531.

Obode, O. C., Adebayo, A. H., Omonhinmin, C. A. & Yakubu, O. F. 2020. A systematic review of medicinal plants used in Nigeria for hypertension management. *International Journal of Pharmaceutical Research,* 12.

Odediran, S. A., Elujoba, A. A. & Adebajo, A. C. 2014. Influence of formulation ratio of the plant components on the antimalarial properties of MAMA decoction. *Parasitology research,* 113**,** 1977-1984.

Ogongo, P., Kariuki, T. M. & Wilson, R. A. 2018. Diagnosis of schistosomiasis mansoni: an evaluation of existing methods and research towards single worm pair detection. *Parasitology,* 145**,** 1355-1366.

Ohikhena, F. U., Wintola, O. A. & Afolayan, A. J. 2016. Research article toxicity assessment of different solvent extracts of the medicinal plant, phragmanthera capitata (Sprengel) Balle on Brine Shrimp (Artemia salina). *International Journal of Pharmacology,* 12**,** 701-710.

Okuda, T. & Ito, H. 2011. Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Molecules,* 16**,** 2191-2217.

Oladimeji, H., Nia, R. & Essien, E. 2006. *In-vitro* anti-microbial and brine-shrimp lethality potential of the leaves and stem of *Calotropis procera* (ait). *African Journal of Biomedical Research,* 9.

Olasehinde, G., Sholotan, K., Openibo, J. O., Taiwo, O., Bello, O. A., Ajayi, J., Ayepola, O. & Ajayi, A. 2018. Phytochemical and Antimicrobial Properties of *Mangifera indica* Leaf Extracts. *Covenant Journal of Physical & Life Sciences (CJPL),* 6**,** 55-63.

Oliveira, A. B., Dolabela, M. F., Braga, F. C., Jácome, R. L., Varotti, F. P. & Póvoa, M. M. 2009. Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. *Anais da Academia Brasileira de Ciencias,* 81**,** 715-740.

Oluwatuyi, M., Kaatz, G. W. & Gibbons, S. 2004. Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phytochemistry,* 65**,** 3249-3254.

Omarova, A., Tussupova, K., Berndtsson, R., Kalishev, M. & Sharapatova, K. 2018. Protozoan parasites in drinking water: A system approach for improved water, sanitation and hygiene in developing countries. *International journal of environmental research and public health,* 15**,** 495.

Omri, A. E., Han, J., Abdrabbah, M. B. & Isoda, H. 2012. Down regulation effect of *Rosmarinus officinalis* polyphenols on cellular stress proteins in rat pheochromocytoma PC12 cells. *Cytotechnology,* 64**,** 231-240.

Orabi, M. A., Bayoumi, S. A., Sayed, H. M., Huseein, E. A., Khedr, A. I. & Hatano, T. 2020. Simultaneous isolation of gallotannins and a related phenolic from *Mangifera indica* kernels and assessment of their anti-Trichomonas vaginalis activities. *Journal of Applied Pharmaceutical Science,* 10**,** 022-038.

Oren, A. & Garrity, G. M. 2021. Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology,* 71**,** 005056.

Organization, W. H. 2002. WHO traditional medicine strategy 2002-2005. Geneva: World Health Organization.

Organization, W. H. 2010. Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. Geneva: World Health Organization.

Organization, W. H. 2014. A global brief on vector-borne diseases. World Health Organization.

Organization, W. H. 2017. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. World Health Organization.

Osman, E., Koko, W., Mohammed, Z. & Galal, M. 2012. Anticercarial Activity of The Plant *Peganum harmala* (Harmal) Crude Extracts.

Osman, M. 2016. Anti-Malarial Activity Of Khaya Senegalensis. *Pharmaceutical Research,* 6.

Osman, M. E., Yassen, H. H., Deng, J. D., Mustafa, R. O. & Hussein, S. M. 2014. General phytochemical screening and antioxidant activity of some Sudanese medicinal plants. *J Forest Prod Ind,* 3**,** 292-5.

Osman, N. A. M. H. 2005. Bacteriology of Chronic Suppurative Otitis Media Study of Aerobic Bacteria. University of Khartoum.

Ouattara, Y., Sanon, S., Traorã, Y., Mahiou, V., Azas, N. & Sawadogo, L. 2006a. Antimalarial activity of *Swartzia madagascariensis* desv. (Leguminosae), *Combretum glutinosum* guill. & perr. (Combretaceae) and *Tinospora bakis* miers. (Menispermaceae), Burkina Faso medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines,* 3**,** 75-81.

Ouattara, Y., Sanon, S., Traore, Y., Mahiou, V., Azase, N. & Sawadogo, L. 2006b. Antimalarial Activity of *Swartzia Madagascariensis* Desv. (Leguminosae), *Combretum Glutinosum* Guill. & Perr. (Combretaceae) and *Tinospora Bakis* Miers. (Menispermaceae), Burkina Faso Medicinal Plants.

Ouf, S. A., Galal, A. M., Ibrahim, H. S., Hassan, A. Z., Mekhael, M. K., El-Yasergy, K. F., Abd El-Ghany, M. N., Rizk, M. A. & Hanna, A. G. 2020. Phytochemical and antimicrobial investigation of the leaves of five Egyptian mango cultivars and evaluation of their essential oils as preservatives materials. *Journal of Food Science and Technology***,** 1-13.

Oviedo, P., Herrera, O. & Caluff, M. 2012. National list of invasive and potentially invasive plants in the Republic of Cuba-2011. *Bissea: Boletín sobre Conservación de Plantas del Jardín Botánico Nacional de Cuba.,* 6**,** 22-96.

Ozioma, E.-O. J. & Chinwe, O. A. N. 2019. Herbal medicines in African traditional medicine. *Herbal Medicine,* 10**,** 191-214.

Padmaja, R., Arun, P., Prashanth, D., Deepak, M., Amit, A. & Anjana, M. 2002. Brine shrimp lethality bioassay of selected Indian medicinal plants. *Fitoterapia,* 73**,** 508-510.

Paduraru, I., Paduraru, O. & Miron, A. 2008. Assessment of antioxidant activity of Basilici herba aqueous extract-*In vitro* studies. Journal of Farmacia.

Palafox-Carlos, H., Yahia, E., Islas-Osuna, M., Gutierrez-Martinez, P., Robles-Sánchez, M. & González-Aguilar, G. 2012. Effect of ripeness stage of mango fruit (*Mangifera indica* L., cv. Ataulfo) on physiological parameters and antioxidant activity. *Scientia Horticulturae,* 135**,** 7-13.

Pan, S.-Y., Zhou, S.-F., Gao, S.-H., Yu, Z.-L., Zhang, S.-F., Tang, M.-K., Sun, J.-N., Ma, D.-L., Han, Y.-F. & Fong, W.-F. 2013. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine,* 2013.

Pandey, A. & Tripathi, S. 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry,* 2.

Pandey, A. K. 2020. Bio Diversity And Its Utilization Of Folklore Medicinal Plant In Rohilkhand Region Utter Pradesh India.

Parekh, J. & Chanda, S. 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology,* 31**,** 53-58.

Park, S.-N., Lim, Y. K., Freire, M. O., Cho, E., Jin, D. & Kook, J.-K. 2012. Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. *Anaerobe,* 18**,** 369-372.

Parkash, J., Prasad, D., Shahnaz, M. & Dev, D. 2018. Herbs as traditional medicines: A review. *Journal of Drug Delivery and Therapeutics,* 8**,** 146-150.

Parsaeimehr, A., Sargsyan, E. & Javidnia, K. 2010. A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra. *Molecules,* 15**,** 1668-78.

Parsek, M. R. & Greenberg, E. P. 2000. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proceedings of the National Academy of Sciences,* 97**,** 8789-8793.

Parvez, G. M. 2016. Pharmacological activities of mango (*Mangifera Indica*): A review. *Journal of Pharmacognosy and Phytochemistry,* 5**,** 1.

Passi, S., Aligiannis, N., Pratsinis, H., Skaltsounis, A. & Chinou, I. 2009. Biologically active Triterpenoids of *Cephalaria ambrosioides* roots. *Planta Med,* 75**,** 163-167.

Patel, S., Gheewala, N., Suthar, A. & Shah, A. 2009. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line. *Int J Pharm Pharm Sci,* 1**,** 38-46.

Pell, S. K. 2004. Molecular systematics of the cashew family (Anacardiaceae).

Pengelly, A. 2020. The constituents of medicinal plants: an introduction to the chemistry and therapeutics of herbal medicine, Routledge.

Perera De Puga, G. 1996. Ecologie des mollusques d'eau douce d'interet medical et veterinaire a cuba. Paris, EPHE.

Pesavento, G., Calonico, C., Bilia, A., Barnabei, M., Calesini, F., Addona, R., Mencarelli, L., Carmagnini, L., Di Martino, M. & Nostro, A. L. 2015. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control,* 54**,** 188-199.

Peshin, T. & Kar, H. 2017. Isolation and Characterization of β-Sitosterol-3-O-β-D-glucoside from the Extract of the Flowers of *Viola odorata*. *Journal of Pharmaceutical Research International***,** 1-8.

Peskin, A. V. & Winterbourn, C. C. 2000. A microtiter plate assay for superoxide dismutase using a water-soluble tetrazolium salt (WST-1). *Clinica chimica acta,* 293**,** 157-166.

Petersen, P. E. 2003. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. *Community Dentistry and oral epidemiology,* 31**,** 3-24.

Petrolini, F. V. B., Lucarini, R., Souza, M. G. M. D., Pires, R. H., Cunha, W. R. & Martins, C. H. G. 2013. Evaluation of the antibacterial potential of *Petroselinum crispum* and *Rosmarinus officinalis* against bacteria that cause urinary tract infections. *Brazilian Journal of Microbiology,* 44**,** 829-834.

Petruk, G., Del Giudice, R., Rigano, M. M. & Monti, D. M. 2018. Antioxidants from plants protect against skin photoaging. *Oxidative medicine and cellular longevity,* 2018.

Pink, R. M. 2016. Introduction. *Water Rights in Southeast Asia and India.* Springer.

Pinto, N. B., Castro, L. M. D., Azambuja, R. H. M., Capella, G. D. A., Moura, M. Q. D., Terto, W. D., Freitag, R. A., Jeske, S. T., Villela, M. M. & Cleff, M. B. 2019. Ovicidal and larvicidal potential of Rosmarinus officinalis to control gastrointestinal nematodes of sheep. *Revista Brasileira de Parasitologia Veterinária,* 28**,** 807-811.

Pisutthanan, S., Plianbangchang, P., Pisutthanan, N., Ruanruay, S. & Muanrit, O. 2013. Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. *Naresuan University Journal: Science and Technology (NUJST),* 12**,** 13-18.

Piwowarski, J. P., Kiss, A. K. & Kozłowska-Wojciechowska, M. 2011. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background. *Journal of ethnopharmacology,* 137**,** 937-941.

Platt, L., Easterbrook, P., Gower, E., Mcdonald, B., Sabin, K., Mcgowan, C., Yanny, I., Razavi, H. & Vickerman, P. 2016. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. *The Lancet infectious diseases,* 16**,** 797-808.

Ponnamma, S. & Manjunath, K. 2012. GC-MS Analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (nees) T. anders. *Int J Pharm Bio Sci,* 3**,** 570-576.

POONGOTHAI, P. & RAJAN, S. 2013. Antibacterial Properties of *Mangifera indica* flower extracts on Uropathogenic Escherichia coli. *Intl. J. Curr. Microbiol. Appl. Sci,* 2**,** 104-111.

Pott, D. M., Osorio, S. & Vallarino, J. G. 2019. From central to specialized metabolism: An overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. *Frontiers in plant science,* 10.

Poudel-Tandukar, K., Nanri, A., Matsushita, Y., Sasaki, S., Ohta, M., Sato, M. & Mizoue, T. 2009. Dietary intakes of α-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. *Nutrition research,* 29**,** 363-370.

Prabhu, K. & Rajan, S. 2015. Assessment of antiulcer activity of ethanolic extract of *Mangifera indica* seed kernel using acid ethanol induced ulcer model. *Int J Curr Microbiol App Sci,* 4**,** 854-860.

Prado, Y., Merino, N., Acosta, J., Herrera, J. A., Luque, Y., Hernández, I., Prado, E., Garrido, G., Delgado, R. & Rodeiro, I. 2015. Acute and 28-day subchronic toxicity studies of mangiferin, a glucosyl xanthone isolated from *Mangifera indica* L. stem bark. *Journal of Pharmacy & Pharmacognosy Research,* 3**,** 13-23.

Prakash, A. 2012. Antibacterial activity of *Mangifera indica* kernel extracts. *Journal Biotechnol Biomater,* 4**,** 5964.

Quideau, S., Deffieux, D., Douat-Casassus, C. & Pouysegu, L. 2011. Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition,* 50**,** 586-621.

Rabib, H., Elagdi, C., Hsaine, M., Fougrach, H., Koussa, T. & Badri, W. 2020. Antioxidant and antibacterial activities of the essential oil of moroccan *Tetraclinis articulata* (Vahl) masters. *Biochemistry Research International,* 2020.

Rahman, I. 2012. Pharmacological antioxidant strategies as therapeutic interventions for COPD. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1822**,** 714-728.

Rahman, M., Rahaman, M., Islam, M., Hossain, M., Mannan Mithi, F., Ahmed, M., Saldías, M., Akkol, E. K. & Sobarzo-Sánchez, E. 2021. Multifunctional Therapeutic Potential of Phytocomplexes and Natural Extracts for Antimicrobial Properties. *Antibiotics,* 10**,** 1076.

Rajan, S., Suganya, H., Thirunalasundari, T. & Jeeva, S. 2012. Antidiarrhoeal efficacy of *Mangifera indica* seed kernel on Swiss albino mice. *Asian Pacific journal of tropical medicine,* 5**,** 630-633.

Raju, N. V., Sukumar, K., Reddy, G. B., Pankaj, P., Muralitharan, G. & Annapareddy, S. 2019. *In-vitro* studies on antitumour and antimicrobial activities of Methanolic kernel extract of *Mangifera indica* L. cultivar Banganapalli. *Biomedical and Pharmacology Journal,* 12**,** 357-362.

Ramos, E. H., Moraes, M. M., Nerys, L. L. D. A., Nascimento, S. C., Militão, G. C., De Figueiredo, R. C., Da Camara, C. A. & Silva, T. G. 2014. Chemical composition, leishmanicidal and cytotoxic activities of the essential oils from *Mangifera indica* L. var. Rosa and Espada. *BioMed research international,* 2014.

Rang, H. 2007. Rang and Dale's Pharmacology (Churchill Livingstone elsevier). *Philadelphia, PA.*

Rašković, A., Milanović, I., Pavlović, N., Ćebović, T., Vukmirović, S. & Mikov, M. 2014. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. *BMC complementary and alternative medicine,* 14**,** 1-9.

Rasool Hassan, B. 2012. Medicinal plants (importance and uses). *Pharmaceut Anal Acta,* 3**,** e139.

Rawi, S., Al-Hazmi, M. & Seif Al Nassr, F. 2011. Comparative study of the molluscicidal activity of some plant extracts on the snail vector of *Schistosoma mansoni, Biomphalaria alexandrina*. *International Journal of Zoological Research,* 7**,** 169-189.

Reddeman, R. A., Glávits, R., Endres, J. R., Clewell, A. E., Hirka, G., Vertesi, A., Beres, E. & Szakonyine, I. P. 2019. A toxicological evaluation of mango leaf extract (*Mangifera indica*) containing 60% mangiferin. *Journal of toxicology,* 2019.

Reichling, J. 2018. Plant–microbe interactions and secondary metabolites with antibacterial, antifungal and antiviral properties. *Annual plant reviews online***,** 214-347.

Reva, A. 2019. *Enzymology of Gentamicin Biosynthesis.* University of Cambridge.

Riaz, T., Nawaz Khan, S. & Javaid, A. 2010. Management of corm-rot disease of gladiolus by plant extracts. *Natural product research,* 24**,** 1131-1138.

Ribeiro, S., Barbosa, L., Queiroz, J., Knödler, M. & Schieber, A. 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food chemistry,* 110**,** 620-626.

Rios, J.-L. & Recio, M. C. 2005. Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology,* 100**,** 80-84.

Roa-Linares, V. C., Brand, Y. M., Agudelo-Gomez, L. S., Tangarife-Castaño, V., Betancur-Galvis, L. A., Gallego-Gomez, J. C. & González, M. A. 2016. Anti-herpetic and anti-dengue activity of abietane ferruginol analogues synthesized from (+)-dehydroabietylamine. *European journal of medicinal chemistry,* 108**,** 79-88.

Roca-Mezquita, C., Graniel-Sabido, M., Moo-Puc, R. E., Leon-Deniz, L. V., Gamboa-León, R., Arjona-Ruiz, C., Tun-Garrido, J., Mirón-López, G. & Mena-Rejon, G. J. 2016. Antiprotozoal activity of extracts of *Elaeodendron trichotomum* (Celastraceae). *African Journal of Traditional, Complementary and Alternative Medicines,* 13**,** 162-165.

Rodrigues, M. L. & Nosanchuk, J. D. 2020. Fungal diseases as neglected pathogens: A wake-up call to public health officials. *PLoS neglected tropical diseases,* 14**,** e0007964.

Rose, A. H. 2012. *Economic microbiology: primary products of metabolism*, Elsevier.

Rosenbaum, C. D., Carreiro, S. P. & Babu, K. M. 2012. Here today, gone tomorrow… and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine, and piperazines. *Journal of medical toxicology,* 8**,** 15-32.

Roy, A., Jauhari, N. & Bharadvaja, N. 2018. 6 Medicinal plants as. *Anticancer Plants,* 2**,** 109.

Ruesgas-Ramón, M., Figueroa-Espinoza, M. C. & Durand, E. 2017. Application of deep eutectic solvents (DES) for phenolic compounds extraction: overview, challenges, and opportunities. *Journal of Agricultural and Food Chemistry,* 65**,** 3591-3601.

Saad, B., Zaid, H., Shanak, S. & Kadan, S. 2017. Introduction to medicinal plant safety and efficacy. *Anti-diabetes and anti-obesity medicinal plants and phytochemicals.* Springer.

Saadabi, A. M. & Moglad, E. 2011. Experimental evaluation of certain Sudanese plants used in folkloric medicine for their antibacterial activity (in-vitro tests). *J Appl Sci Res,* 7**,** 253-6.

Sady, H., Al-Mekhlafi, H. M., Ngui, R., Atroosh, W. M., Al-Delaimy, A. K., Nasr, N. A., Dawaki, S., Abdulsalam, A. M., Ithoi, I. & Lim, Y. A. 2015. Detection of Schistosoma mansoni and Schistosoma haematobium by real-time PCR with high resolution melting analysis. *International journal of molecular sciences,* 16**,** 16085-16103.

Saeed, M. E., Abdelgadir, H., Sugimoto, Y., Khalid, H. E. & Efferth, T. 2015. Cytotoxicity of 35 medicinal plants from Sudan towards sensitive and multidrug-resistant cancer cells. *Journal of ethnopharmacology,* 174**,** 644-658.

Saetung, A., Itharat, A., Dechsukum, C., Wattanapiromsakul, C., Keawpradub, N. & Ratanasuwan, P. 2005. Cytotoxic activity of Thai medicinal plants for cancer treatment. *Songklanakarin J Sci Technol,* 27**,** 469-478.

Sahoo, R. R. 2012. Antioxidant &antimicrobial efficacy of Ficus religiosa L. & Ficus benghalensis L. PLANT.

Sahrawat, A., Pal, S. & Shahi, S. 2013. Antibacterial activity of *Mangifera indica* (mango) leaves against drug resistant bacterial strains. *International Journal of Advanced Research,* 1**,** 82-86.

Salati, S., Doudi, M. & Madani, M. 2018. The biological synthesis of silver nanoparticles by mango plant extract and its anti-Candida effects. *Journal of Applied Biotechnology Reports,* 5**,** 157-161.

Saleem, M., Tanvir, M., Akhtar, M. F., Iqbal, M. & Saleem, A. 2019. Antidiabetic potential of *Mangifera indica* L. cv. Anwar Ratol leaves: medicinal application of food wastes. *Medicina,* 55**,** 353.

Salehi, B., Lopez-Jornet, P., Pons-Fuster López, E., Calina, D., Sharifi-Rad, M., Ramírez-Alarcón, K., Forman, K., Fernández, M., Martorell, M. & Setzer, W. N. 2019. Plant-derived bioactives in oral mucosal lesions: A key emphasis to curcumin, lycopene, chamomile, aloe vera, green tea and coffee properties. *Biomolecules,* 9**,** 106.

Salem, M. Z., Zayed, M. Z., Ali, H. M. & Abd El-Kareem, M. S. 2016. Chemical composition, antioxidant and antibacterial activities of extracts from Schinus molle wood branch growing in Egypt. *Journal of wood science,* 62**,** 548-561.

Salgueiro, L., Martins, A. & Correia, H. 2010. Raw materials: the importance of quality and safety. A review. *Flavour and Fragrance Journal,* 25**,** 253-271.

Samuelsson, G. & Bohlin, L. 2017. Drugs of natural origin: a treatise of pharmacognosy, CRC Press Inc.

Santhirasegaram, V., Razali, Z., George, D. S. & Somasundram, C. 2015. Effects of thermal and non-thermal processing on phenolic compounds, antioxidant activity and sensory attributes of chokanan mango (*Mangifera indica* L.) juice. *Food and Bioprocess Technology,* 8**,** 2256-2267.

Sanusi, B. M., Garba, A., Muhammad, A., Mohammed, A. & David, O. 2011. Phytochemical screening and antimicrobial efficacy of aqueous and methanolic extract of *Mangifera indica* (mango stem bark).

Sarker, S. D. & Nahar, L. 2012. An introduction to natural products isolation. *Natural products isolation.* Springer.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. & Latha, L. Y. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines,* 8.

Satyal, P., Jones, T. H., Lopez, E. M., Mcfeeters, R. L., Ali, N. A. A., Mansi, I., Al-Kaf, A. G. & Setzer, W. N. 2017. Chemotypic characterization and biological activity of Rosmarinus officinalis. *Foods,* 6**,** 20.

Sawadogo, W. R., Schumacher, M., Teiten, M.-H., Dicato, M. & Diederich, M. 2012. Traditional West African pharmacopeia, plants and derived compounds for cancer therapy. *Biochemical pharmacology,* 84**,** 1225-1240.

Sawangjaroen, N. & Sawangjaroen, K. 2005. The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: Blastocystis hominis. *Journal of Ethnopharmacology,* 98**,** 67-72.

Scartezzini, P. & Speroni, E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of ethnopharmacology,* 71**,** 23-43.

Schulze-Kaysers, N., Feuereisen, M. & Schieber, A. 2015. Phenolic compounds in edible species of the Anacardiaceae family–a review. *RSC Advances,* 5**,** 73301-73314.

Schwartz, C. & Fallon, P. G. 2018. Schistosoma "eggs-iting" the host: granuloma formation and egg excretion. *Frontiers in immunology,* 9**,** 2492.

Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofziger, T. H., Currens, M. J., Seniff, D. & Boyd, M. R. 1988. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer research,* 48**,** 4827-4833.

Seigler, D. S. 2012. *Plant secondary metabolism*, Springer Science & Business Media.

Sekretar, S., Schmidt, S., Vajdak, M., Zahradnikova, L. & Annus, J. 2004. Antioxidative and antimicrobial effects of some natural extracts in lard. *Czech journal of food sciences,* 22**,** 215.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. & De, B. 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *International journal of pharmaceutical sciences review and research,* 3**,** 91-100.

Seol, G. H. & Kim, K. Y. 2016. Eucalyptol and its role in chronic diseases. *Drug discovery from mother nature***,** 389-398.

Sepehri, Z., Javadian, F., Khammari, D. & Hassanshahian, M. 2016. Antifungal effects of the aqueous and ethanolic leaf extracts of *Echinophora platyloba* and *Rosmarinus officinalis*. *Current medical mycology,* 2**,** 30.

Sermakkani, M. & Thangapandian, V. 2012. GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian J Pharm Clin Res,* 5**,** 90-94.

Severino, J. F., Goodman, B. A., Kay, C. W., Stolze, K., Tunega, D., Reichenauer, T. G. & Pirker, K. F. 2009. Free radicals generated during oxidation of green tea polyphenols: electron paramagnetic resonance spectroscopy combined with density functional theory calculations. *Free Radical Biology and Medicine,* 46**,** 1076-1088.

Shah, K., Patel, M., Patel, R. & Parmar, P. 2010. *Mangifera indica* (mango). *Pharmacognosy reviews,* 4**,** 42.

Shahat, A. A. & Marzouk, M. S. 2013. Tannins and related compounds from medicinal plants of Africa. *Medicinal Plant Research in Africa.* Elsevier.

Sharma, N. & Kumar, G. 2015. Exploration of Some Ethnomedicinal Plants Used by Natives of Hamirpur District in Himachal Pradesh. *Asian Agri-History,* 19.

Sharma, R. R. 2012. Preliminary phytochemical screening of some indigenous medicinal plant leaves extract in regulation of antidiabetic activity. *Sci Res Rep,* 2**,** 307-310.

Shayoub, M. E. A., ., A. S. K., , S., M. El., A. A, Asma. Noureldaim Mahmoud, , A. M., Elhassan, ., A. M. E. A., ., A. D. H. D., ., A.-O. S. A. J., , H. & Osman, M. 2016. Anti-Malarial Activity of *Khaya Senegalensis*. *Pharmaceutical Research,* 6.

Sheikhaldin, E. A. 2014. Comparison between INAA and ICP-OES: Analysis of Sudanese Medicinal Plants. University of Khartoum.

Sheng-Ji, P. 2001. Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharmaceutical biology,* 39**,** 74-79.

Sher, A. 2009. Antimicrobial activity of natural products from medicinal plants. *Gomal Journal of Medical Sciences,* 7**,** 72-78.

Sherma, J. 1971. Paper Chromatography. Vol. II in the series Paper Chromatography and Electrophoresis (Zweig, G. and Whitaker, JR, eds.). Academic Press, New York.

Shibahara, A., Yamamoto, K., Shinkai, K., Nakayama, T. & Kajimoto, G. 1993. Cis-9, cis-15-octadecadienoic acid: a novel fatty acid found in higher plants. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism,* 1170**,** 245-252.

Shimada, K., Fujikawa, K., Yahara, K. & Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of agricultural and food chemistry,* 40**,** 945-948.

Shimoi, K., Masuda, S., Shen, B., Furugori, M. & Kinae, N. 1996. Radioprotective effects of antioxidative plant flavonoids in mice. *Mutation Research/Fundamental and Molecular mechanisms of mutagenesis,* 350**,** 153-161.

Shirley, D.-A. & Moonah, S. 2016. Fulminant amebic colitis after corticosteroid therapy: a systematic review. *PLoS neglected tropical diseases,* 10**,** e0004879.

Shirley, D.-A. T., Farr, L., Watanabe, K. & Moonah, S. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open forum infectious diseases, 2018. Oxford University Press US, ofy161.

Shivakumar, B., Ramaiah, M., Hema, M., Vijay Kumar, M. & Vaidya, V. 2012. Quantitative determination of total content of phenol, flavonoid and tannin in leaf extract of *Barlaria Buxifolia* Linn. *Am. J. PharmTech. Res,* 2**,** 418-422.

Shuai, L. & Luterbacher, J. 2016. Organic solvent effects in biomass conversion reactions. *ChemSusChem,* 9**,** 133-155.

Siddhuraju, P. & Becker, K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of agricultural and food chemistry,* 51**,** 2144-2155.

Siddig, H. S., Mohammed, I. A., Mohammed, M. N. & Bashir, A. M. 2017. Prevalence of intestinal parasites among selected group of primary school children in Alhag Yousif Area, Khartoum, Sudan. *International Journal of Medical Research & Health Sciences,* 6**,** 125-131.

Siddiqui, I. A. & Sanna, V. 2016. Impact of nanotechnology on the delivery of natural products for cancer prevention and therapy. *Molecular nutrition & food research,* 60**,** 1330-1341.

Silva, S., Alves, N., Silva, P., Vieira, T., Maciel, P., Castellano, L. R., Bonan, P., Velozo, C. & Albuquerque, D. 2019. Antibacterial Activity of *Rosmarinus officinalis*, *Zingiber officinale*, *Citrus aurantium* bergamia, and *Copaifera officinalis* Alone and in Combination with Calcium Hydroxide against Enterococcus faecalis. *BioMed research international,* 2019.

Singariya, P., Kumar, P. & Mourya, K. 2011. *In-vitro* Bio-efficacy of Stem extracts of Ashwagandha against Some Pathogens. *Journal of Current Pharmaceutical Research. 2011d,* 8**,** 25-30.

Singh, M., Khatoon, S., Singh, S., Kumar, V., Rawat, A. K. S. & Mehrotra, S. 2010. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. *Pharmacognosy research,* 2**,** 254.

Singh, R., Singh, S., Kumar, S. & Arora, S. 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food and chemical toxicology,* 45**,** 1216-1223.

Sleet, R. & Brendel, K. 1983. Improved methods for harvesting and counting synchronous populations of Artemia nauplii for use in developmental toxicology. *Ecotoxicology and environmental safety,* 7**,** 435-446.

Smith, D., Davern, K., Board, P., Tiu, W., Garcia, E. & Mitchell, G. 1986. Mr 26,000 antigen of *Schistosoma japonicum* recognized by resistant WEHI 129/J mice is a parasite glutathione S-transferase. *Proceedings of the National Academy of Sciences,* 83**,** 8703-8707.

Sofowora, A. 1996. Medicinal plants and traditional medicine in Africa, Karthala.

Somani, S. J., Modi, K. P., Majumdar, A. S. & Sadarani, B. N. 2015. Phytochemicals and their potential usefulness in inflammatory bowel disease. *Phytotherapy research,* 29**,** 339-350.

Song, L.-G., Wu, X.-Y., Sacko, M. & Wu, Z.-D. 2016. History of schistosomiasis epidemiology, current status, and challenges in China: on the road to schistosomiasis elimination. *Parasitology research,* 115**,** 4071-4081.

Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I. & Bahorun, T. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular mechanisms of mutagenesis,* 579**,** 200-213.

Soto-Vaca, A., Gutierrez, A., Losso, J. N., Xu, Z. & Finley, J. W. 2012. Evolution of phenolic compounds from color and flavor problems to health benefits. *Journal of Agricultural and Food Chemistry,* 60**,** 6658-6677.

Soulaimani, B., El Hidar, N., El Fakir, S. B., Mezrioui, N., Hassani, L. & Abbad, A. 2021. Combined antibacterial activity of essential oils extracted from *Lavandula maroccana* (Murb.), *Thymus pallidus* Batt. and *Rosmarinus officinalis* L. against antibiotic-resistant Gram-negative bacteria. *European Journal of Integrative Medicine,* 43**,** 101312.

Spanova, M. & Daum, G. 2011. Squalene–biochemistry, molecular biology, process biotechnology, and applications. *European journal of lipid science and technology,* 113**,** 1299-1320.

Srivastava, M. 2010. *High-performance thin-layer chromatography (HPTLC)*, Springer Science & Business Media.

Staniszewska, M., Bondaryk, M., Swoboda-Kopec, E., Siennicka, K., Sygitowicz, G. & Kurzatkowski, W. 2013. *Candida albicans* morphologies revealed by scanning electron microscopy analysis. *Brazilian Journal of Microbiology,* 44**,** 813-821.

Stead, D. A. 2000. Current methodologies for the analysis of aminoglycosides. *Journal of Chromatography B: Biomedical Sciences and Applications,* 747**,** 69-93.

Steffen, W., Crutzen, P. J. & Mcneill, J. R. 2007. The Anthropocene: are humans now overwhelming the great forces of nature. *AMBIO: A Journal of the Human Environment,* 36**,** 614-622.

STOCKER-Wörgötter, E. 2008. Metabolic diversity of lichen-forming ascomycetous fungi: culturing, polyketide and shikimate metabolite production, and PKS genes. *Natural product reports,* 25**,** 188-200.

Stockert, J. C., Blázquez-Castro, A., Cañete, M., Horobin, R. W. & Villanueva, Á. 2012. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta histochemica,* 114**,** 785-796.

Stojiljkovic, J., Trajchev, M., Nakov, D. & Petrovska, M. 2018. Antibacterial activities of rosemary essential oils and their components against pathogenic bacteria. *Advances in Cytology & Pathology*.

Stothard, J. R., Chitsulo, L., Kristensen, T. & Utzinger, J. 2009. Control of schistosomiasis in sub-Saharan Africa: progress made, new opportunities and remaining challenges. *Parasitology,* 136**,** 1665-1675.

Stupar, M., Grbić, M. L., Džamić, A., Unković, N., Ristić, M., Jelikić, A. & Vukojević, J. 2014. Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. *South African Journal of Botany,* 93**,** 118-124.

Sturrock, H. J., Gething, P. W., Ashton, R. A., Kolaczinski, J. H., Kabatereine, N. B. & Brooker, S. 2011. Planning schistosomiasis control: investigation of alternative sampling strategies for *Schistosoma mansoni* to target mass drug administration of praziquantel in East Africa. *International health,* 3**,** 165-175.

Sudha, T., Chidambarampillai, S. & Mohan, V. 2013. GC-MS analysis of bioactive components of aerial parts of *Fluggea leucopyrus* Willd.(Euphorbiaceae). *Journal of applied pharmaceutical science,* 3**,** 126.

Sulaiman, I. S. C., Basri, M., Chan, K. W., Ashari, S. E., Masoumi, H. R. F. & Ismail, M. 2015. *In vitro* antioxidant, cytotoxic and phytochemical studies of *Clinacanthus nutans* Lindau leaf extracts. *African Journal of Pharmacy and Pharmacology,* 9**,** 861-874.

Sultana, B., Hussain, Z., Asif, M. & Munir, A. 2012. Investigation on the antioxidant activity of leaves, peels, stems bark, and kernel of mango (*Mangifera indica* L.). *Journal of food science,* 77**,** C849-C852.

Suntar, I. 2019. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews***,** 1-11.

Svoboda, K. P. & Deans, S. 1992. A study of the variability of rosemary and sage and their volatile oils on the British market: their antioxidative properties. *Flavour and Fragrance Journal,* 7**,** 81-87.

Szumny, A., Figiel, A., Gutierrez-Ortíz, A. & Carbonell-Barrachina, Á. A. 2010. Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. *Journal of Food engineering,* 97**,** 253-260.

Taddaow, K., Supap, S., Yingmance, T. & Kanokporn, S. 2018. Antibacterial Effects of Thai Mango (*Mangifera indica* Linn) Leaves Against Acne-inducing Bacteria. *Science International Journal,* 30**,** 449-453.

Talba, A., Suleiman, M., Raji, M. & Oniye, S. 2014. Phytochemical screening and *in-vitro* antibacterial activity of *Mangifera indica* (Mango) kernel on *Aeromonas caviae*. *IOSR Journal of Pharmacy,* 4**,** 45-50.

Tapiero, H., Tew, K., Ba, G. N. & Mathe, G. 2002. Polyphenols: do they play a role in the prevention of human pathologies? *Biomedicine & pharmacotherapy,* 56**,** 200-207.

Tarranum, A., Chauhan, R., Kumar, R. & Chauhan, M. 2015. Comparison of Physicochemical Properties of Some Edible Oils. *Advertisement Tariff***,** 28.

Tchuente, L.-A. T., Rollinson, D., Stothard, J. R. & Molyneux, D. 2017. Moving from control to elimination of schistosomiasis in sub-Saharan Africa: time to change and adapt strategies. *Infectious Diseases of Poverty,* 6**,** 42.

Tempone, A., Borborema, S. T., De Andrade Jr, H., De Amorim Gualda, N., Yogi, A., Carvalho, C. S., Bachiega, D., Lupo, F., Bonotto, S. & Fischer, D. C. H. 2005. Antiprotozoal activity of Brazilian plant extracts from isoquinoline alkaloid-producing families. *Phytomedicine,* 12**,** 382-390.

Thawabteh, A., Juma, S., Bader, M., Karaman, D., Scrano, L., Bufo, S. A. & Karaman, R. 2019. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. *Toxins,* 11**,** 656.

Thetiot-Laurent, S. A. L., Boissier, J., Robert, A. & Meunier, B. 2013. Schistosomiasis chemotherapy. *Angewandte Chemie International Edition,* 52**,** 7936-7956.

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A. & Dzobo, K. 2018. Natural products for drug discovery in the 21st century: innovations for novel drug discovery. *International journal of molecular sciences,* 19**,** 1578.

Thompson, R. & Smith, A. 2011. Zoonotic enteric protozoa. *Veterinary parasitology,* 182**,** 70-78.

Tittikpina, N. K., Ejike, C. E., Estevam, E. C., Nasim, M. J., Griffin, S., Chaimbault, P., Kirsch, G., Atakpama, W., Batawila, K. & Jacob, C. 2016. Togo to go: Products and compounds derived from local plants for the treatment of diseases endemic in Sub-Saharan Africa. *African Journal of Traditional, Complementary and Alternative Medicines,* 13**,** 85-94.

Tiwari, R. & Rana, C. 2015. Plant secondary metabolites: a review. *International Journal of Engineering Research and General Science,* 3**,** 661-670.

Tom, H. A. M. 2016. Prevalence of Hospital Aquired Infection in Postoperative Wound and Antibiotic Resistance at Kassala Teaching Hospital, Kassala Stata, Sudan (2014–2016). University of Gezira.

Tomi, K., Kitao, M., Konishi, N., Murakami, H., Matsumura, Y. & Hayashi, T. 2016. Enantioselective GC–MS analysis of volatile components from rosemary (*Rosmarinus officinalis* L.) essential oils and hydrosols. *Bioscience, biotechnology, and biochemistry,* 80**,** 840-847.

Tona, L., Kambu, K., Ngimbi, N., Cimanga, K. & Vlietinck, A. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. *Journal of ethnopharmacology,* 61**,** 57-65.

Tor-Anyiin, T., Akpuaka, M. & Oluma, H. 2011. Phytochemical and antimicrobial studies on stem bark extract of *Sterculia setigera*, Del. *African Journal of Biotechnology,* 10**,** 11011-11015.

Trease, G. E. & Evans, W. C. 1983. Pharmacognosy.

Trias, R., Bañeras, L., Badosa, E. & Montesinos, E. 2008. Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International journal of food microbiology,* 123**,** 50-60.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. & Yangsabai, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines,* 5**,** 93.

Tyagi, T. & Agarwal, M. 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and phytochemistry,* 6**,** 195-206.

Ul-Haq, I., Ullah, N., Bibi, G., Kanwal, S., Ahmad, M. S. & Mirza, B. 2012. Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iranian journal of pharmaceutical research: IJPR,* 11**,** 241.

Ulbricht, C., Abrams, T. R., Brigham, A., Ceurvels, J., Clubb, J., Curtiss, W., Kirkwood, C. D., Giese, N., Hoehn, K. & Iovin, R. 2010. An evidence-based systematic review of rosemary (*Rosmarinus officinalis*) by the Natural Standard Research Collaboration. *Journal of dietary supplements,* 7**,** 351-413.

Ultee, A., Bennik, M. & Moezelaar, R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. *Applied and environmental microbiology,* 68**,** 1561.

Uritu, C. M., Mihai, C. T., Stanciu, G.-D., Dodi, G., Alexa-Stratulat, T., Luca, A., Leon-Constantin, M.-M., Stefanescu, R., Bild, V. & Melnic, S. 2018. Medicinal plants of the family Lamiaceae in pain therapy: A review. *Pain Research and Management,* 2018.

Vaghasiya, Y., Patel, H. & Chanda, S. 2011. Antibacterial activity of *Mangifera indica* L. seeds against some human pathogenic bacterial strains. *African Journal of Biotechnology,* 10**,** 15788-15794.

Van Rensburg, C., Anderson, R., Joone, G., Myer, M. & O'sullivan, J. 1997. Novel tetramethylpiperidine-substituted phenazines are potent inhibitors of P-glycoprotein activity in a multidrug resistant cancer cell line. *Anti-cancer drugs,* 8**,** 708-713.

Vanhaecke, P., Persoone, G., Claus, C. & Sorgeloos, P. 1981. Proposal for a short-term toxicity test with Artemia nauplii. *Ecotoxicology and environmental safety,* 5**,** 382-387.

Varakumar, S., Kumar, Y. S. & Reddy, O. V. S. 2011. Carotenoid composition of mango (*Mangifera indica* L.) wine and its antioxidant activity. *Journal of Food Biochemistry,* 35**,** 1538-1547.

Vardhan, P. V. & Shukla, L. I. 2017. Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. *International journal of radiation biology,* 93**,** 967-979.

Vazquez-Olivo, G., Antunes-Ricardo, M., Gutierrez-Uribe, J. A., Osuna-Enciso, T., León-Felix, J. & Heredia, J. B. 2019. Cellular antioxidant activity and *in vitro* intestinal permeability of phenolic compounds from four varieties of mango bark (*Mangifera indica* L.). *Journal of the Science of Food and Agriculture,* 99**,** 3481-3489.

Verma, S., Yadav, S. & Singh, A. 2015. *In vitro* Antibacterial Activity and Phytochemical Analysis of *Mangifera indica* L flower. Extracts against Pathogenic. Microorganisms. *Journal of Pharmacology & Clinical Toxicology,* 3**,** 1053.

Vermerris, W. & Nicholson, R. 2007. *Phenolic compound biochemistry*, Springer Science & Business Media.

Vijayakumar, M., Priya, K., Nancy, F., Noorlidah, A. & Ahmed, A. 2013. Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using Artemisia nilagirica. *Industrial Crops and Products,* 41**,** 235-240.

Viruel, M., Escribano, P., Barbieri, M., Ferri, M. & Hormaza, J. 2005. Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Molecular Breeding,* 15**,** 383-393.

Wahid, A. 2012. *In vitro Phytochemical and biological Investigation of plant Lannea coromandelica (Family: Anacardiaceae).* East West University.

Waksmundzka-Hajnos, M., Sherma, J. & Kowalska, T. 2008. *Thin layer chromatography in phytochemistry,* CRC Press.

Wang, C., Zhang, W., Wong, J. H., Ng, T. & Ye, X. 2019. Diversity of potentially exploitable pharmacological activities of the highly prized edible medicinal fungus Antrodia camphorata. *Applied microbiology and biotechnology,* 103**,** 7843-7867.

Wang, P., Goggins, W. B. & Chan, E. Y. 2018a. A time-series study of the association of rainfall, relative humidity and ambient temperature with hospitalizations for rotavirus and norovirus infection among children in Hong Kong. *Science of the Total Environment,* 643**,** 414-422.

Wang, W., Li, N., Luo, M., Zu, Y. & Efferth, T. 2012. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. *Molecules,* 17**,** 2704-2713.

Wang, Z.-D., Liu, Q., Liu, H.-H., Li, S., Zhang, L., Zhao, Y.-K. & Zhu, X.-Q. 2018b. Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. *Parasites & vectors,* 11**,** 28.

Wangchuk, P., Yeshi, K. & Jamphel, K. 2017. Pharmacological, ethnopharmacological, and botanical evaluation of subtropical medicinal plants of Lower Kheng region in Bhutan. *Integrative medicine research,* 6**,** 372-387.

Wangkheirakpam, S. 2018. Traditional and folk medicine as a target for drug discovery. *Natural Products and Drug Discovery.* Elsevier.

Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K. & Schempp, C. Á. 2007. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine,* 14**,** 508-516.

Wichtl, M. 2004. Herbal drugs and phytopharmaceuticals*: a handbook for practice on a scientific basis*, Medpharm GmbH Scientific Publishers.

Win, D. T. 2005. Oleic acid-The anti-breast cancer component in olive oil. *Au Jt,* 9**,** 75-78.

Winborn, J. & Kerrigan, S. 2019. Quantitative analysis of desomorphine in blood and urine using solid phase extraction and gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci,* 1106-1107**,** 43-49.

Wolfe, K., Wu, X. & Liu, R. H. 2003. Antioxidant activity of apple peels. *Journal of agricultural and food chemistry,* 51**,** 609-614.

Wollinger, A., Perrin, E., Chahboun, J., Jeannot, V., Touraud, D. & Kunz, W. 2016. Antioxidant activity of hydro distillation water residues from *Rosmarinus officinalis* L. leaves determined by DPPH assays. *Comptes Rendus Chimie,* 19**,** 754-765.

Yakubu, M. & Salimon, S. 2015. Antidiarrhoeal activity of aqueous extract of Mangifera indica L. leaves in female albino rats. *Journal of ethnopharmacology,* 163**,** 135-141.

Yan, X. 2015. *Population structure of Staphylococcus aureus in China.*

Yang, L., Wang, K., Li, H., Denstedt, J. D. & Cadieux, P. A. 2014. The influence of urinary pH on antibiotic efficacy against bacterial uropathogens. *Urology,* 84**,** 731. e1-731. e7.

Yao, X. T., Ling, P. X., Jiang, S., Lai, P. X. & Zhu, C. G. 2013. Analysis of the essential oil from *Gaillardia pulchella* Foug. and its antioxidant activity. *Journal of oleo science,* 62**,** 329-333.

Yu, F.-R., Lian, X.-Z., Guo, H.-Y., Mcguire, P. M., Li, R.-D., Wang, R. & Yu, F.-H. 2005. Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *J Pharm Pharm Sci,* 8**,** 528-35.

Yu, F., Takahashi, T., Moriya, J., Kawaura, K., Yamakawa, J., Kusaka, K., Itoh, T., Morimoto, S., Yamaguchi, N. & Kanda, T. 2006. Traditional Chinese medicine and Kampo: a review from the distant past for the future. *Journal of International Medical Research,* 34**,** 231-239.

Yu, X. M., Ramiandrasoa, F., Guetzoyan, L., Pradines, B., Quintino, E., Gadelle, D., Forterre, P., Cresteil, T., Mahy, J. P. & Pethe, S. 2012. Synthesis and biological evaluation of acridine derivatives as antimalarial agents. *ChemMedChem,* 7**,** 587-605.

Yu, Y., Shen, M., Song, Q. & Xie, J. 2018. Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. *Carbohydrate polymers,* 183**,** 91-101.

Yusuf, N. A., Yusri, Y. M., Ismail, N. & Vythilingam, I. 2007. Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia. *Tropical biomedicine,* 24**,** 55-62.

Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T. & Hossain, N. 2017. A review on antibiotic resistance: alarm bells are ringing. *Cureus,* 9.

Zargari, A. 1997. Iranian medicinal plants. *Tehran: Tehran University Publications*.

Zhong, M., Liu, Y., Liu, J., Di, D., Xu, M., Yang, Y., Li, W., Chen, Y. & Liu, J. 2014. Isocorydine derivatives and their anticancer activities. *Molecules,* 19**,** 12099-12115.

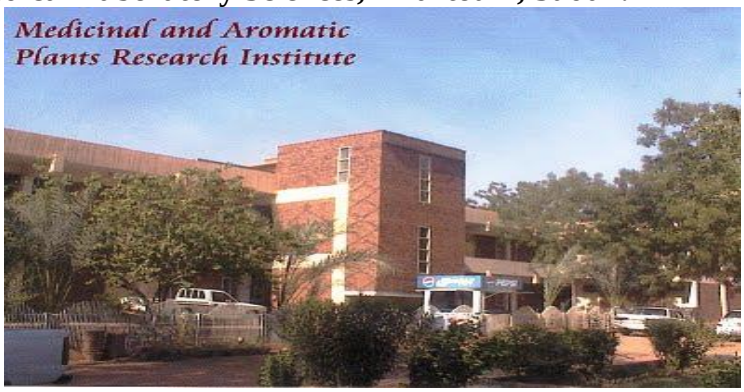
Znati, M., Jabrane, A., Hajlaoui, H., Harzallah-Skhiri, F., Bouajila, J., Casanova, J. & Jannet, H. B. 2012. Chemical composition and *in vitro* evaluation of antimicrobial and anti-acetylcholinesterase properties of the flower oil of *Ferula lutea*. *Natural product communications,* 7**,** 1934578X1200700738.

Zoral, M., Futami, K., Endo, M., Maita, M. & Katagiri, T. 2017. Anthelmintic activity of *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) infections in Cyprinus carpio. *Veterinary parasitology,* 247**,** 1-6.

Appendices

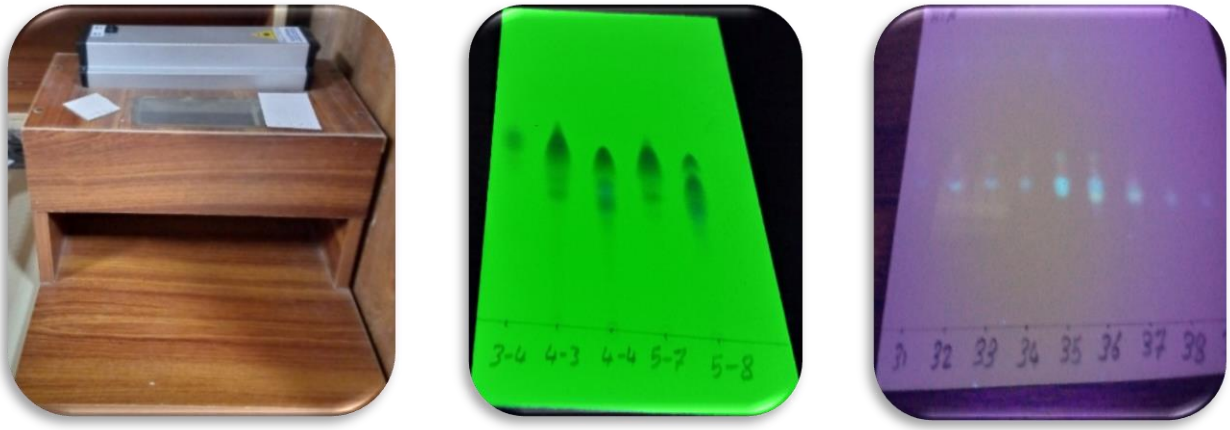
Appendix (1): International Center for Chemical and Biological Sciences (ICCBS), H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-Pakistan.

Appendix (2): International University of Africa (IUA), Faculty of Pure and Applied Sciences and Faculty of Medical Laboratory Sciences, Khartoum, Sudan.



Appendix (3): National Centre for Research (NCR), Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

(A) (B) (C)

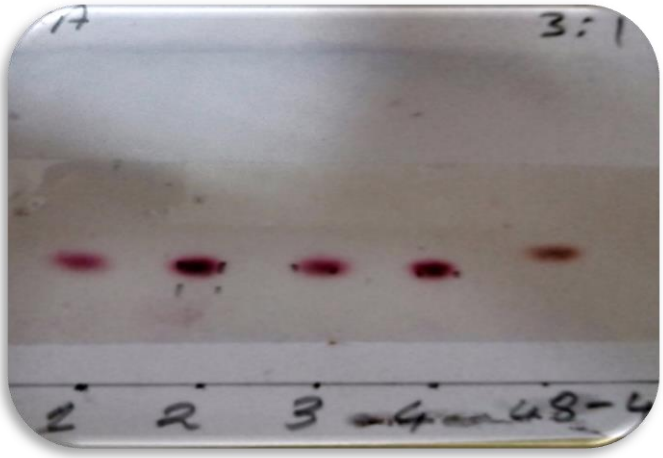


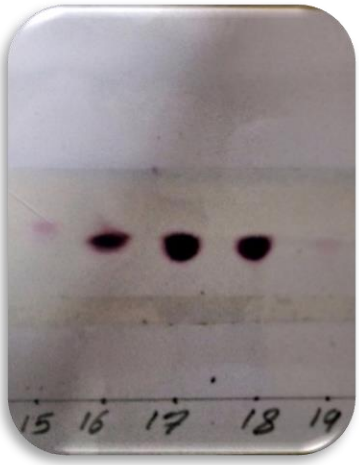
Appendix (4): (A) UV light at (B) 245 nm and (C) 365 nm.

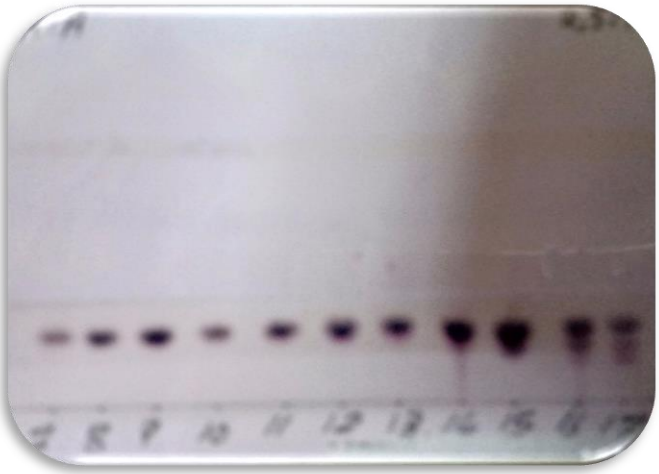
(A) (B) (C)

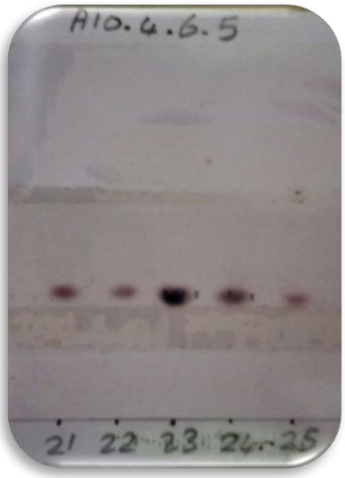
Appendix (5): Column chromatography to obtain purified bioactive compounds as (A) silica gel column, (B) C-18 column and (C) Sephadex column.

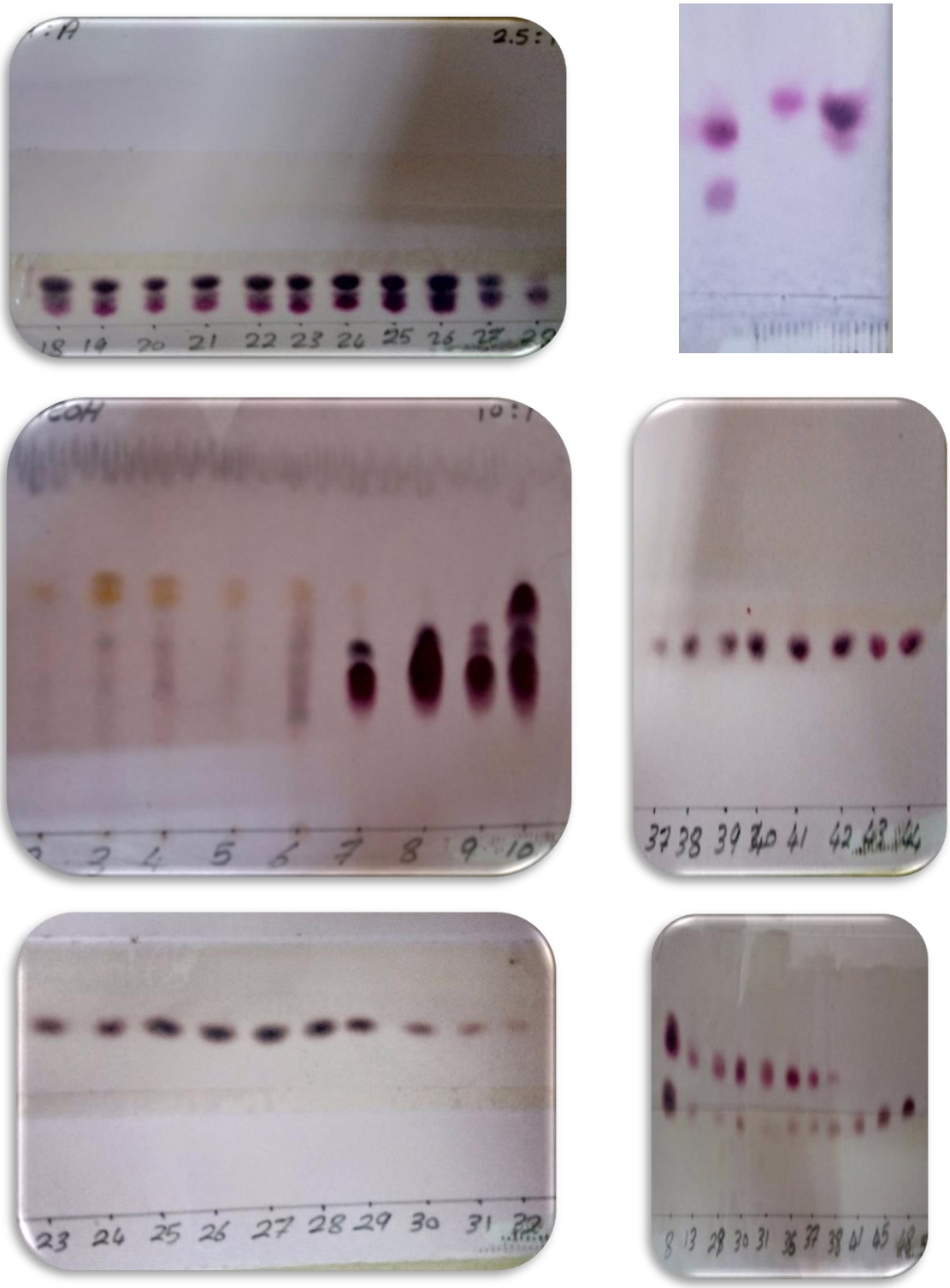
Appendix (6): High Performance Liquid Chromatography (HPLC).







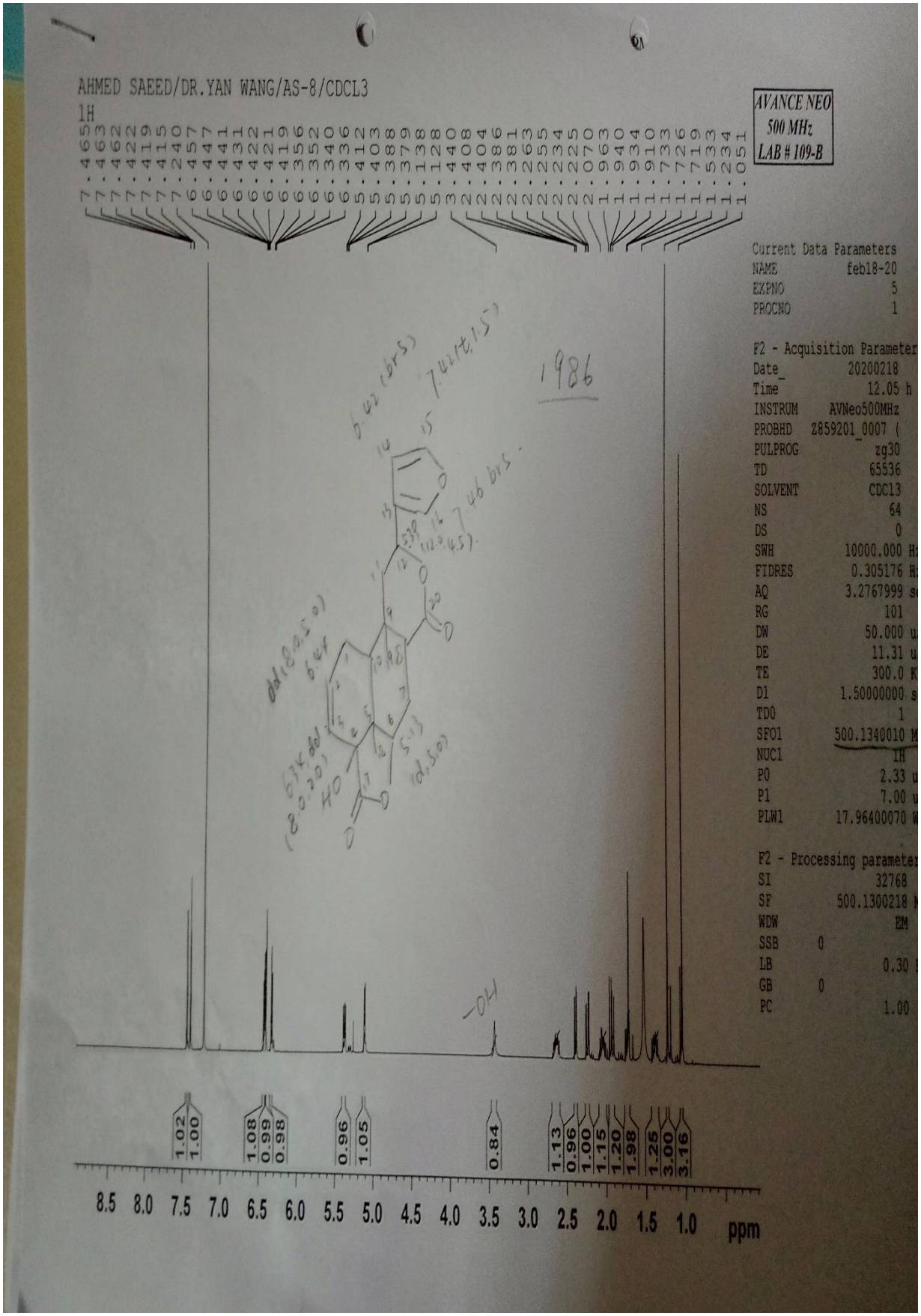




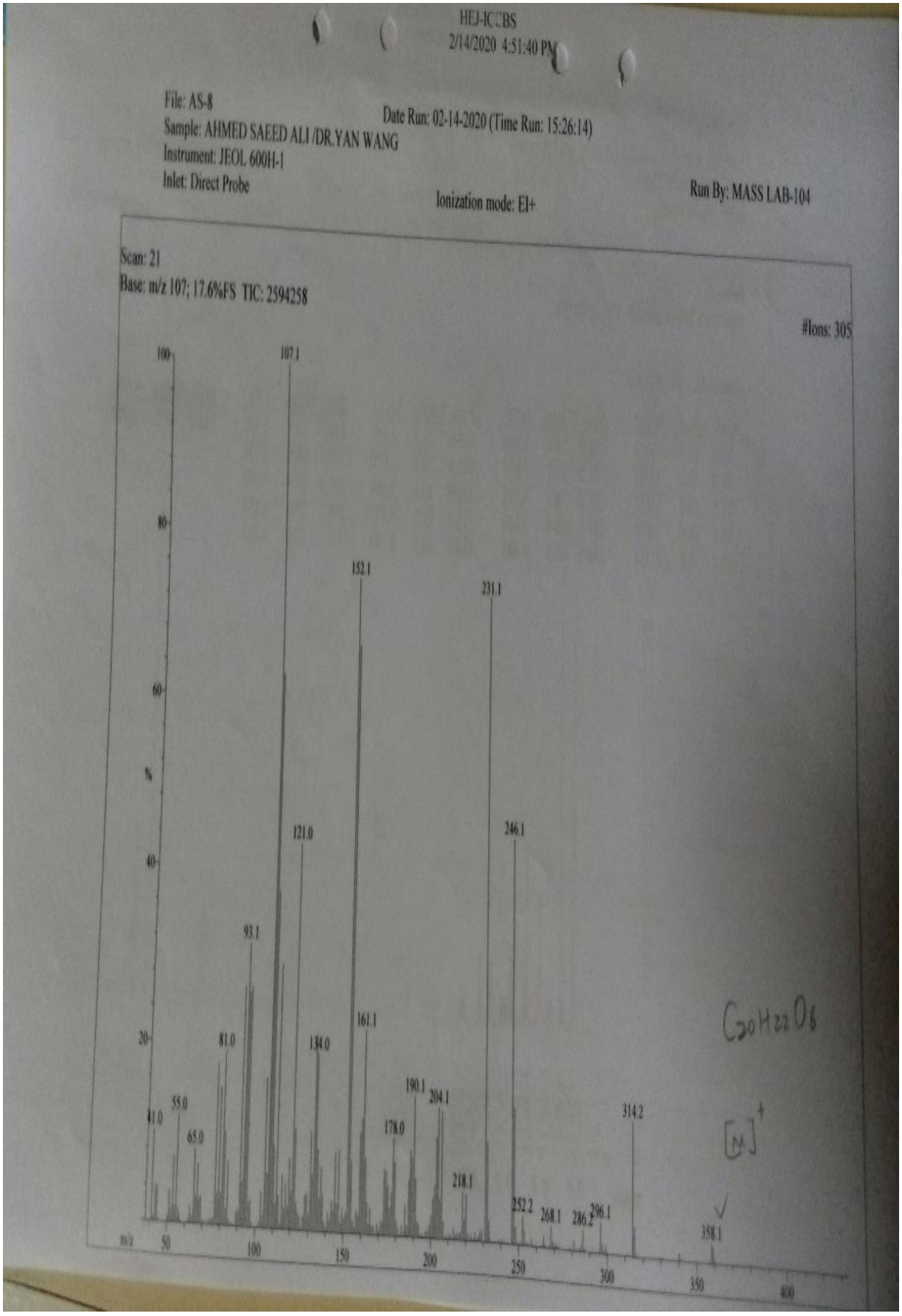
Appendix (7): TLC plates showing compounds.

Appendix (8): Isolated compounds.

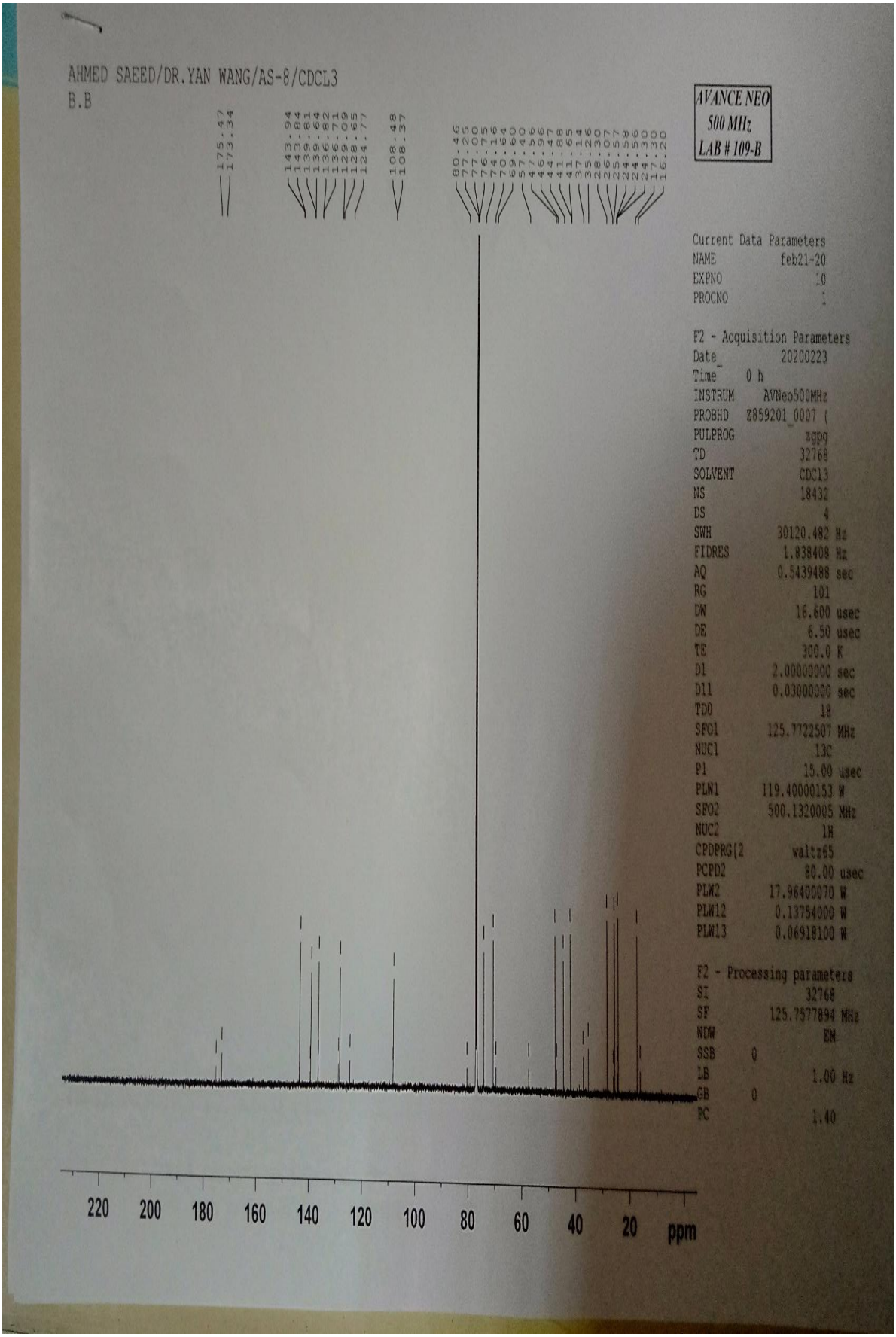
Appendix (9): TLC check isolated compounds before sumpted NMR.



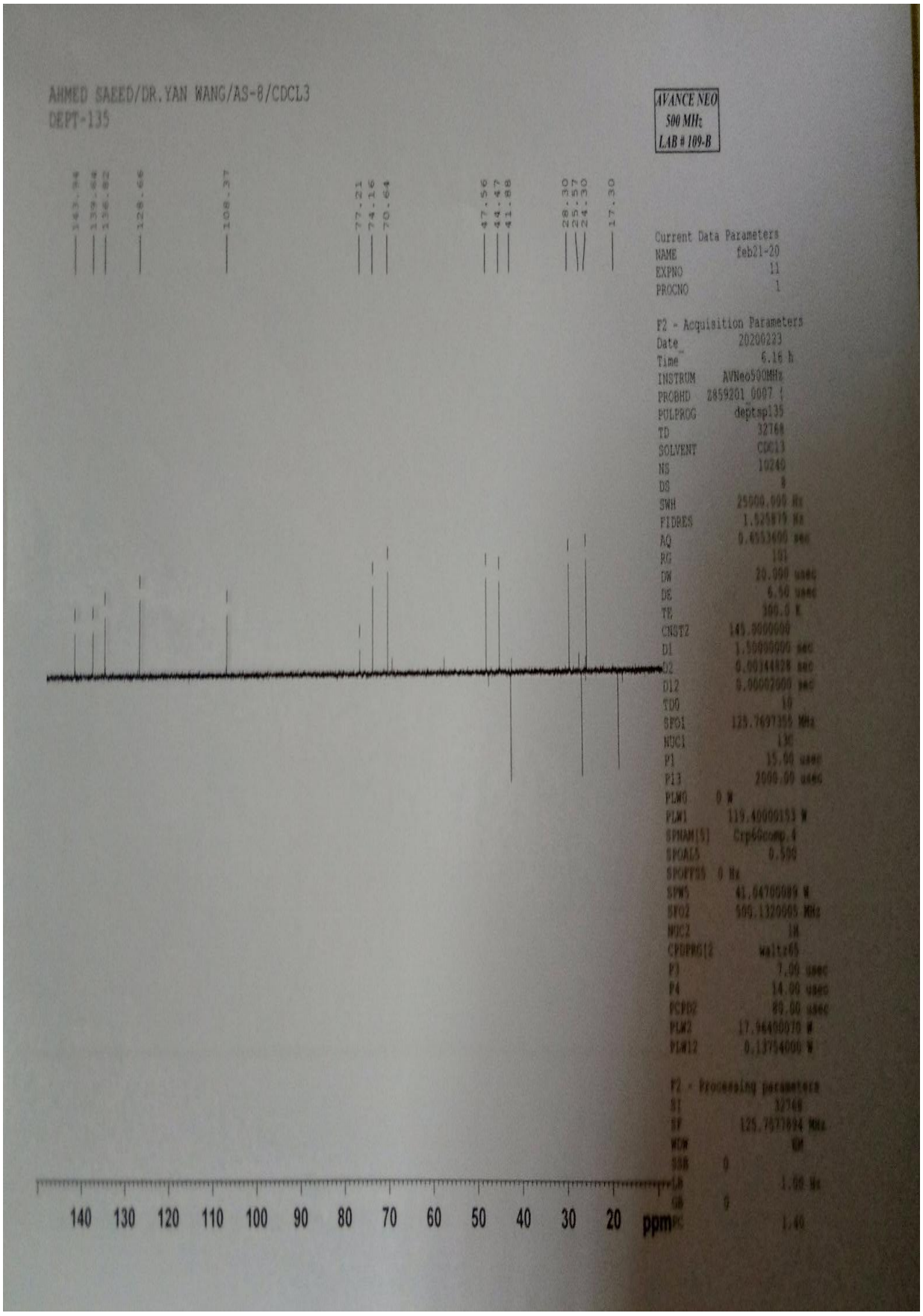
Appendix (10A): ^{1}H-NMR spectrum of the isolated new compound in $CDCL_3$.



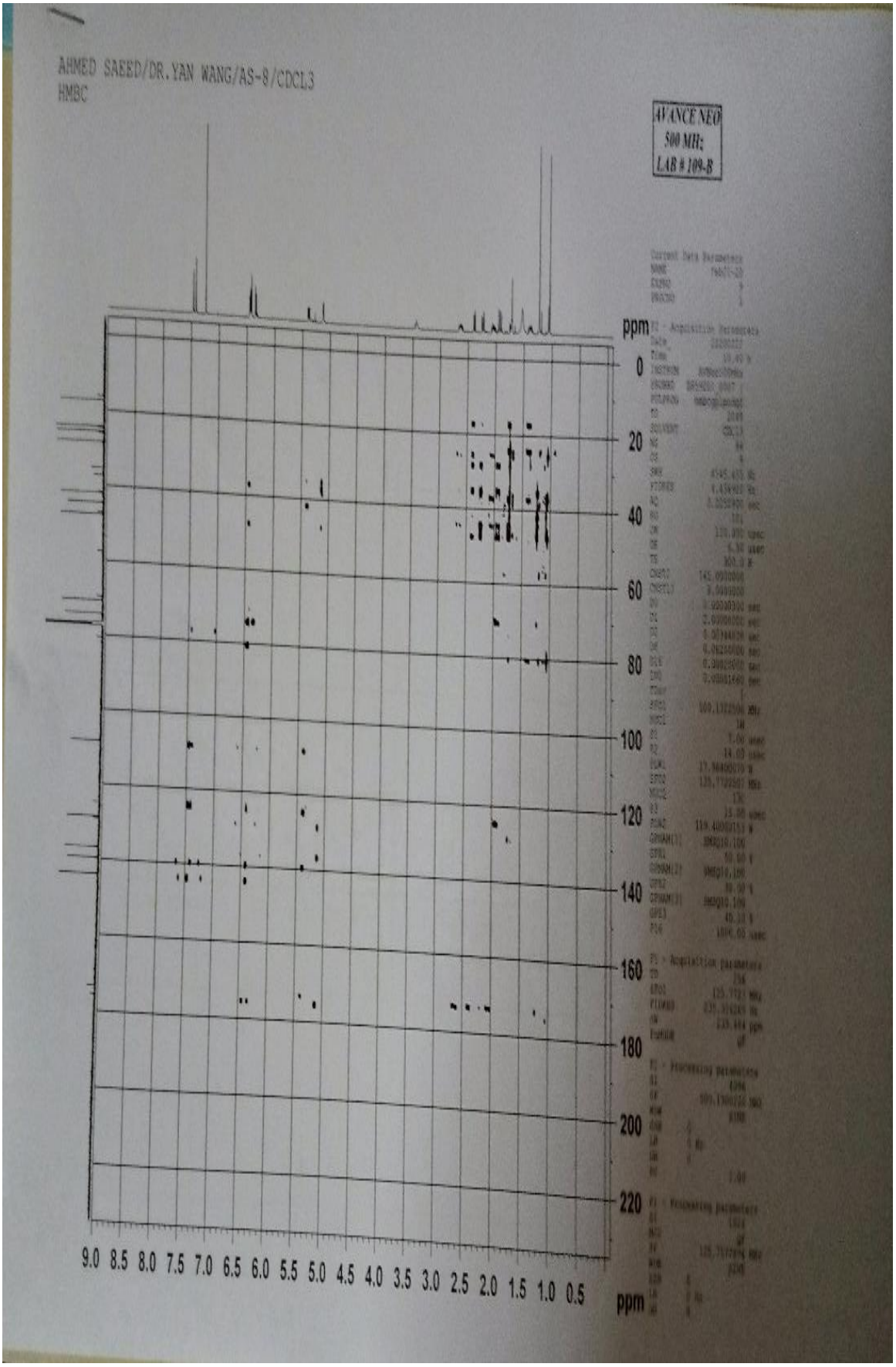
Appendix (10B): The Mass spectrum of the isolated new compound in $CDCL_3$.



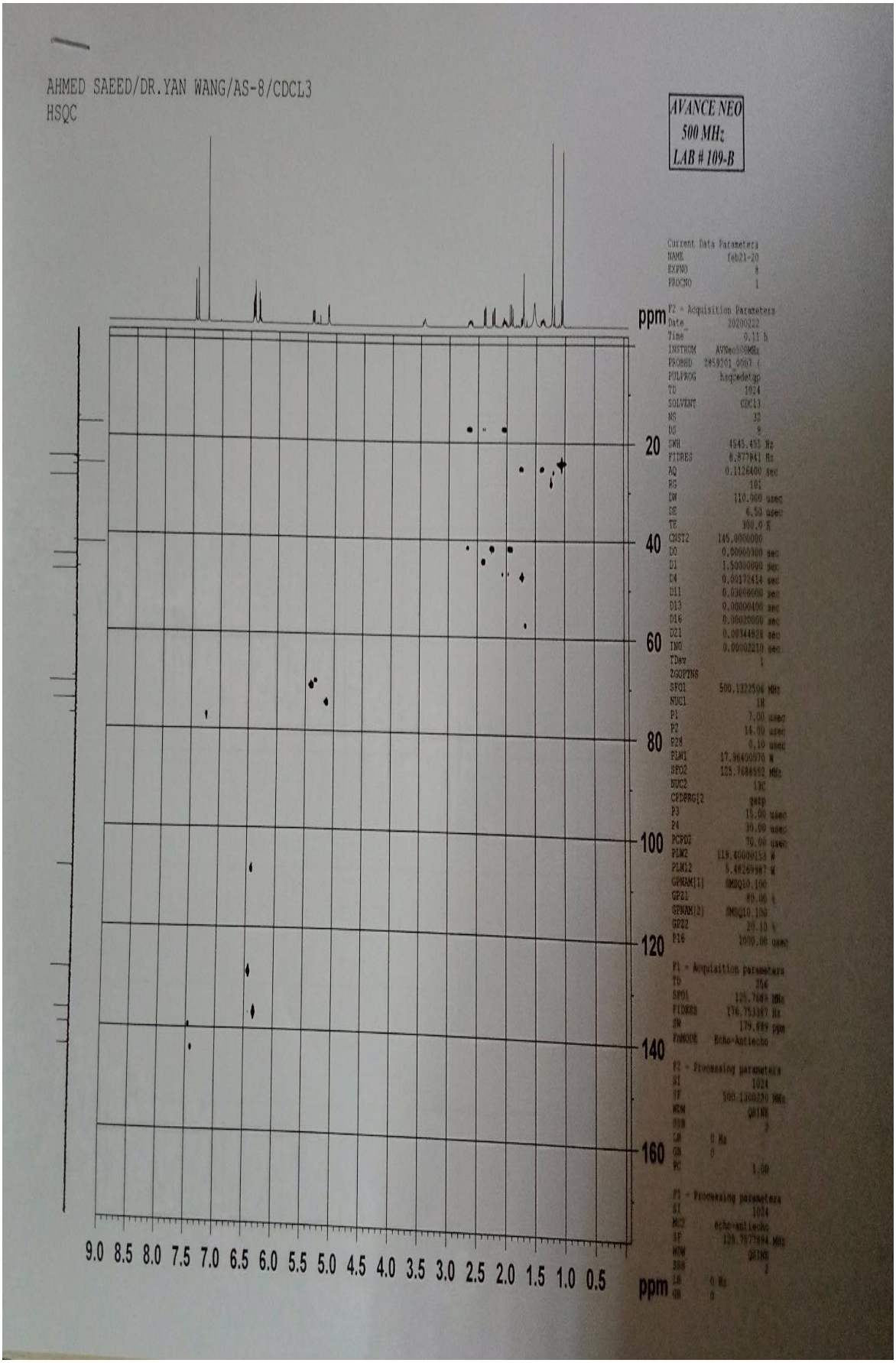
Appendix (10C): ^{13}C-NMR spectrum of the isolated new compound in $CDCL_3$.



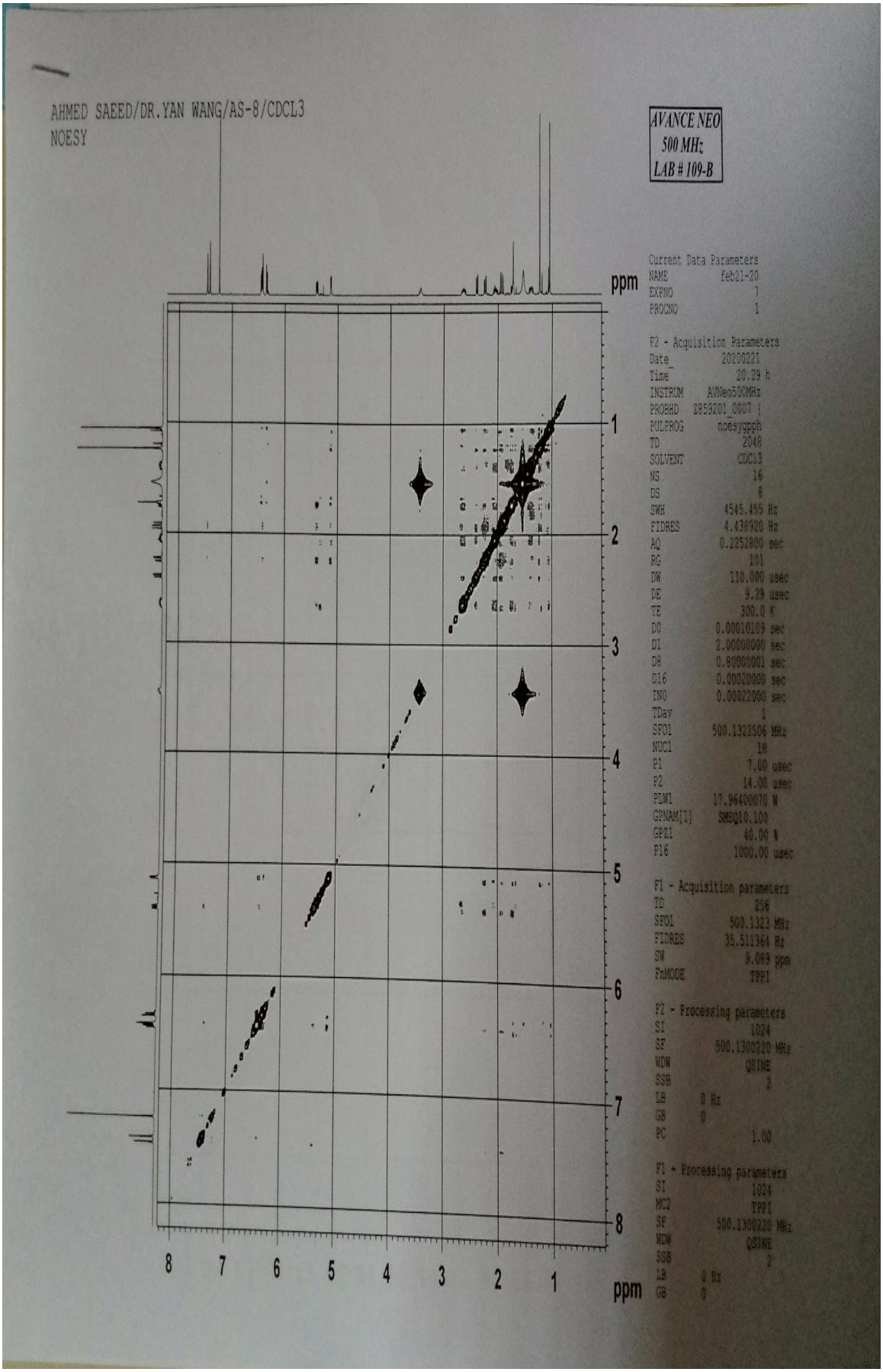
Appendix (10D): DEPT NMR spectrum of the isolated new compound in $CDCL_3$.



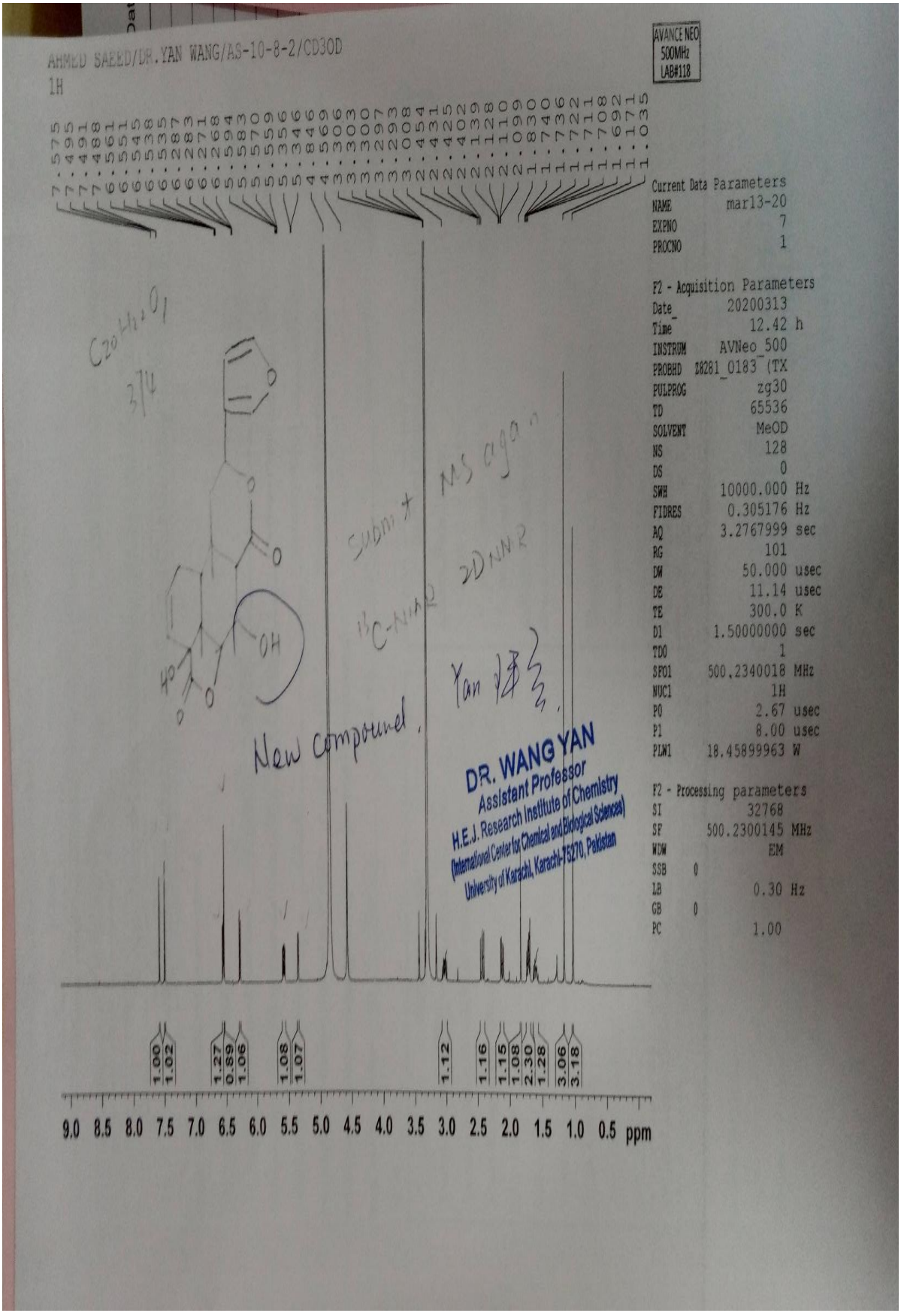
Appendix (10E): HMBC NMR spectrum of the new compound in $CDCL_3$.



Appendix (10F): HSQC NMR spectrum of the new compound in $CDCL_3$.



Appendix (10G): NOESY NMR spectrum of the new compound in $CDCL_3$.



Appendix (11): ^{1}H-NMR spectrum of the isolated new compound in CD_3OD.