INTERNATIONAL UNIVERSITY OF AFRICA
DEANSHIP OF GRADUATE STUDIES, SCIENTIFIC RESEARCH AND PUBLICATIONS
FACULTY OF PURE AND APPLIED SCIENCES
DEPARTMENTOF APPLIED AND INDUSTRIAL CHEMISTRY

CHEMICAL COMPOSITION, PHYSICOCHEMICAL CHARACTERIZATION AND POTENTIAL APPLICATION OF OILS FROM SOME SELECTED SUDANESE MEDICINAL PLANTS

A Thesis submitted in fulfillment of the requirements for the award of degree of Doctor of Philosophy (Organic Chemistry)

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March, 2021

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SUPERVISORS' DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of PhD of Science in Industrial Chemistry.

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I hereby declare that the work in this thesis is my own except for the quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature

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Date :

Dedication

Special dedication of this grateful feeling to my beloved parents;

Beloved fiancé;

Wonderful supervisors'

Loving siblings;

Supportive families;

For their love, support, sacrifices and best wishes.

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My sincere expression also extends to the laboratory staffs and the office staffs that play an important role in ready to render help during the period of my project work and for their kind co-operation towards the completion of my project. Last but not least I wish to avail myself of this opportunity, express a sense of gratitude and love to my beloved parents, fiancé, siblings, and friends, for their assistance, support, strength, patience and help. Thank you.

ABSTRACT

Cockroaches (*Periplaneta Americana*) are the most abundant and obnoxious non-biting insect pest in residential buildings, hospitals, hostels and restaurants. Cockroaches are considered to be among natures' most adaptable creatures and have been living on the planet for at least 250 million years which could pose a serious health issues in many countries including Sudan. Their inclination for destruction and spreading pathogenic organism and diseases has earned mans' loathing. Therefore, searching for the repellent agents is one of the effective ways to control insects. The aims of this study were: firstly to determine physicochemical properties of three seed oils, namely *Ocimum basilicum* (A), *Adansonia digitata* (B) and *Moringa oleifera* (C). Thereafter, to determine the chemical composition of essential oils from two medicinal plants, namely *O. basilicum* (D) and *Cyperus rotundus* (E). Subsequently, to evaluate the oils (volatiles and fixed) in synergistic combination against cockroaches repellent. Finally, to formulate natural repellent product from the oils. In addition to propose potential industrial applications form the oils. The essential oils were distilled by steam distillation, while the seed-oils obtained by soxhlet extraction methods. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyses the chemical compositions of essential oils and fatty acid compositions of seed oils. The physicochemical properties of the oils were assessed by standard and established methods. Ebeling Choice-Box test with a little modification used in repellence test. The lipid content were 18.01%, 33.83% and 42.87% for A, B and C, respectively. While, the essential oils content were 0.78% and 0.73%, almost forty one and forty-four compounds were detected in essential oils; the predominant constituents were methyl cinnamate (25.32%) and (-)-Isolongifolol (7.63%) for D and E, respectively. The Pale yellow with camphor odor-; the reddish yellow with characteristic odor-; and the golden yellow with characteristic odor oils were extracted from the seeds of A, B, and C. The obtained fixed oils have the following properties: freezing point, -2, -14 and 0 °C; melting point, 5, 8 and 21 °C; boiling point, 215, 227 and 225 °C; refractive index (25 °C), 1.485, 1.436 and 1.447; iodine value, 108.6, 98.3 and 96.6 g/100 g of oil; peroxide value, 4.6 , 4.3 and 7.6 meq. O_2/kg of oil; free fatty acids, 0.20, 0.34 and 0.07%; acid value, 4.0 , 6.8 and 1.4 mg of KOH/g of oil; saponification value, 164.2, 180.7 and 185.2 mg KOH/g of oil; unsaponifiable matter, 1.6, 1.7 and 3.2; moisture and volatile value, 4.97, 14.79 and 4.91(wt.%); density, 0.914, 0.867 and 0.900 g/cm^3; viscosity, 10.29, 35.03 and 60.99 mm^2/s; specific gravity, 0.921, 0.874 and 0.907; the major fatty acid were linolenic- (43.92%), oleic- (51.74%) and linoleic acid (30.63%)), respectively. In bioassay test, the repellency of the oils against cockroaches increase with concentration of oils increased (depends on oil concentration) and the IC_{50} and IC_{90} values were 6.0 % and 14.2 %, respectively for oils formulation. In conclusion, the experiment of this study showed promising results for essential oils as the repellent against the American cockroaches. Therefore, may warrant further study to identify the bioactive compound(s).

المستخلص

الصراصير (*Periplaneta Americana*) هي أكثر الآفات الحشرية غير القاضمة البغيضة وفرة في المباني السكنية والمستشفيات والداخليات والمطاعم . و تعتبر الصراصير من بين أكثر المخلوقات الطبيعية قابلية للتكيف وقد عاشت على هذا الكوكب منذ ما لا يقل عن 250 مليون عام مما قد يشكل مشاكل صحية خطيرة في العديد من البلدان بما فيها السودان . ميلها للتدمير ونشر الأمراض و الكائنات المسببة للأمراض أكسبها كراهية الناس و لذلك فإن البحث عن عوامل طاردة واحدة من الطرق الفعالة للسيطرة على هذه الحشرات. هدفت الدراسة أولاً تحديد الخواص الفيزيوكيميائية لزيوت البذور الثلاثة: بذور الريحان (A)، بذور التبلدي (B)، بذور المورينقا اوليفيرا (C). بعد ذلك تحديد التركيب الكيميائي للزيوت الطيارة من اثنين من النباتات الطبية وهما الريحان (D) و السعدة (E)، لاحقاً تقييم الزيوت (المتطايرة والثابتة) في تركيبة فعالة ضد طارد الصراصير .وأخيرا صياغة طارد حشري طبيعي من الزيوت. بالإضافة إلى اقتراح التطبيقات الصناعية المحتملة للزيوت. تم تقطير الزيوت الطيارة عن طريق التقطير بالبخار، في حين تم الحصول على زيوت البذور عن طريق طرق الاستخلاص بالسوكسليت. تم استخدام جهاز كروماتوجرافيا الغاز- مطياف الكتلة (GC-MS) لتحليل التركيبات الكيميائية للزيوت الأساسية وتركيبات الأحماض الدهنية من زيوت البذور . كما تم تقييم الخواص الفيزيائية والكيميائية للزيوت من خلال الطرق القياسية المعترف بها. اختبار Ebeling Choice-Box مع بعض التعديلات البسيطة استخدم في اختبارالطارد. وجد ان نسبة الدهون 18.01 ٪ ، 33.83 ٪ و 42.87 ٪ ل (A) و (B) و (C) علي التوالي. بينما كان محتوى الزيوت الطيارة 0.78 ٪ و 0.73 ٪ ، تقريباً تم اكتشاف واحد وأربعين وأربعة وأربعين مركب في الزيوت الطيارة. المكونات الغالبة منها هي سينامات الميثيل (25.32 %) و (-)- ايزولينوفيلول (7.63%) لكل من (D) و (E) علي التوالي.اللون الأصفر الشاحب مع رائحة الكافور و اللون الأصفر المحمر مع رائحة مميزة واللون الأصفر الذهبي مع الرائحة المميزة تم استخراجها من بذور (A) و (B) و (C). الزيوت الثابتة التي تم الحصول عليها لها الخصائص التالية: نقطة التجمد -2 و -14 و 0، درجة مئوية و نقطة انصهار 5 ، 8 و 21 درجة مئوية، و نقطة الغليان 215 و 227 و 225 درجة مئوية و معامل الانكسار (25 درجة مئوية) 1.485 و 1.436 و 1.447، و قيمة اليود و 108.6 و 98.3 و 96.6 جم / 100 غرام من الزيت ؛ و قيمة البيروكسيد 4.6 و 4.3 و 7.6 ميلمكافئ / O_2 كغ من الزيت ؛ الأحماض الدهنية الحرة 0.20 و 0.34 و 0.07 ٪ ؛ و قيمة الحمض 4.0 و 6.8 و 1.4 ملغ من / KOH جم من الزيت ؛ وقيمة التصبن 164.2 و 180.7 و 185.2 ملغ / KOH جم من الزيت ؛ و المواد غير القابلة للتصبن 1.6 و 1.7 و 3.2 ؛ و الرطوبة وقيمة التطاير4.97 و 14.79 و 4.91 (بالوزن) ؛ و الكثافة 0.914 و 0.867 و 0.900 جم / سم 3 ؛ و اللزوجة 10.29 و 35.03 و 60.99 مم 2 / ثانية ؛ و الثقل النوعي 0.921 و 0.874 و 0.907 ؛ الأحماض الدهنية الرئيسية لينولينيك (43.92 ٪) ، أوليك (51.74 ٪) وحمض اللينوليك (30.63 ٪)) ، على التوالي. في اختبار الفحص الحيوي ، يزداد طرد الزيوت للصراصير مع زيادة تركيز الزيت (يعتمد على تركيز الزيت) وكانت قيم IC_{50} و IC_{90} 6.0% و 14.2% علي التوالي لتركيبة الزيت. في الختام أظهرت هذه الدراسة نتائج واعدة للزيت العطري باعتباره طاردًا للصراصير الأمريكية. و بالتالي تستدعي إجراء المزيد من الدراسة لتحديد المركبات النشطة حيوياً.

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LIST OF SYMBOLS

-	Subtract
%	Percentage
~	Tilde
+	Add
<	Less-than
=	Equals
>	Greater-than
±	Plus-minus
≤	Less than or equal to
≥	More than or equal to
°C	Degree Celsius
°C/min	Degree Celsius per minute
µg/mL	Micrograms per milliliter
µL/mL	Microliter per milliliter
µm	Micrometer
A	Statistical alpha
Bar	Unit of pressure
CFU/mL	Colony forming unit per milliliter
cm	Centimeter
eV	Electron volt
g	Gram
g/cm^3	Gram per cubic centimeter
g/g	Gram per gram
g/kg	Gram per kilogram
g/L	Gram per liter
g/mol	Gram per mol
GHz	Gigahertz
h	Hour
Hz	Hertz
KHz	Kilohertz
KV	Kilovolt
L	Liter
lbs	Pounds
Lx	Lux
m	Meter
M	Molarity
m/z	Mass-to-charge ratio
meq/kg	Mill equivalents per kilogram

mg	Milligram
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
MHz	Megahertz
min	Minutes
mL	Milliliter
mL/kg	Milliliter per kilogram
mL/min	Milliliter per minute
mm	Millimeter
mTorr	Millitorr
N	Normality
nm	Nanometer
ppm	Parts per million
ppt	Parts per trillion
psi	Pound-force per square inch
s	Seconds
V	Volt
v/v	Volume per volume
W	Watt
w/v	Mass per volume ratio
wt%	Weight percentage
x	Multiple
δ	Delta
λ	Gamma
λ_{max}	Lamda maximum
μL	Microliter
π	Pi
π^*	Pi star
cm^{-1}	Reciprocal centimeter

LIST OF ABBREVIATIONS

AOAC	Association of Analytical Chemists
AOCS	American Oil Chemists' Society
AED	Atom emission detector
AIDS	Acquired immunodeficiency syndrome
ANOVA	One way analysis of variance
ATCC	American type culture collection
B. subtilis	*Bacillus subtilis*
CO_2	Carbon dioxide
DAD	Diode array detector
DEPT	Distortionless enhancement by polarization transfer
DMAE	Diffused microwave multi-mode cavity system
DMSO	Dimethyl sulfoxide
ECD	Electron capture detector
EI-MS	Electron Ionization-Mass Spectrometry
ELSD	Evaporate light scanning detector
FFA	Free fatty acid
FID	Flame ionization detector
FMAE	Focused microwave single-mode cavity system
FTIR	Fourier Transform Infrared
FTIRD	Fourier Transformed Infrared Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectroscopy
IR	Infrared
LC	Liquid chromatography
LSD	Least significant difference
MAE	Microwave-assisted extraction
MS	Mass Spectrometry
MSD	Mass selective detector
N	Nitrogen
NA	Nutrient agar
NIST	National Institute of Standards and Technology
O	Oxygen
PV	Peroxide value
SFE	Supercritical fluid extraction
Soxhlet	Solvent semi-continuous extraction
SPSS	Statistical Package for the Social Sciences
TCD	Thermal conductivity detector
TLC	Thin Layer Chromatography

UNICEF	United Nations Children's Fund
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet Spectroscopy
UV-Vis	Ultraviolet–Visible Spectroscopy
WHO	World Health Organization
UFA	Unsaturated Fatty Acid
SFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Throughout the ages, Nature has catered to the basic needs of humans, not the least of which is the provision of medicines for the treatment of a wide spectrum of diseases. Plants, in particular, have played a dominant role in the development of sophisticated traditional medicine systems (Cragg et al., 2009). Many natural compounds extracted from plants exhibit important biological activities. Among these diverse natural compounds, essential oils extracted from aromatic used as bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal, and plants are attracting special attention (Yu et al., 2011). Since the middle ages, essential oils have been widely used in cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural, and food industries (Bakkali et al., 2008). In the last few years, interest and research into the potential of essential oils and as alternative for several industrial applications has grown tremendously.

Seeds of plants are considered as one of the major sources for many important phytochemicals, such as oils and its components. Since long time ago, people world-wide have been used the seeds oils for several different purposes such as medicine, food, as well as fuel. Nowadays, the developing science and technology had also studied for various properties of the plant seed oil, in terms of replacing the existing petroleum with biodiesel, production of polyurethanes coatings, as a modulator in rumen fermentation properties and the encapsulation of the oil for nano-emulsion (Ali et al., 2013; Dhar et al., 2012).

Sudan is gifted with a wide variety of herbal medicine and these medicine have served as the primary healthcare for locals since ages (Mustaffa et al., 2011 and Effendy et al., 2012). From a global survey report by WHO, it shows that Sudan was one of the nine countries that contributed a large amount of sales in herbal medicine worldwide between the year of 1999 to 2001 (Figure 1.1). At the same time, referring to World Bank report, they predicted that during 2050, the global market for herbal products would be about 5 US trillion dollars (Rasadah and Ali, 2008).

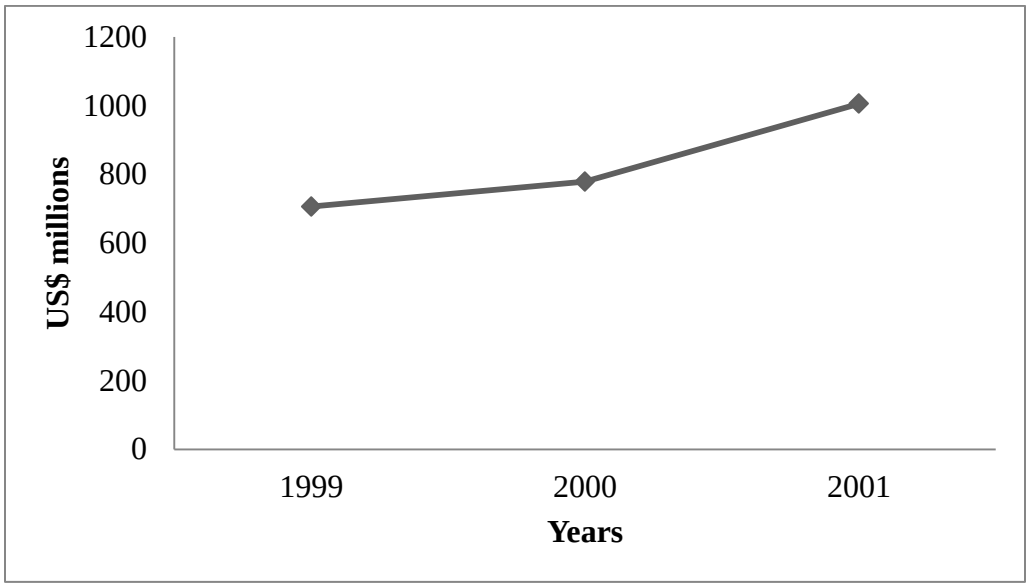

Figure 1.1: Growth in the sales of herbal medicine of nine representative countries from 1999-2001 (Bhutan, Canada, the Czech Republic, Iran, Madagascar, Malaysia, Pakistan, Sudan and Sweden)

Adapted from: Effendy et al. (2012)

In relation to this, the government urged researchers, academicians and industry operators to grab the opportunity by speeding up their research and development activities in medicinal plants to find new leads and could market them worldwide (Rasadah and Ali, 2008).

1.2 PROBLEM STATEMENT

Cockroaches are the most abundant and obnoxious non-biting insect pest in residential buildings, hospitals, hostels and restaurants. They feed indiscriminately on human food and sewage and so have copious opportunity to disseminate human pathogens. When cockroaches run over food, they may leave filth or oily liquid that has offensive and sickening odour which ruin food or render it unacceptable. Pathogenic bacteria including *Salmonella spp, Shigella spp, Campylobacter* spp, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have been isolated from cockroaches (Yahaya and Clement, 2010).

Efforts have been made to develop formulas with properties which are effective in destroying any targeted insects. Commercial insecticides commonly include active ingredients, which are not only harmful to targeted insects but also to human beings and animals. Insecticides in aerosol spray forms, when used in a confined environment, may prove to be toxic to humans and household pets when used at a level of concentration proven harmful. Undesirable effects may include neurotoxic reactions, suffocation, and the noxious odour may be unpleasant and may cause headaches to individuals with increased sensitivity, children, babies and the elderly. The stored products are protected against attack by cockroaches and many others pest in many countries. Meanwhile, environmentally compatible stored-product control agents are surely needed to replace synthetic pesticides that are insecticidal harmful to environment and not effective due to the increasing difficulty of managing pesticide effect resistance (Donald et al., 2009). Highly efficient cockroach repellents had gained a huge demand in the market recently. Several chemicals were studies for repellent action against cockroaches, such as methyl neodacanamide, *N,N*-diethylphenylacetamid, propylneodecanamide, methyl neotridecanamide, alkyl neoalkanamides, eugenol and citral.

Sharififard et al. (2016) reported the conventional insecticides are used as main tool to control cockroach infestations but there are many concerns about the harmful side

effects of these chemical compounds. In addition, the insecticide use is restricted in places such as food preparation areas, restaurants, storage buildings and apartments. These restrictions of chemical insecticide application increase demand for safer alternatives against cockroach infestations. Different level of resistance to many compounds of chemical insecticides including organochlorine, organophosphorus and carbamate insecticides have been documented in many field collected strains of cockroaches from Iran. Therefore, application of these insecticides should be stop and replace with other safer compounds.

Therefore, supposed to be the insecticidal compositions has effective killing properties for targeted insects and non-poisonous to users. However, non-poisonous insecticides that are available commercially have little efficacy and does not serve the required purposes. Essential oils are commonly use in such compositions as the active ingredient of the insecticides. The uses of some of the essential oil are not effective due to the essential oils being too slow-acting in killing the insects. Besides that, the productions of certain insecticides containing essential oils are cost-prohibitive and subsequently they are fewer developments in this aspect. Some of the compositions containing essential oils may contain inadequate lethal amount to be effective enough to kill or control the targeted insects.

Accordingly, new formulations are needs to provide a repellent, which incorporates non-harmful essential oils, which can use by humans and animals. The compositions should be produce using natural products to reduce toxicity and the process should be cost-effective. Besides that, the formulation should be effective in repelling targeted insects, has a longer lasting effect and of lesser toxicity than traditional repellents.

It is worth to be mention that, recently, some reports has published or reported for some Sudanese plants for their constituents and certain biological activities. Nevertheless, none of the previous studies had attempted to investigate the repellency potential of

essential oils (especially *Cyperus rotundus* and *Ocimum basilicum*) in single or synergistic combination against cockroaches.

Moreover, the Sudanese plants' seed oils of *O. basilicum, M. oleifera and A. digitata* L, so far not been physicochemically investigated widely to suit world market requirements, especially if ever oils is to be commercially produced in Sudan for export. Such studies will help in selecting the right types to grow for a specific market and knowing the oil properties will help to identify the end uses and their potential industrial applications.

However, the cockroach repellent property of some essential oils in combination with fixed oils not been studied previously. Moreover, the wide uses of *Ocimum basilicum, Cyeprus rotundus, Moringa oleifera* and *Adansonia digitata* in traditional medicine interested us now to investigate for several applications and to determine the bioactive compound(s) or their properties for a possible sources of natural products with potential uses for several industrial applications such as food flavours, repellency agents etc.

Due to the increasing dilemma of insecticides resistance, adverse effects and high costing have led researchers to explore natural resources especially plant materials (such as essential and fixed oils) as an alternative source of repellent. In relation to this, the study about essential and fixed oils from various plants in Sudan has been done extensively by the researchers to discover their beneficial potential. Many of the essential oils from the plants have shown their potential as repellent. However, as far as the articles could be ascertained, there is no yet studies about repellent activity of essential and fixed oils in combination from Sudanese medicinal plants especially *O. basilicum, C. rotundus, M. oleifera* and *A. digitata*. Therefore, a part of this study was highlighting the insect activity of oils from Sudanese medicinal plants namely *O. basilicum, C. rotundus, M. oleifera* and *A. digitata* independently and in synergistic combination.

Therefore, the purposes of this study were firstly to determine the chemical composition of essential oils from *O. basilicum,* and *C. rotundus.* Then to investigate the physicochemical properties of *M. oleifera, A. digitata* L. and *O. basilicum* seedoils. Thereafter, to evaluate the repellency activities of the oils in combinations against cockroaches repellency, and subsequently to formulate a potential natural repellency agent from oils. Finally to propose potential industrial application for the oils according to their properties.

1.3 OBJECTIVES OF THE STUDY

1. To extract oils (volatile and fixed) from *O. basilicum, A. digitata, M. oleifera, C. rotundus*
2. To determine the chemical composition of the essential (volatile) oils
3. To study the physicochemical properties of the seed (fixed) oils
4. To investigate the repellency activity of the (volatile and fixed) oils against cockroach.
5. To formulate a potential natural repellency agent.
6. To propose several industrial applications according to the properties, characteristic and chemical compositions of the oils.

1.4 SCOPE OF THE STUDY

The essential oils were obtained from leaves of *O. basilicum,* and rhizome of *C. rotundus* by using steam distillation extraction. While, the seed-oils were obtained from seed of *O. basilicum, M. oleifera* and *A. digitata* by Soxhlet using n-hexane. The essential oils were analyzed and determined by performing chromatography and spectroscopy technique via Gas Chromatography-Mass Spectroscopy (GC-MS). All the injected liquid samples were prepared at similar concentration and solid samples at similar weight. The

essential oils were analyzed by using GC-MS to determine their chemical compositions. The physicochemical properties' determination of seed oils such as physical state, color and odour, freezing, melting and boiling points, refractive index analysis, acid value, free fatty acid, iodine value, peroxide value, saponification, unsaponifiable matter analysis, density, moisture and volatile matter analysis etc, were studied according to standard methods proposed by American Oil Chemists' Society (AOCS), Association of Official Analytical Chemists (AOAC) and some reported literature. Moreover, the fatty acid compositions of the seed oils determined by using GC-MS.

For the cockroaches' repellency test, the Ebeling choice-box method with slight modification was used to evaluate the repellent test. The test performed by different concentrations of essential and fixed oils individually and in combinations.

The formulation of repellent product was prepared from the oils (essential and fixed) according to their repellent activities. Finally, proposed several products for different industrial applications according to the properties, characteristic and chemical compositions of the oils.

1.5 SIGNIFICANCE AND CONTRIBUTION OF THE RESEARCH

The results of this study would contribute a new, inexpensive and alternative cockroach repellent agent from Sudanese medicinal plants. In addition, it is hoped that this research will help the researshers in area of natural products to development and gain an insight into the effectiveness of our local herbal as most of them are prepared in combination of more than one ingredient.

The chemical compound identification of oils (essential and fixed) presented in this study contributes towards a significant study for industrial application since there is lack of a previous reports. The identified compounds of all oils gives a clearer image on the abundance of the bioactive compounds in each oil.

1.6 OVERVIEW OF THE THESIS

In detail, the Chapter one reveals the research motivation with the support of facts and figures from reporting studies. The objectives of the research were pointed out with an expended study scope. The brief significance or the contributions of the study were performed together with the necessity to contribute towards a research.

The Chapter two discusses the review of the literature or the background on the plants material *C. rotundus, O. basilicum, M. oleifera* and *A. digitata*, pass methods for the extraction, chromatography and spectroscopy techniques adapted to achieve the aim of the study. Several different methods were studied and were correlated with the current study. This method addresses some of the fundamental and practical methods that are suitable for the progress on the research.

As for chapter three, detail explanation of the material and methods were written according to the way of the analysis being conducted. The brief descriptions of the study are as drawn in the methodology flowchart whereby it represents the two different methods adapted.

Chapter four is allocated for results and discussion of the obtained data according to the objectives whereby the yield of the extraction methods was discussed. The data in the analysis of the oils were listed and reviewed according to different plants. Lastly, the data of the biological study of repellency was evaluated to provide a scientific supporting data with the concentration level for the suitable repellent formulation or proposed industrial applications.

The Chapter five represents the concluding chapter of the overall work and it summarizes the research work and point out the result in accordance with the objective of the study. Some recommendations were also suggested to expand the study in several terms.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW

The purpose of presenting this chapter was to review and analyze the critical points of current knowledge as well as theoretical and methodological relation via specific subtopics whereby it could be an aid in the current study. The main topics discussed were on the plant material and extraction, chromatography and spectroscopy techniques and physicochemical properties, cockroaches, insect repellent, types of repellent tests.

2.2 NATURAL PRODUCTS

For centuries, a natural product has used in many uses and can be described as invaluable resources and useful for our mother nature. In our daily life, it can be used as medicine or even using in cooking as flavour or food additives. The natural product plays avital role in progression of human life.

According to Oladeji et al. (2012), a natural product is a chemical compound or substance that produced by a living organism. They are found in nature and usually has a pharmacological or biological activity that can be used in pharmaceutical drug discovery and drug design. It can be considered as a natural product also can be prepared by total synthesis. Natural product compound has some form of biological activity. It has an active principle such as a structure that can act as a lead compound in order to form a chain of compound. In order to improve potency, selectivity or pharmacokinetic parameters, lead compound is used. Lead compound is a chemical compound that has pharmacological or biological activity. Its chemical structure was used as a starting point for chemical modifications.

A surge of interest in medicinal and aromatic plants have been seen by the industry, life and health sciences and general public in lasta couple of decades. The extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles. These plants have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Shan et al., 2007).

Herbs and spices have used not only as food ingredients but also as a remedy for old people in the past. Scientific data have been accumulated in many research showing that many spices and related essential oils medicinal properties are useful in the prevention of diseases or the relieve of their symptoms.

Consumer demand has increased for processed products that keep more of their original characteristics (Özcan et al., 2005). The safety aspects of chemical preservatives have been doubted since they are known for a contribution of many carcinogenic and teratogenic attributes as well as residual toxicity.For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives have been intensified (Omidbeygi et al., 2007).

2.2.1 Sources of Natural Products

Natural products can be obtained from five different sources; the plant kingdom, the microbial world, the marine world, animal sources and venoms or toxins. A rich source such as morphine, cocaine, digitalis, quinine, tubocurarine, nicotine and muscarine, which called as lead compounds, caught be found in the plant kingdom. Plant does provide a large amount of rich, complex and highly varied structures. It is unlikely to be synthesized in laboratories. They survive if potent compounds contained in which

deter animals or insects from eating them where evolution has already carried out a screening process itself.

Prebiotic, microbial, plants and animal source's compound occurred naturally, have attracted mankind from ancient times where the task, color and odor of isolated compounds could be used for various purposes which have been extracted from a variety of flowers, plants and insects. Drugs and lead compounds were discovered in bacteria and fungi. These microorganisms have evolved in giving their hosts an advantage over their competitors in the microbial world by produced anenormousvariety of antimicrobial agents. Marine worlds were also believed in contributes to the wealthy of biologically potent chemicals with attractive inflammatory, antiviral and anticancer activity. Figure 2.1 showed the schematic flow diagram formation of natural products.

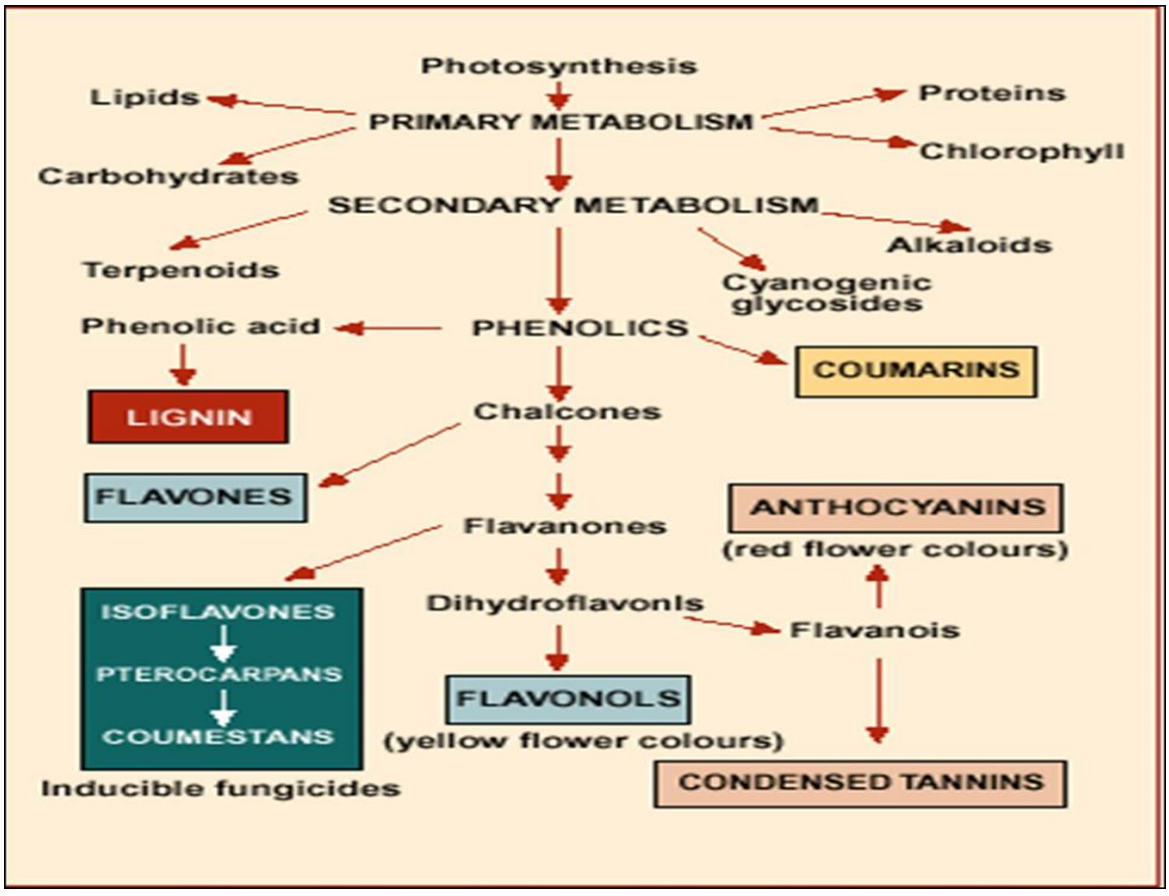

Figure 2.1: Classification of Natural Products

Source:https://www.google.com/search?q=diagram+Classification+of+Natural+Products&hl=en-US&source =lnms&tbm

2.2.2 Significance of Natural Product

Nowadays we used natural products as a basic need of life, such as cloth, food, and building in our daily life. These natural products are been often used and have high market value due to their comparable efficacy, present in low toxicity and environmental safely (Nerio et al., 2010). Researchers had recognized that many natural products, generally those derived from plant, could exhibit health benefits (Petronilho et al., 2012). Natural products have often been the unique means to treat injuries and disease and been utilized for human use since ancient times. Studies show that natural products have a biological activity for use in pharmaceutical industry. Generally they have made a great impact in pharmaceutical drug discovery and drug design (Cullagh, 2008). In recent time, several researches indicated their potential uses as drug in curing awful disease such as Type II Diabetes (Bedekar et al., 2010).

2.3 ESSENTIAL OILS

Essential oils are defined as concentrated, hydrophobic liquid, which contained a volatile aroma compound from plants and called as aromatic herbs or aromatic plants. They are also known as volatile or ethereal oils or simply as the "oil of" the plant material from which they were extracted (Trade Policy Analysis and Development Agency, 2011). It carries a distinctive scent or essence of the plant in which their oil is "essential" in the sense. Essential oils are mixtures of more than 200 compounds that can be grouped into 2 fractions; (i) a volatile fraction constitutes of 90-95% of the whole oil and contains monoterpenes and sesquiterpenes hydrocarbon and their oxygenated derivatives along with aliphatic aldehydes, alcohols and esters and (ii) a non-volatile residue that constitutes from 5 to 10% of the whole oil and contains hydrocarbons, fatty

acids, sterols, carotenoids, waxes, coumarins, psoralens and flavonoids (Lucchesi et al., 2004; Orphanides et al., 2011).

Several methods for extracting essential oils include uses of supercritical fluid CO_2 (SFE) or microwaves and mainlylow or high pressure distillation employing boiling water or hot steam (Bakkali et al., 2008).

Essential oils occur commonly in the families such as Lamiaceae, Verbenaceae, Valerianaceae, Araliaceae, Umbelliferae, Myrtaceae, Cistaceae, Violaceae, Thymelaceae, Anacardiaceae, Rutaceae, Burseraceae, Cneoraceae, Meliaceae, Magnoliaceae, Santalaceae, Betulaceae, Juglandaceae, Myricaceae, Salicaceae, Asteraceae, Ericaceae, Poaceae, Araceae, Pandanaceae, Cyperaceae, Zingiberaceae and Orchidaceae (Bhat et al., 2005). In recent years, essential oils have been of research interest in view of their biological activities that have medicinal properties useful in prevention of diseases. They also possess different pharmacological activities (Tognolini et al., 2006). An interest potential application of essential oils is preventing fungal growth, and mycotoxins yield in the cereals and grain based food mainly was monoterpenes and sesquiterpenes (Dambolena et al., 2010). Other than that, essential oils are also believed has potentially antioxidant properties (Hussain et al., 2008). Chemical composition of essential oils influenced their biological activities (Carović-Stanko et al., 2010).

In nature, for plants, their essential oils playaprominent role in the protection as antibacterials, antivirals, antifungals, insecticides also against herbivores by reducing their appetite. They also may attract some insects to favour dispersion of pollens and seeds, or repel undesirable others. They are liquid, volatile, lipid and rarely coloured, lipid soluble and soluble in organic solvents with a lower density than that of water (Bakkali et al., 2008). Essential oils can be obtained from all plant organs, i.e.buds, flowers, leaves, stem, twigs, seeds, fruits, roots, wood or bark.

Extraction products vary in quality, quantity and composition influenced by the climate, soil composition, geographical area, plant organ, age and vegetative cyclestage. Therefore in order to obtain essential oils of constant composition, they have to be extracted under the same conditions; same organ of the plant growing in the same soil under sameclimate and season. The numerous constituents of the essential oils were distributed among few groups on the basis of their chemical structure which is monoterpenes hydrocarbon, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, phenylpropanoids, phenols and hydrocarbons identified (Tognolini et al., 2006).

Essential oil is the natural product which can be derived from secondary plant substances that comprised of many compounds including monoterpenes, sesquiterpenes and terpenoids which function as the aromatic characteristics of many plants (Appel and Tanley, 2000). Previous studies show that essential oils can be used as insecticides because of their low toxicity to wildlife, however essential oils could be toxic to non-target insects initially but after a few days, insects that come in contact with the treated environment would not be affected as they would be with traditional insecticides. Therefore, essential oils would be excellent contact sprays, but they would offer poor residual protection (Isman, 2006).

2.4 Sudanese Medicinal Plants

The medicinal plants and herbs have been used for many years in the treatment of various diseases in animals and human beings. Now-a-days, utilization of these medicinal plants is increasing (Tipu et al., 2006). The synthesis of a new drug is too expensive, wasting products in the process; the drug is not stable and may be creating toxic as by products. Furthermore, the environmental problems associated with synthetic new drugs have to be considered. Plants are rich sources for a wide variety of phytochemicals such as terrene's, alkaloids, terpenoids, saponins, flavonoids, phenols and tannins, which have been reported to have biological activities (Sahgal et al., 2009). The presence of these phytochemicals in plants makes it to be a possible source of raw materials for many

pharmaceutical industries as therapeutics sources. By this safer way and cheaper in price, natural drug can be originated. Nevertheless, several medicinal plants for different reasons have not received sufficient scientific studies and sometimes are classified as 'forgotten plants' (Abdel Rahman et al., 2011). The growing interest in medicinal plants or herbs as a source of new pharmaceuticals and the increasing demand for herbal products in the world encourage us to revise some Sudanese plants by evaluating their applicability and benefits using advanced scientific approaches to increase our information about their biological effects and the responsible phytochemicals from them.

2.4.1. Ocimum basilicum

The genus *Ocimum*, of *Ocimum basilicum* (Family: *Lamiaceae* formerly *Labiatae*), collectively called basil has long been recognized worldwide as a diverse, rich source of essential oils and an important culinary herb. Basils show great variation in both morphology diversity such as inflorescence, leaf and essential oil components (Blank et al., 2004, Makri and Kintzios 2008, Telci et al., 2006; Nour et al., 2009). *Ocimum* comprises 150 species 50 to of herbs and shrubs, widely distributed in tropical, subtropical and temperate regions of Asia, Africa, and Central and South America (Gupta, 2006), particularly regarding plant growth, morphology, physical appearance and essential oil content and composition, most commercial basil cultivars available in the market belong to the species *O. basilicum*. The taxonomy of *O. basilicum* is further complicated by the existence of numerous varieties, cultivars and chemotypes within the species that do not differ significantly in morphology. The composition of volatile oil constituents was used to characterize the diversity among the most economically important *O.* species (Abduelrahman et al., 2009). The essential oils of basils were used as food flavorant, medicines and in perfumery industry. Figure 2.2: Illustration the *O. basilicum* Plant (a) and its seeds (b).

(a) **(b)**

Figure 2.2: Illustration (a) *O. basilicum* Plant and (b) seeds

Source: https://www.google.complantsoftheworldonline.org

Researchers, previously reported on the chemical variability of composition of the essential oils of several accessions of basil collected from different parts of the world and grown in Sudan or in Malaysia; these accessions were classified into seven and four distinct groups respectively, according to the major chemical constituents of the essential oil (Abduelrahman et al., 2009, Azhari et al., 2010). Also these researchers have reported some biological activities of these oils; and were showed very interesting biological activities such as being bactericidal (Azhari et al., 2009a), mosquito repellent (Azhari et al., 2009b) and larvicidal (Azhari et al., 2009c) etc. It is used in the traditional medicine to sooth pain, treat vomiting and stress and commonly as insect repellent (Aidaross et al., 2005). Although the plants have been reported to have insect repellent properties, there is no substantive data to indicate their level of effectiveness and the concentration that offer complete protection from mosquito bites. Table 2.1 shows some chemical compositions of *O. basilicum* essential oils reported in the literature.

Table 2.1: Chemical compositions of *O. basilicum* essential oils reported in the literature.

Compound			Percentage %			
α-Pinene	0.23	0.002	0.09-0.37	0.19	na	0.6
Camphor	0.64	na	0.08-1.19	0.20	1.8	0.3
Citral	23.51	na	0.21-0.51	na	na	na
Geraniol	34.89	0.259	0.11-13.1	0.03	na	na
Cineole	0.05	na	0.4-10.72	10.18	na	9.9
β-Pinene	0.19	0.002	0.14-0.73	0.57	0.25	1.0
Citronellal	0.59	na	na	na	na	na
Eugenol	1.33	na	0.1-10.96	13.66	2.2	20.7
Vanillin	0.27	na	na	na	na	35.1
Linalool	2.21	0.003	9.12-72.59	43.78	35.99	na
myrcene	na	0.006	0.14-0.78	0.76	na	1.0
eucalyptol	na	0.001	0.40	na	7.57	na
β-ocimene	na	0.007	0.19	0.57	na	1.6
α-ylangene	na	0.007	na	0.08	na	na
terpinen-4-ol	na	0.030	0.09-0.37	0.18	na	0.2
β-caryophyllene	na	0.087	0.35	0.08	6.54	0.2
β-copaene	na	0.021	na	na	na	na
α-β-bergamotene	na	0.117	1.1-2.19	0.10	na	5.0
α-humulene	na	0.031	0.11-0.18	0.36	na	0.5
germacrene d	na	78.01	0.08	na	na	2.7
methyl eugenol		6	0.27-18.39	0.09	6.37	0.4
Limonene		na	0.7-0.49	0.23	na	na
References	(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, *some data modified as mean average from origin source (a): Saha et al. (2013), (b): Ozcan and Chalchat, (2002), (c): Ladwani et al. (2018), (d): Pripdeevech et al. (2010), (e): Mehdizadeh et al. (2016) and (f): Pirasa et al. (2018).

Basil is well-known for its folk medicinal value and is accepted officially in a number of countries. The leaves and flower of basil are used in folk medicine as a tonic and vermifuge, and basil tea is good for treating dysentery, nausea and flatulence. The oil of the plant is beneficial for the alleviation of spasm, rhinitis mental fatigue, could, and as a first aid treatment for wasp stings and snakebites. It has been used as a folk remedy for boredom and convulsion. Basil cures headache, improves digestion and is also good for toothache, earache and for curing epistaxis when used with camphor. Infusion of plant is effective in cephalgia, gouty joints, fever, otitis and snake bite. The plant is effective in treatment of stomach problems, fever, cough, gout and given internally to treat cystitis, nephritis and in internal piles. Infusion of basil seed is used to treat gonorrhea, chronic diarrhea and dysentery. Plant is also used to keep away insects and snakes (Ch et al., 2015).

The seed (fixed) oil composition constitutes another characteristic which contributes to the rich diversity of the plants. Generally, the oils were classified as non-drying, semi-drying or drying oils. Drying oils are higher in unsaturated fatty acids that will polymerize when exposed to the oxygen in air, usually in the presence of a catalyst. The result is an increase in the molecular weight including cross-linking. The drying index indicates how well or how poorly certain drying oil will oxidize. The drying index of drying oil, semi-drying oil and non-drying oil is >70, 50-70 and<50, respectively (Nour et al., 2009). Its known that, the potential industrial applications for the seed (fixed) oil depends on its physicochemical properties. Therefore, it is more necessary and important to determine the physicochemical properties of the oil for its potential industrial applications. Fatty acid compositions of some *O. basilicum* seed oil reported in the literature were shown in Table 2.2.

Table 2.2: Fatty acid composition of *O. basilicum* seed oil reported in the literature

Fatty acids		**Composition (%)***					
Palmitic	C16:0	6.8-8.8	4.90	1.6-7.5	8-9.2	6.23-10.16	5-13
Palmitoletic	C16:1	0.2-0.3	0.07	0.1	0.2	na	na
Stearic	C18:0	2.0-2.8	2.50	0.7-3.8	3.6-3.8	2.97-4.88	2-3
Oleic	C18:1	8.7-11.6	7.55	0.9-11.0	10.3-12.3	6.22-19.92	6-10
Linoleic	C18:2	18.3-21.7	20.20	1.8-19.1	23.4-26.0	16.73-24.93	12-32
Linolenic	C18:3	57.4-62.5	63.80	6.1-50.1	49.3-52.4	42.45-61.85	49-62
Arachidic	C20:0	0.2	0.25	0.3	0.2-0.3	na	na
	SFA	9-11.8	7.89	2.6-7.9	11.9-13.3	10.38-14.76	11.5
	MUFA	8.9-11.9	7.86	1-11.1	10.5-12.5	6.22-19.92	8.0
	PUFA	75.7-84.2	84.3	7.9-69.2	72.7-78.4	59.18-86.78	77.5
	UFA	84.6-96.1	92.2	8.9-80.3	85.6-88.1	81.03-93.07	85.5
References		(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, *some data modified as mean average from origin source (a): Angers et al. (1996), (b): Ghaleshahi et al. (2019), (c): Amin et al. (2017), (d): Kakaraparthi et al. (2015), (e): Mostafavai et al. (2019) and (f): Nour et al. (2009).

2.4.2. Cyperus rotundus

Kingdom: Plantae; Subkingdom: Tracheobionta; Superdivision: Spermatophyta; Division: Magnoliophyta; Class: Liliopsida; Subclass: Commelinidae; Order: Cyperales; Family: *Cyperaceae*; Genus: *Cyperus* L; Species: *Cyperus rotundus* (Nalini *et al*., 2014). It is commonly known as Nagarmotha is found throughout India. It belongs to the family Cyperaceae (Kumar et al., 2017; Bajpay et al., 2018; Kamala et al., 2018; Raut and Gaikwad, 2006). *C. rotundus* (nutgrass) contains a strong inhibitor of AChE, which possibly acts as an agent of plant's war against herbivore animals, and other plants trying to grow in the same habitat (Sharma and Gupta, 2007). Figure 2.3 illustration of the *C. rotundus* rhizome. *C. rotundus* contained flavonoids, tannins, glycosides, furochromones, monoterpenes, sesquiterpenes, sitosterol, alkaloids saponins, terpenoids, essential oils, starch, carbohydrates, protein, separated amino acids and many other secondary metabolites. The previous works also showed that the plant exerted antiparasitic, insecticidal, repellent, antibacterial, antioxidant, anticancer, central nervous, neuroprotective, antiinflammatory, antipyretic, analgesic, hypolipidemic, weight control, antiplatelet, gastrointestinal, hepatoprotective, antidiabetic, anti-dysmenorrhea, dermatological and many other effects (Al-Snafi, 2016). The *C. rotundus* have been reported to contain oils, alkaloids, glycosides, saponins, flavonoids, tannins, starch and carbohydrates. It also contains proteins and traces of Mg, V, Cr, Mn and Co. The rhizomes of *C. rotundus* have been used in ancient medicine in India for fever, dysentery, pruritis, pain, vomiting and various blood disorders (Raut and Gaikwad, 2006). Table 2.3 shows some chemical compositions of *C. rotundus* essential oils reported in the literature.

Figure 2.3: Illustration of the *C. rotundus* rhizome
Source: https://www.google.complantsoftheworldonline.org

Table 2.3: Some chemical compositions of *C. rotundus* essential oils reported in the literature.

Compound	**Composition (%)***					
α-pinene	0.06	2.20	2.87	3.0-10.8	0.82-10.8	2.02
Sabinene	0.02	na	0.43	na	na	na
β –pinene	0.13	3.90	2.13	5.3-11.3	0.67-11.3	1.62
p-cymene	0.08	na	0.18	1.7-0.6	0.6-1.7	0.39
Limonene	0.09	na	0.28	2.0 5.7	2.0-5.7	0.39
1,8-cineole	0.34	na	0.36	na	0.67-1.5	
Pinocarvone	0.04	na	7.92	2.2-0.4	0.4-2.2	2.11
Terpinen-4-ol	0.02	na	0.59	0.9-1.0	0.9-1.0	0.50
Myrtenal	0.04	na	na	na	na	1.33
Verbenone	0.05	na	1.55	0.6-1.1	0.43-1.1	2.01
(E)-carveol	0.08	na	0.48	0.4	0.4-1.23	0.04
α-copaene	0.44	na	0.40	0.5	0.5-1.44	1.62
β-elemene	0.20	3.70	0.64	0.8-0.5	0.35-0.8	0.15
Cyperene	11.83	na	7.83	1.6-2.6	1.6-37.9	na
β-caryophyllene	2.11	37.9	3.08	0.8-0.6	0.6-6.7	0.68
Caryophellene oxide	na	0.20	2.86	5.4-2.6	0.2-5.4	
α-muurolene	0.36	na	na	na	0.26-0.34	na
α-ylangene	Na	na	na	1.9	na	na
References	(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, *some data modified as mean average from origin source (a): Chang et al. (2012), (b): Aghassi et al. (2013), (c): Al-Snafi (2016), (d): Lawal and Oyedeji, (2009) (e): Hu et al. (2017) and (f): Eltayeib and Um Ismaeel (2014).

2.4.3. Moringa oleifera

Kindom: Plantae Order: *Brassicales* Family: *Moringaceae* Genus: *Moringa* Species: *M.oleifera* (Wikipedia. org. *Moringa*). A strong and rapidly growing *Moringa oleiera* Lamarck (fam. *Moringaceae*) tree is widely cultivated due to its high adaptability to environmental conditions. It's considered as one of the most useful trees in the world because almost all parts of this plant can be used as in food, in medicines and for industrial purposes.

M. oleifera (Moringaceae) is a fast-growing softwood tree indigenous to sub-Himalayan tracts of Northern India. It is one of 13 species within the same genus, and has become the most diffuse in tropical and subtropical areas at up to 2000 m (Leone et al., 2016). M. oleifera seed oil as a sustainable source of renewable energy for bio diesel production (Mathur, 2014). The methanolic extract of M. oleifera and Stachytarpheta indica leaves was evaluated for repellent effect against Aedes aegypti mosquito (Mgbemena et al., 2015). Table 2.4 shows fatty acid composition of some M. oleifera seed oil reported in the literature.

In many countries, there are huge efforts to spread the use and cultivation of *M. oleifera*, since it is a significant source of fats, proteins, β-carotene, vitamin C, iron, potassium, and other nutrients with low toxicity of seeds and leaves. For these reasons, some parts of this plant have drawn much attention and have been studied for its various biological activities, including antiatherosclerotic, immune-boostin, anticardiovascular diseases, antiviral, antioxidant and antimicrobial, ani-inflammatory properties and tumor suppressive effects in skin papillomagensis, hepatocarcinoma cancer, colon cancer, and myeloma (Barakat and Ghazal, 2016). Figure 2.4: shows of the *M. oleifera* Plant seeds.

Figure 2.4: Illustration of the *M. oleifera* seeds
Source: https://www.google.complantsoftheworldonline.org

Table 2.4: Fatty acid composition of *M. oleifera* seed oil reported in the literature

Fatty acids		**Composition (%)***					
Lauric	C12:0	na	0-0.3	na	Na	na	na
Myristic	C14:0	0.78	0.3-1.5	0.2	0.72	na	0.13
Palmitic	C16:0	10.64	25-46	7.8	6.1	5.66-6.46	6.46
Palmitoletic	C16:1	na	0.82–3.44	2.9	1.2	1.43-1.92	0.09
Stearic	C18:0	6.07	2.68–6.00	7.6	4.6	4.79-7.94	5.88
Oleic	C18:1	22.51	67.79–79.50	70	78.7	73.30-79.58	71.21
Linoleic	C18:2	na	0.83	0.9	Na	0.58-0.59	0.06
Linolenic	C18:3	na	0.36	0.5	1.8	0.15-0.17	0.18
Arachidic	C20:0	2.21	2.14-4.08	4.2	2.3	1.57-5.1	3.62
Behenic	C22:0	1.03	4.57–7.10	6.2	4.5	2.62-3.62	6.41
Lignoceric	C24:0	na	0.54	na	na	na	na
Gondic	C20:1	na	na	na	na	na	na
	SFA	na	17.24–23.79	49.1	18.3	15.00-22.83	na
	MUFA	na	71.71–80.70	na	79.9	na	na
	PUFA	na	0.41–2.20	na	1.8	na	na
	UFA	na	na	50.9	79.9	77.14-84.98	na
References		(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, *some data modified as mean average from origin sources (a): Adegbe et al. (2016), (b): Leone et al. (2016), (c): Ghazali and Abdulkarim, (2011), (d): Ogunsina et al. (2014), (e): Barakat and Ghazal (2016) and (f): Lalas and Tsaknis (2002)

2.4.4. Adansonia digitata L.

Kindom: Plantae Order: *Malvales* Family: *Malvaceae* Genus: *Adansonia* Species: *A .digitata* (Wikipedia. org. *Adansonia*).

Baobab (*Adansonia digitata* L.) is a large iconic tree indigenous to Africa where it is found in many countries. It is an emblematic, culturally important and physically majestic sub-tropical tree. The baobab has been referred to as "arbre a palabre", meaning the place in the village where the elders meet to resolve problems. In the past decade, it has attracted the interest of several pharmaceutical companies and researchers due to its various traditional uses (medicinal, nutritional and cosmetic). Recently, the European commission authorized the import of baobab fruit pulp as a novel food and it was approved in 2009 by the Food and Drug Administration as a food ingredient in the United States of America. Figure 2.5 shows of the *A. digitata* seeds.

Figure 2.5: Illustration of the *A. digitata* seeds

Source: https://www.google.complantsoftheworldonline.org

Due to the high demand for commercial baobab products in European and United States, this tree with its edible fruits needs to be conserved and treasured (Kamatou et al., 2011). It is reported that it is an excellent antioxidant due to the vitamin C content which is seven to ten times higher than the vitamin C content of oranges. Baobab has numerous biological properties including antimicrobial, anti-viral, anti-oxidant and anti-inflammatory activities amongst others (Kamatou et al., 2011). It have so many medicinal and non-medicinal uses it is used in the treatment of bronchial asthma, dermatitis, sickle cell anemia, diuretic, anti-diabetic, diarrhea, dysentery, laxative, hiccough in children, anti-oxidant, antiinflammatory, antidote for poison, anti-trypanosome uses (Sundaramble et al., 2015). The various parts of the plant (leaves, bark and seeds) are used as a panacea, that is, to treat almost any disease and specific documented uses include the treatment of malaria, tuberculosis, fever, microbial infections, diarrhea, anemia, dysentery, toothache, etc., Several plant parts have interesting anti-oxidant and anti-inflammatory properties (Rahul et al., 2015). Development of plant based alternative compounds for mosquito control has gained importance now-a-days, in view of increasing resistance in mosquito vectors to existing insecticides. The larvicidal and repellent activities of benzene, chloroform, hexane and methanol leaf extracts of Indian medicinal plant, *A. digitata* were investigated against malarial vector, Anopheles stephensi (Krishnappa et al., 2012). Table 2.5 shows some fatty acid composition of *A. digitata* seed oil reported in the literature.

Table 2.5: Fatty acid composition of *A. digitata* seed oil reported in the literature

Fatty acids		**Composition (%)***					
Lauric	C12:0	na	0-0.3	na	na	na	na
Myristic	C14:0	0.78	0.3-1.5	0.1	0.168	0.2	1.01
Palmitic	C16:0	18.0-30.0	25-46	28.8	21.76	24.2	29.57
Palmitoletic	C16:1	na	0.3-1.7	0.25	na	na	0.27
Stearic	C18:0	2.0-9.0	na	4.4	8.85	4.6	36.28
Oleic	C18:1	30.0-42.0	21-59	25.1	36.40	35.8	31.41

Linoleic	C18:2	20.0-35.0	12-29	36.0	25.50	30.7	27.31
Linolenic	C18:3	na	0-8	0.5	2.60	1.0	6.65
Arachidic	C20:0	na	0.5-1.0	0.7	0.17	1.3	0.14
Behenic	C22:0	na	na	na	0.33	0.7	na
Lignoceric	C24:0	na	na	na	0.21	0.2	na
Gondic	C20:1	na	na	na	na	0.9	na
	SFA	33	na	34.6	31.49	31.7	31.7-50
	MUFA	36	na	28.7	36	37	24-31.7
	PUFA	31	na	36.7	31	31.7	26-37
	UFA	na	26.89	na	67.15	na	na
References		(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, *some data modified as mean average from origin sources: (a): Vermaak et al. (2011), (b): Bamalli et al. (2014), (c): Komane et al. (2017), (d): Babiker et al. (2017), (e): Osman, (2004) and (f): Ayaz et al. (2014).

2.5 EXTRACTION TECHNIQUES OF OILS

The term of extraction is known to be solid-liquid extraction of natural products from plants or microorganisms (Kaufmann and Christen, 2002). In the field of natural products such as pharmaceutical industry, defines the separation of medicinally active constituents of the plant from other inert constituents through selective procedures involving various solvents (Meghna and Minal, 2013). The extraction was also classified into different ideas by Sticher (2008), whereby it is desorption and diffusion of components towards the matrix, solubility of compounds in solvent and finally the collection of extracts.

For the extraction, various conventional methods had been used for many decades but now the modern methods (non-conventional methods) are also being widely used due to some drawbacks that occur in the conventional methods. It would be due to the longer extraction duration, higher solvent volumes, degradation of thermo labile components (Gupta et al., 2012; Meghna and Minal, 2013 and Sticher, 2008), higher energy, inconsistent extraction, poor safety (Gupta et al., 2012) and requirement for labor (Sticher, 2008).

Therefore, the modern methods plays and important role in enhancing those limitations besides increasing extraction yield, enhancing the quality and efficiency of samples (Gupta et al., 2012 and Meghna and Minal, 2013), elimination of undesirable and insoluble components of plant and reduction in effort of sample cleanup (Gupta et al., 2012). Chemat et al. (2012), worked on the 'Green Extraction of Natural Products' with the definition of providing an extraction procedure with ideal consumption of raw materials, improving and optimization of current methods, using non-dedicated equipment and discovering alternative solvents.

Azmir et al. (2013) and Gupta et al. (2012), pointed that solvent is the major parameter that influences the extraction procedure. The selection of solvents should be according to the policy of 'like dissolves like' whereby the polar compounds have a higher probability in extracting polar constituents and vice versa. The mixture of solvents such as the hydro alcoholic (water and alcohol) was reported to produce higher yields of extraction (Gupta et al., 2012). The polarity, toxicity towards living things, cost, efficiency of molecular interaction energies between solute and solvent and mass transfer is the key factor in choosing the suitable solvent (Azmir et al., 2013). From the view of various extraction methods, extraction temperature, pressure and duration also have the possibilities in contributing towards the difference in the properties and yield of the extracts.

2.5.1 Conventional Extraction Techniques

The conventional extraction method includes the decoction, hydrodistillation, maceration, percolation and soxhlet extraction. Each of the technique was described according to its principle and mechanism together with operating procedures. One of the parameters that differentiate the various conventional methods is the temperature (Handa et al., 2008).

A) Decoction

Decoction, a hot aqueous based extraction is conducted through the application of heat whereby this extraction is suitable for water soluble and thermally stable constituents (Handa et al., 2008). The extraction was conducted in an open-type cylindrical vessel usually made up of stainless steel. The bottom of the reactor vessel is fitted with filter cloth and discharge valve whereas the outer part is wrapped with steam jacket. The plant material is boiled under steam heat supplied into the jacket in a fixed volume of water usually around 1:4, 1:8 or 1:16 w/v of sample and water for a specific duration. The ratio is determined according to the physical feature of the material whereby it is soft, moderately hard or very hard. High volume of water is needed as the hardness increases. Boiling was done by the volume reduced to one-fourth and the mixtures were then cooled and filtered from the marc (extracted plant material) into the holding tank. In order to remove fine impurities, the extract was passed through sparkler filter and concentrated with spray drying to obtain a dry and powdered extract (Handa et al., 2008).

B) Hydrodistillation

The mechanism of hydrodistillation involves certain physicochemical processes that are the hydrodiffusion, hydrolysis and decomposition by heat. Hydrodiffusion in terms of extraction are the flow of solvent and solute across the plant membranes and the rate of this diffusion are influenced by the solubility of the matrix constituents in the solvent whereas the concept of hydrolysis is the chemical reaction between the constituents and solvent. Heat is also applied in the reaction to accelerate the reaction. All the physicochemical process described gives simultaneous effect in the process whereby the high heat causes higher solubility of constituents through higher diffusion rate across the membrane of matrix (Azmir et al., 2013 and Handa et al., 2008).

Some of the examples of hydrodistillation are the water, water and steam and direct steam distillation. To perform the extraction, the plant material is filled in the still

compartment with water and heat supplied to boil it. In the case of steam, direct steam was flown into the sample container and cooling was provided to condense steam vapor, water and constituents. The mixtures were collected and water was removed to obtain the extract (Azmir et al., 2013). The removal of water could be done by adding anhydrous sodium sulfate to absorb the moisture (Yamini et al., 2008) and the schematic diagram on the extraction is as in Figure 2.6.

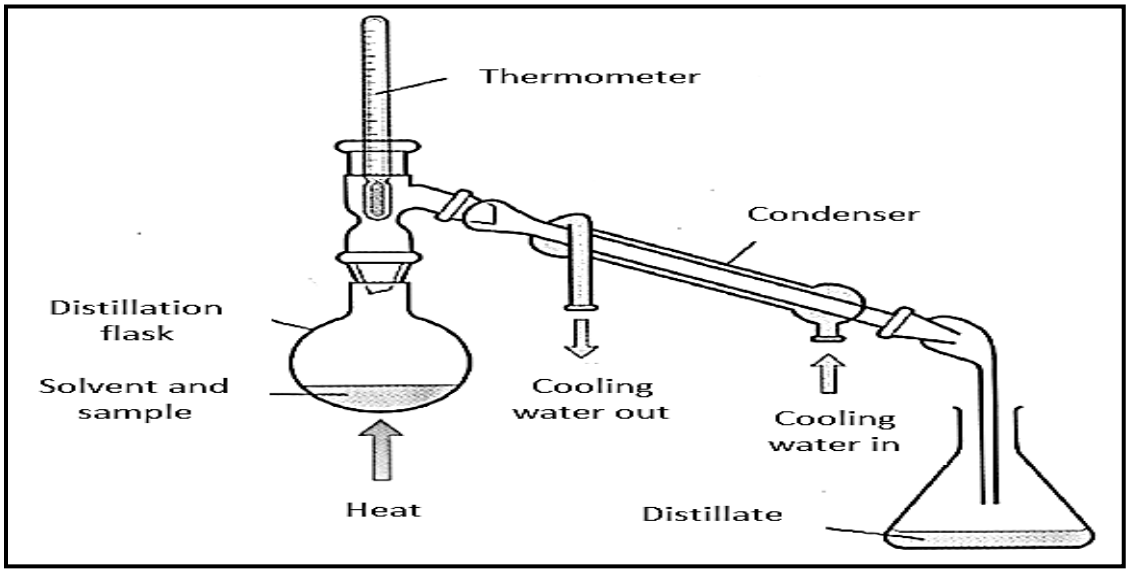

Figure 2.6: Schematic figure of hydrodistillation
Source: Scottish Sensory Centre (2013)

C) Maceration

The principle of maceration involves soaking and agitation of plant material in order to obtain the extract from solid materials through a leaching process (Handa et al., 2008). The soaked plant material cellular wall were softened by the *menstruum* (solvent) and penetrates in, to extract the soluble constituents. The agitation technique facilitates the diffusion of solvents into the samples and replaces the concentrated solution from the sample with new solvents (Azmir et al., 2013). This process is known to be a batch process (Meghna and Minal, 2013). According to Handa et al. (2008) this process is influenced by several factors; transport rate of menstruum into the sample and out of insoluble material, the solubility rate of active principle and surface area of plant material for mass transfer.

Handa et al. (2008) and Azmir et al. (2013) had described the small scale extraction steps of maceration whereby, the uniformly ground parts of the plant were soaked in selected menstruum in a tightly stoppered container and were exposed to occasional shaking or agitation at room temperature. The liquid was strained off after achieving soaking duration via filtration technique to remove the marc (damp plant residue) and impurities. The marc was pressed during filtration in order to maximize the yield of extraction. The filtrate was concentrated to remove the menstruum leaving behind dry extract.

D) Percolation

Percolation is a batch process that is often used to extract the active components for the extraction of alcoholic and fluid extracts. The principle of this extraction is on the equilibrium concept whereby the sample was macerated in solvent so that the active constituents attain equilibrium by leaching. This technique is also based on the mass transfer principle according to the capacity and utility of the percolator (a narrow, both ends opened, cone shaped vessel). The application of gravity was also involved towards the weight of the column of liquid (Handa et al., 2008).

The extraction image is as in Figure 2.7 whereby it has been conducted in the percolator whereby just like maceration, the plant samples were soaked in menstruum in a closed container for required duration and were transferred into the percolator whereby both ends of the percolator were closed. The maceration was further continued in the percolator. The bottom end of the percolator was opened, allowing the percolate (solvent extract) to drip. Required amount of menstruum is added to wash the marc whereby the sample particle has high probability getting exposed to the passing menstruum and the marc was pressed to remove any solution. The enriched solution was further concentrated by filtration and evaporation to obtain the extracts (Handa et al., 2008).

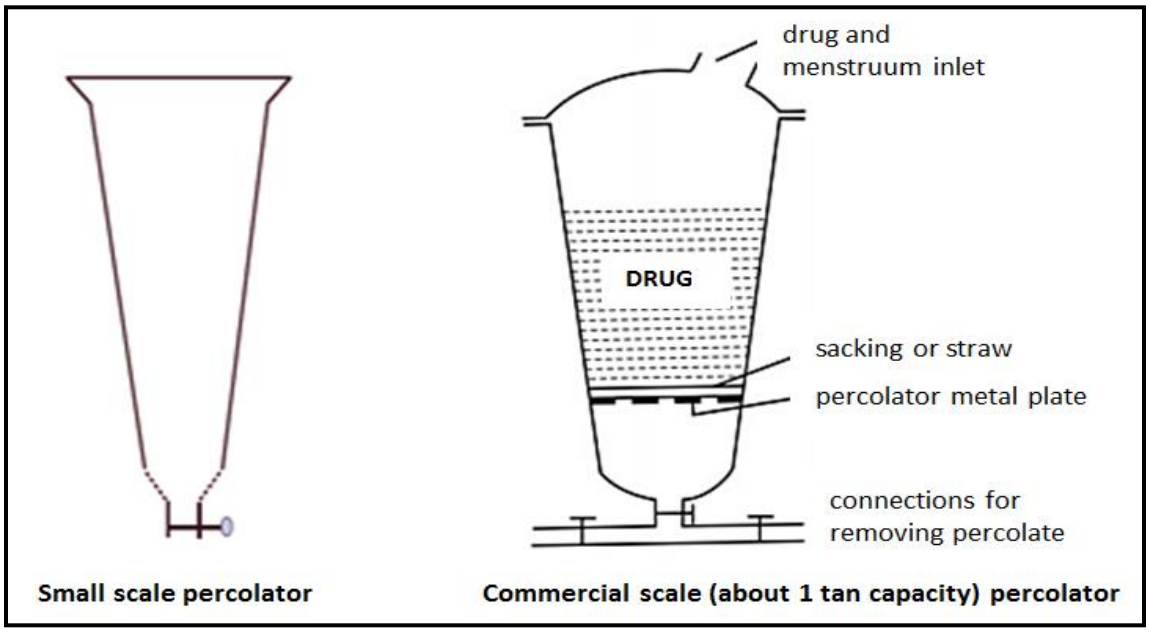

Figure 2.7: Schematic figure of percolator for percolation extraction

Source: Handa *et al*. (2008)

E) Hot Solvent Semi-Continuous (Soxhlet) Extraction

The principle and mechanism involved in soxhlet extraction are heat and recycling of solvents (Handa et al., 2008). Continuous heat is supplied to the solvents to evaporate and distill to extract the thermo labile components. Azmir et al. (2013) had reported that this extraction was first invented by Franz Ritter Von Soxhlet, a German chemist, in 1897 for the extraction of lipids. But now, the usage of this technique had expanded up to the level of extraction of various constituents.

In laboratory scale, the finely grounded sample was placed into the sample holder, thimble, and was placed into the soxhlet chamber. The holder was then attached to the distillation flask and choice of solvent was added in via soxhlet chamber at least two cycles of volumes. The condenser was attached at the top end of the soxhlet and continuous supply of water is ensured. The heat was supplied to the extracting solvent and the vapor condenses in the distillation condenser and drips into the soxhlet chamber washing the soluble constituents. The solvent together with the extract flows back into

the distillation flask as they reach the siphon level (Azmir et al., 2013 and Handa et al., 2008). Continuous heat evaporates the fresh solvent again to the solid bed of the plant for several cycles leaving behind the solute (extract) to continue the extraction cycle up to the ensured time of complete extraction whereby solution in the siphon tube is completely colorless (Solanki and Nagori, 2012). The final extract is the distillation still that is rich in active constituents to be concentrated. The setup of the apparatus is illustrated as in Figure 2.8.

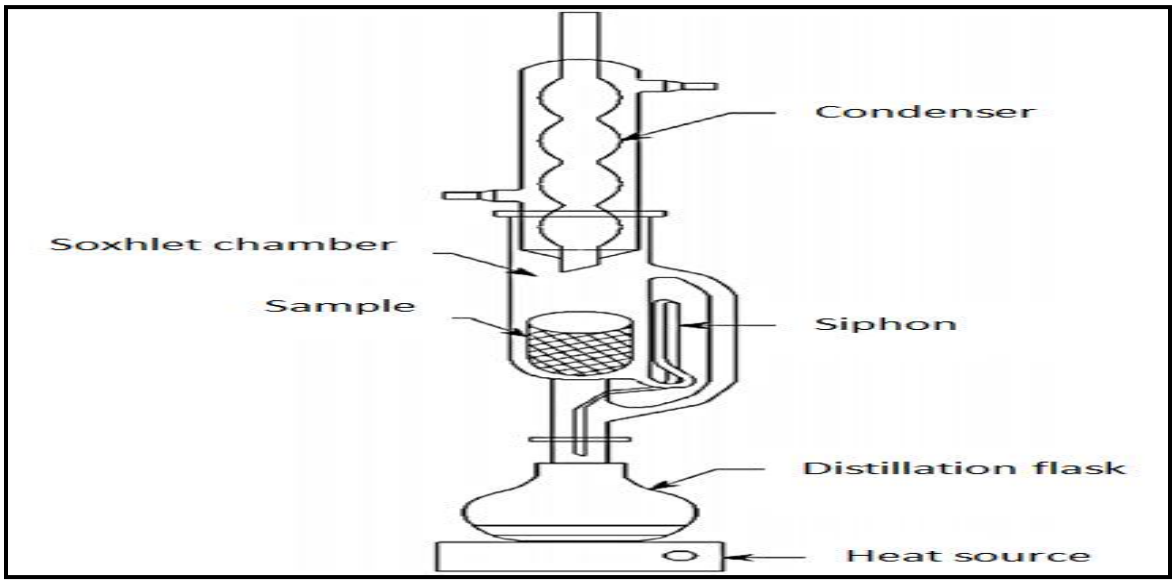

Figure 2.8: Schematic figure of conventional soxhlet extraction

Source: Dutta et al. (2014)

F) Steam distilliation

Steam distillation (SD) as one of the important mechanisms has a great role in oil recovery in thermal methods and so it is important to simulate this process experimentally and theoretically (Daryasafar et al., 2014). Steam distillation is carried out by passing dry steam through the plant material whereby the steam volatile compounds

are volatilized, condensed and collected in receivers. Steam distillation has been in use for essential oil extraction for many years (Pushpangadan and George, 2012). (SD) has been applied to flavour extraction in different juices for identification purposes. Methods involving heating the sample can modify the flavour composition quantitatively and qualitatively. Moreover, odour-active compounds in fruits often present glucosidic precursors which are converted to the free aroma compound during heating processes, or at low pH values. For that reason, distillation under reduced pressure is of great interest in juice analysis (Heredia and Vicario, 2013).

(SD) is a process used for the recovery of volatile compounds with high boiling point, from inert and complex matrices, solid or liquid, using saturated or superheated steam as separation and energy agent. This process is used for the extraction of essential oil from plants. In practice, the process uses water and/or steam as extracting agent to vaporize or liberate the volatile compounds from the raw material. The compounds are volatilized by absorbing heat from the steam, and are then transported to the steam where they are diffused. The resulting vapor phase is cooled and condensed prior to separating the water from the organic phase based on their immiscibility. In this process, two products are obtained: volatile oil and hydrosol. The volatile oil is in the upper phase and the hydrosol (water and some hydrolyzed compounds) is in the bottom phase of the decanter (Prado et al., 2015).

(SD) is one of the principal methods for the manufacture of essential oils and fragrances. Essential oils are mostly mixtures of terpenoid substances. They have relatively high boiling temperatures (frequently above 200°C). Their recovery by ordinary distillation at atmospheric pressure is impractical because they undergo thermal decomposition at such high temperatures. They can be recovered by vacuum distillation, but a more economical approach is steam distillation (Berk, 2013).

Essential oils are practically insoluble in water. The total vapor pressure of a mixture of immiscible substances is equal to the sum of the vapor pressures of the pure components. Consequently, such a mixture will boil at a temperature lower than the boiling point of each of the components. A mixture of an essential oil and water will therefore boil at a temperature below 100 °C at atmospheric pressure. This is the basic principle on which steam distillation is based. Saturated steam is bubbled through the material containing essential oils (juices, extracts, spices, herbs, etc.). The essential oils are volatilized into the steam and entrained towards the condenser. When the vapors are condensed, a liquid consisting of two immiscible layers is obtained, from which the essential oil is separated by centrifugation or by decantation. A variant of steam distillation is hydrodistillation, wherein a mixture of water and the aromatic material (e.g., a spice, a herb, a flower, etc.) is distilled, the vapor is condensed, and the essential oil is recovered from the condensate. Yet another variant is so-called "solvent-free extraction, which is not at an extraction process all but rather a laboratory distillation technique. The material is heated by microwave, whereby the essential oil is vaporized by the *in situ* water and recovered from the distillate (Berk, 2013).

Steam-distillation has been used for a long time to produce dairy aromas from dairy products. However, high temperatures may affect the composition of the aroma obtained, because of degradation of heat-sensitive flavor components. Another disadvantage is that hydrophobic flavor components may be retained by the matrix of the original product, leading to a different composition of the aroma. As steam distillates contain a considerable amount of water (up to 90%), a weak flavor is obtained (Sibeijn and Wouters, 2009).

(SD) is the method most commonly used to produce flavouring substances on a commercial basis. Extraction by means of liquid carbon dioxide, under low temperature and high pressure, is a more expensive alternative that provides a more natural organoleptic profile. Essential oils obtained by distillation and those produced by solvent

extraction also have different organoleptic profiles and chemical compositions; these differences in composition will in turn influence their antimicrobial properties. Literature reports have indicated that essential oils extracted from herbs with hexane exhibit superior antimicrobial activities compared with the corresponding essential oils obtained by steam distillation (Tassou et al., 2012).

Steam distillation process happened when light fractions of crude oil are separated by injecting the steam into the crude oil. Observation of the produced vapors of matured steam floods proves the fact that steam can carry a large amount of light hydrocarbons in the steam distillation process (Daryasafar et al., 2014). Figure 2.9 shows Schematic figure of Steam distillation apparatus.

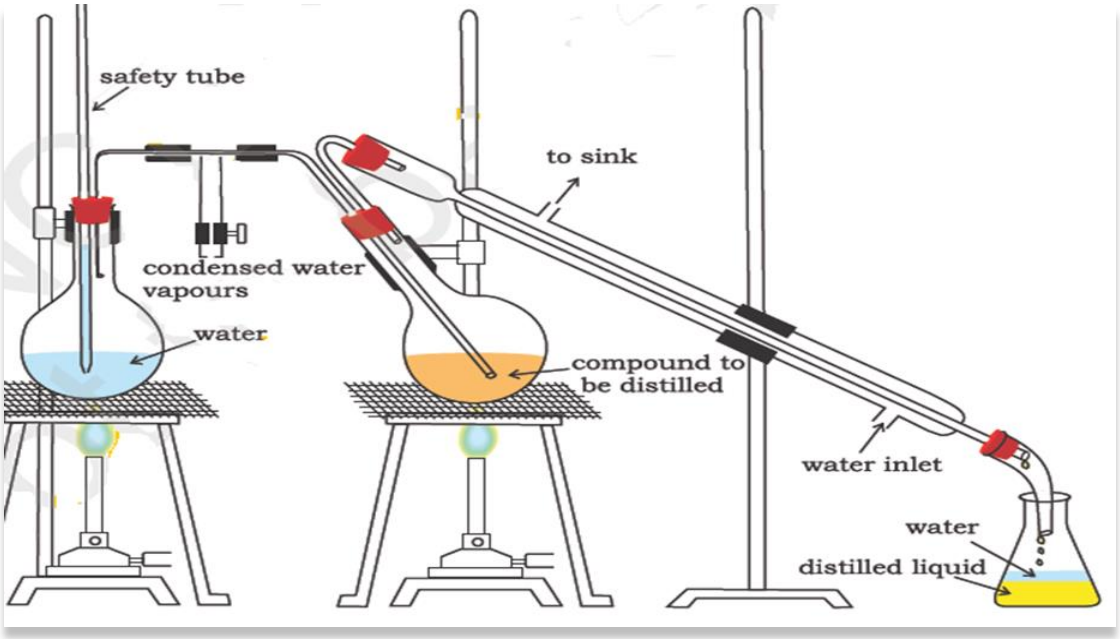

Figure 2.9: Schematic figure of Steam distillation

Source: Dutta et al. (2014)

2.5.2 Non-Conventional Extraction Technique

Microwave-assisted, supercritical fluid (Kaufmann and Christen et al., 2002; Sticher, 2008; Gupta et al., 2012) and ultrasound-assisted extraction are the non-conventional technique (Sticher, 2008). The differences between the operating parameters of these methods are the agitation, magnetic field, pressure, radiation and temperature.

(i) Microwave-Assisted Extraction (MAE)

This microwave-assisted extraction (MAE) works based on the principle of heat through microwave energy with the frequency level of 0.3-300 GHz (Kaufmann and Christen, 2002). This energy does also possess electric that causes heat and magnetic power that causes dipolar spin and ionic transmission (Kaufmann and Christen, 2002). Heat is transferred directly to the solvent mixture and magnetic dipole rotation of the solvent and matrix molecules speeds up the extraction within the time limit of usually less than 30 min (Kaufmann and Christen, 2002 and Sticher, 2008). The heating causes the moisture evaporates from the matrix and breaks the cell wall, releasing constituents into the sample (Meghna and Minal, 2013).

The two different techniques of MAE (Figure 2.10) are the extraction via closed vessels under controlled pressure and temperature (diffused microwave multi-mode cavity system (DMAE) and open vessels at atmospheric pressure (focused microwave single-mode cavity system (FMAE). The factor that differentiates between these two systems are the boiling point of the solvent and atmospheric pressure whereby FMAE system is limited to the boiling point of the solvent at atmospheric pressure while PMAE system can be operated above the boiling point of the solvent at suitable pressure. Thus, it is reported that the PMAE are more efficient due to high extraction speed (Sticher, 2008). Kaufmann and Christen (2002), suggested that the application of the PMAE is for the digestion or acid mineralization or extraction under pressurized conditions above the boiling point of the solvent. For the operation of PMAE system, the samples together

with extraction solvent are placed into the extraction Teflon vessels located on the rotating carousel. Since there are several single vessels, the solvents may vary. The temperature was set depending on the boiling point and the volume of the solvent. Vessels cool to room temperature before opening and filtration of mixture done to obtain the extract. The operation of FMAE uses quartz vessels attached to a vapor condenser for reflux process to take place. The removals of the extract are much favorable through this system compared to PMAE due to safety handling with application of cooling system.

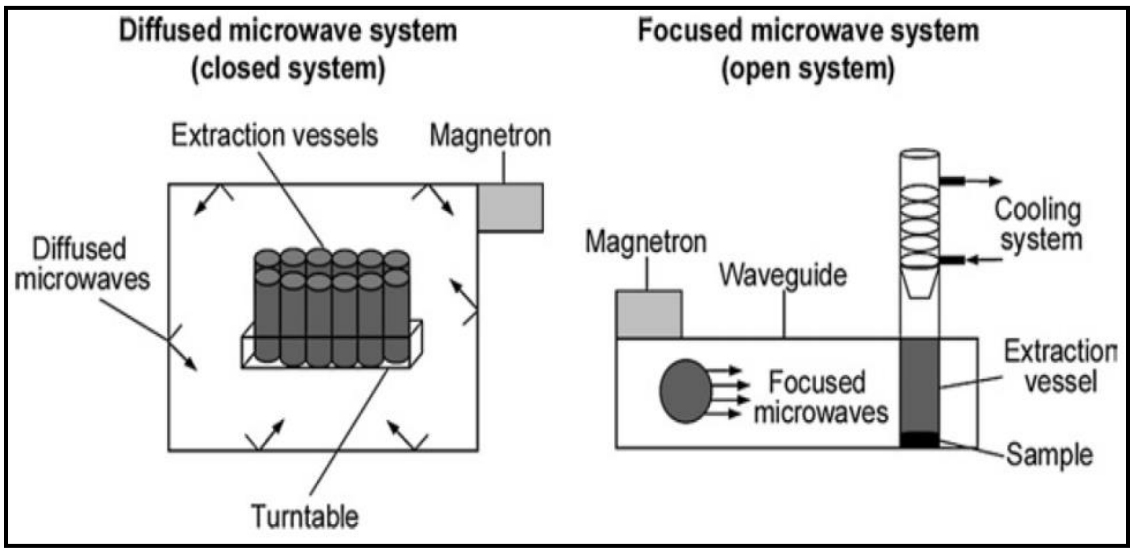

Figure 2.10: Schematic figure of MAE

Source: Sticher (2008)

(ii) Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is a liquid extraction process whereby the normal liquid solvent phase is replaced by supercritical fluid, a substance above its critical point (Sticher, 2008). The main factor influences the SFE are the temperature, pressure, flow rate of CO_2, amount, moisture content and particle size of sample, additional co-solvent and solvent-to-feed-ratio. The generally SFE works on the principle of conducting extraction closer to ambient temperature. Sticher (2008) had reported that

carbon dioxide (CO_2) is the most common supercritical fluid being used due to its low critical temperature. Handa et al. (2008) added that CO_2 is favored physical properties, low cost, environmentally safe and easily available. For certain extraction, CO_2 is not suitable due to its polarity limitations and thus the addition of co-solvent or modifier such as organic solvent are needed (Handa et al., 2008).

The SFE extraction can be either in a static mode or dynamic mode whereby in dynamic mode the fresh solvent were continuously supplied into the extraction vessel at higher temperature and pressure (Kaufmann and Christen, 2002). According to the Figure 2.11 extraction fluid of preferred pressure supplied through the pump into the extraction vessel held above the critical temperature of the fluid. The pressure is controlled by the restriction device (Kaufmann and Christen, 2002). The supplied fluid flows into the extraction vessel and into the sample matrix and flows out through the restrictor and ended in the collection vessel (Stricher, 2008). The removal of the fluid from the solute is done through depressurization of supercritical fluid (Azmir et al., 2013).

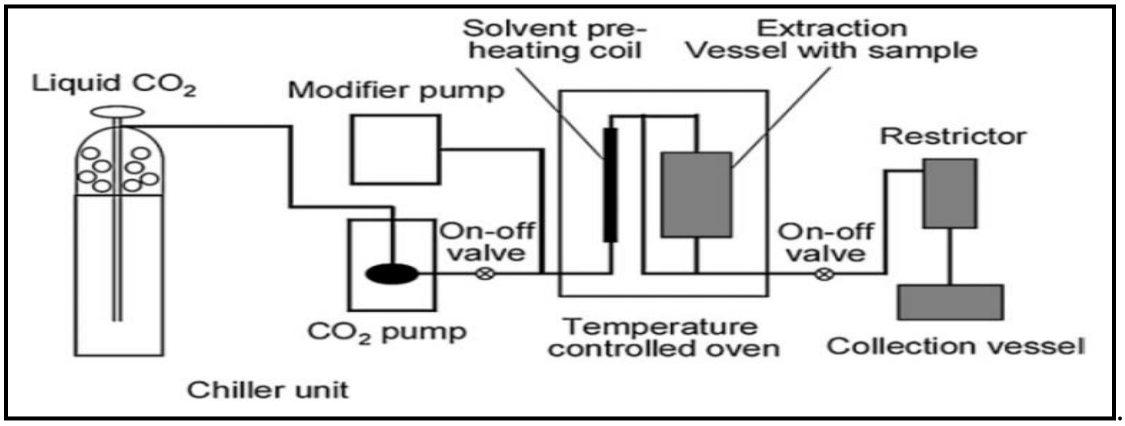

Figure 2.11: Schematic figure of SFE

Source: Sticher (2008)

(iii) Ultrasound-Assisted Extraction (UAE)

Gupta et al. (2012) reported that the principle and mechanism of ultrasound or sonication work based on the high intensity and frequency sound wave interaction with plant material and Azmir et al. (2013) added that the reaction involves the physical phenomena of diffusion of solvent across the cell wall and washing of contents in the matrix. The frequencies of this extraction range from 20-2000 kHz (Handa et al., 2008). This energy wave forms microcavities and microjets that contribute towards rupturing and breaking of plant cell wall whereby the particles of the solid and liquid vibrates and accelerates; and thus enables the constituents to be easily diffused into the solvent. It is also said that, higher energy of ultrasound weakens the intramolecular forces of the molecule creating bubbles that further facilitate the release of compounds and enhance the mass transfer through diffusion of solvent into the sample by preventing the plant tissue from getting saturated (Gupta et al., 2012; Azmir et al., 2013 and Meghna and Minal, 2013). Meghna and Minal (2013) added that the movement of the waves causes expansion and compression phase that the energy pulls apart and pushes the molecules together respectively. Ultrasonic bath (closed extractor fitted with an ultrasonic horn transducer) and ultrasonic horn transducer are the different type of ultrasound extractor (Meghna and Minal, 2013). The setup image is as in Figure 2.12. The extraction steps involved are the addition of solvent according to the ratio of liquid material into the sample container and the placement of the container into the ultrasonic device. The extraction condition; temperature, duration, and ultrasound power were also set on the device prior to extraction (Samaram et al., 2014).

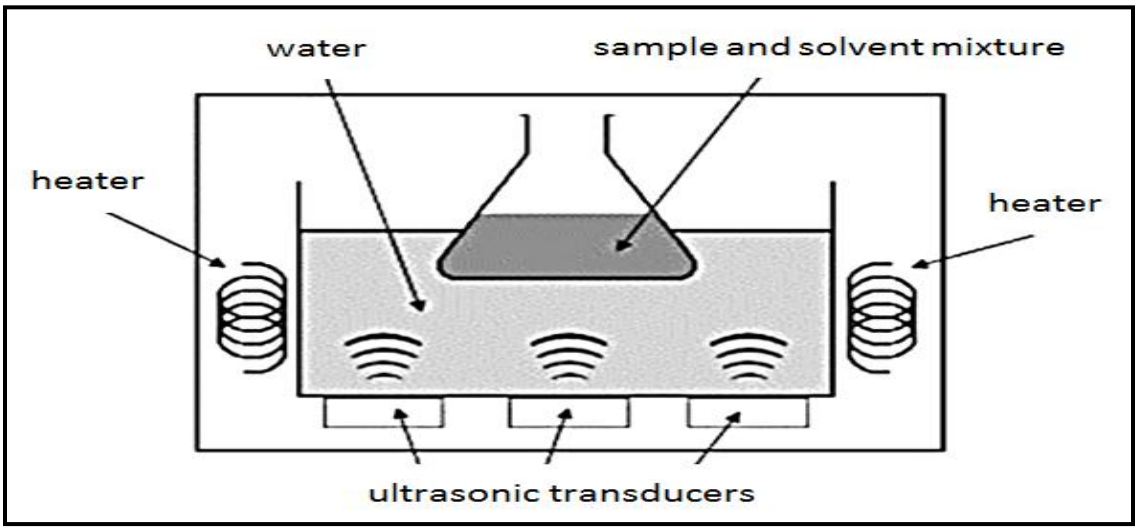

Figure 2.12: Schematic figure of UAE

Source: Samaram *et al*. (2014)

Even though there are many extraction techniques that could be adapted, each of them has its own pros and cons that play an important role during the selection of proper techniques. Several comparisons had been reviewed to review the types of extraction methods and its conditions that had been used in the extraction of the studied plants. Table 2.6 was tabulated to review the types of extraction methods and its conditions that had been used in the extraction of the plant extracts.

Table 2.6: Pros and cons of conventional and non-conventional extraction techniques

Extraction Technique	Advantage	Disadvantage	Reference
Conventional Methods			
Decoction	Suitable for thermally stable and aqueous soluble compounds Suitable for tough and fibrous plant	High energy for heating	Handa et al., 2008
Hydrodistillation	Extraction before hydration of samples	Less suitable for thermo labile compounds	Azmir et al., 2013
Maceration	Favorable for thermo labile components[b] Recovery of solvents[a]	High volume of solvents[b]	Handa et al., 2008[a] andMeghna and Minal, 2013[b]
Percolation	Favorable for thermo labile components and dilute products (tinctures) Recovery of solvents	High volume of solvents Longer extraction duration Energy-consuming	Handa et al., 2008
Soxhlet	Easy to handle Favorable for many natural constituents[b] Promotes fresh solvents for extraction[b] Maintains heat[b] No extract filtration[b] Extraction of large amount of sample[b] Low volume of solvent[a]	Not suituble for heat sensitive pigmeants[a] No agitation[a] Thermal decomposition of certain compounds[a] Longer extraction duration[a]	Ngamwonglumlert et al. 2017 Handa et al., 2008[a]; Meghna and Minal, 2013[b]

Superscript (a and b) represents the corresponding reference.

Table 2.6: *Continues*

Extraction Technique	Advantage	Disadvantage	Reference
Non-conventional Methods			
MAE	Shorter extraction duration[b] Low volume of solvent[b]	Hazardous to flammable organic solvents[a] Low reproducibility Excessive temperature and pressure causes solute degradation[c] Filtration or centrifugation of extracts[c] Poor yield when constituents and solvents are non-polar or volatile[c]	Kaufmann and Christen, 2002[a] Meghna and Minal, 2013[b] and Sticher, 2008[c]
SFE	Higher yield due to repeated reflux[a] Easier separation of solute from fluid[a] Recycling of fluid minimizes waste[a] Low temperature and volume of solvent[c] No solvent residues[c] Low cost and toxicity[c] Reduce thermal degradation [c] Favorable for volatile components[c]	Low yield at low temperature[b]	Azmir et al., 2013[a]; Handa et al., 2008[b] and Sticher, 2008[c]
UAE	Shorter extraction duration[b] High extraction efficiency[b] Extraction of organic and inorganic compounds[b]	Depends on sonication time and power[c] Produces heat[c] High energy converts drug constituents into free radicals[abcd]	Azmir et al., 2013[a]; Gupta et al., 2012[b]; Meghna and Minal, 2013[c] and Handa et al., 2008[d]

	Low volume of solvent and temperature for thermally unstable compounds[c]	
	Suitable for various solvent[c]	

Superscript (a, b and c) represents the corresponding reference.

2.6 CHROMATOGRAPHIC TECHNIQUES

Various chromatographic techniques are used as an aid in the separation and identification of constituents from a group of mixtures.

2.6.1. Gas Chromatography (GC)

The gas chromatography (GC) technique is reported for the identification and characterization of volatile compounds, whereas the non-volatile compounds need to undergo a derivatization process before being analyzed via GC (Tistaert et al., 2011). GC works based on the principle of vaporizing the compounds by heat without decomposition to separate and analyzed the compounds. The vaporized analyte were carried by carrier gas, mobile phase, passing through the chromatography column, stationary phase. The choice of column is based on the type of samples polarity. As the analyte passes, they separate into its constituents according to characteristic rates and the eluted compounds were detected in time sequence. The flow of the analysis is as shown in Figure 2.13. This chromatography commonly attached with a sensitive detector either thermal conductivity (TCD), flame ionized (FID), mass selective (MSD), electron capture (ECD), nitrogen phosphorus (NPD), fourier transformed infrared, (FTIRD) and atom emission (AED) detector for identification purpose (Tistaert et al., 2011). The obtained data are in the form of qualitative and quantitative whereby various library suggestions could be obtained from the mass detector and the intensity of the compound in terms of peaks area could be obtained from the percentage area report.

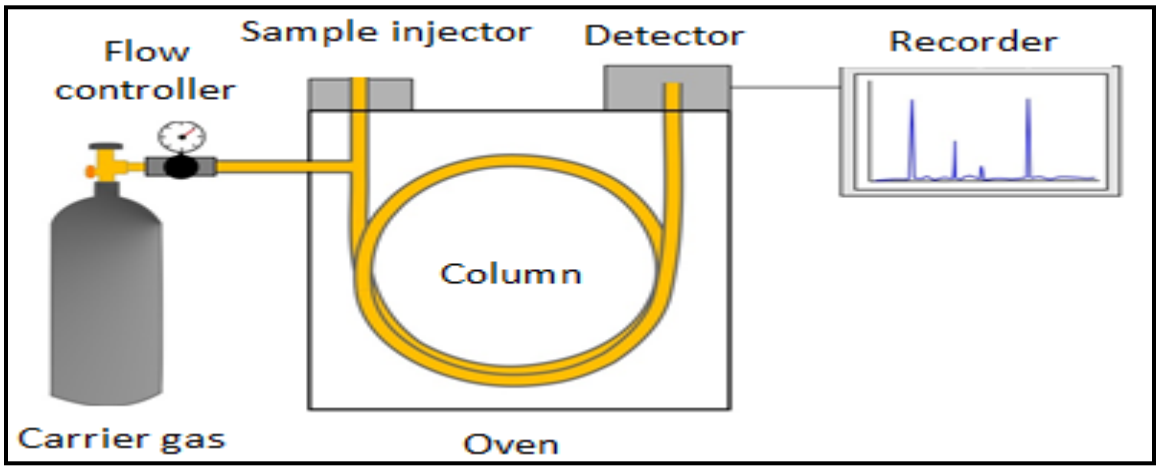

Figure 2.13: Schematic figure of GC
Source: Tistaert et al. (2011)

2.6.2 Thin Layer Chromatography (TLC)

Thin layer chromatography, an adsorption technique, is a chromatography method that is used for screening of compounds present in samples of natural products (Tistaert et al., 2011), stability tests of samples and quality control of final products (Shahid, 2012). This chromatography works based on two parameters that are the mobile and stationary phase whereby the stationary phase behaves as the adsorbent. The principle involved was the adsorptive and/or desorptive capacities that vary for each plant constituents. Ekiert et al. (2010) states that; the composition of the mobile phase influences the separation of the compounds. Capillary action is involved whereby the moving mobile phase carries the constituents according to its polarity towards the mobile phase causing separation of mixtures into single compounds.

In conducting the analysis, the mobile phase was prepared in a specific ratio and was transferred into a closed developing chamber and the atmosphere was allowed to saturate for a few min. The plant mixtures were spotted on the plate and the line was marked as a baseline. The spots were allowed to dry and the plate was transferred into the developing chamber and allowed to develop. The plate was removed and dried when the solvent reaches the solvent front line.

The analysis of the separation of the constituents of the mixture could be visualized under various detection methods. The heating process, ultraviolet light (fluorescent compounds) and detection reagents helps with visibility of certain targeted group of compounds. The visualized compounds were marked and the R_f (retention factor) value was calculated according to the ratio between the distance constituents travelled under standard chromatography conditions.

2.6.3 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) (Figure 2.14) works based on the application flow of mobile phase in high pressure (up to 400 bar) in a packed column. HPLC could be applied in separation, identification and quantitation of constituents present in a sample that could be dissolved by the liquid solvent.

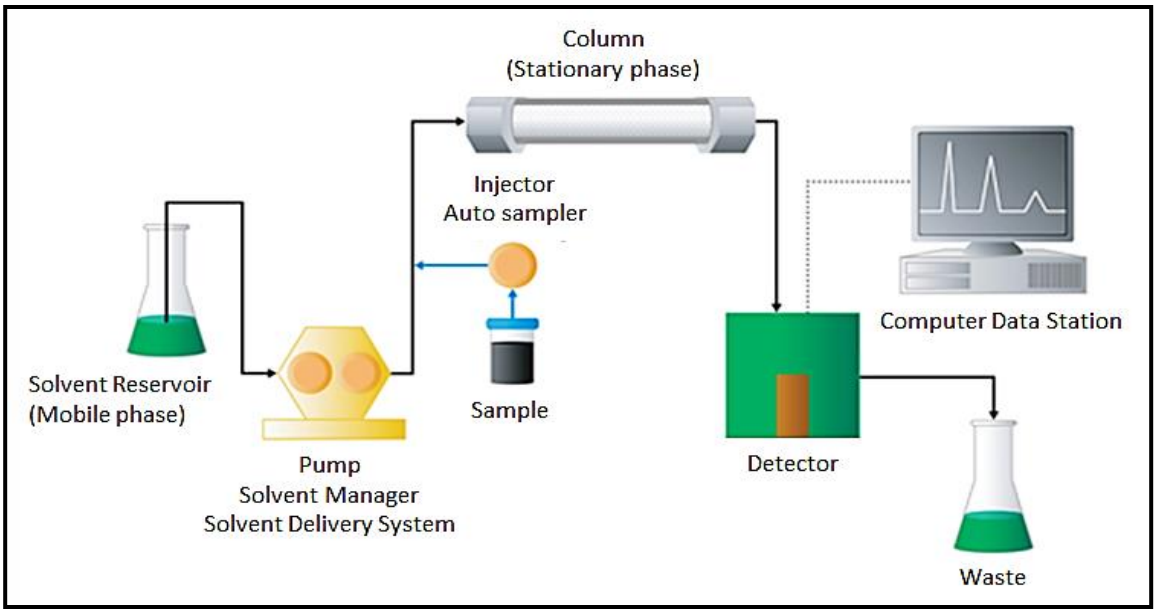

Figure 2.14: Schematic figure of HPLC
Source: Waters (2014)

HPLC has high sensitivity as low concentrations (ppt) constituents could be traced. The operation of HPLC starts with the flow of solvent, mobile phase, passing

across the high pressure pump to generate a specified flow rate. Auto sampler introduces the sample into the flowing mobile phase and passes through the column, stationary phase containing chromatographic packing material. The eluted chromatogram bands were detected by detector wired into the computer data system. Several detectors could work with HPLC such as the UV absorbance (for UV light active compound), fluorescence (for fluorescent compound), and evaporate light scanning detector (ELSD) (as universal detector) (Waters, 2014). Table 2.7 shows pros and cons of various chromatography techniques.

Table 2.7: Pros and cons of various chromatography techniques

Chromatography Technique	Advantage	Disadvantage	Reference
TLC	Fast[a] Simple[ab] Less costly[ab] Easily optimized parameters (sample application and plate development)[a] Little sample clean-up and equipment[b]	Require specific adsorbent for certain detection reagent[a] Low reproducibility and resolution[a] Require high concentration of constituents in sample[a] Parameters (sample spotting, vapors environment in chamber and unstable colorization of detection reagent) are difficult to be controlled[a]	Tistaert et al., 2011[a] and Shahid, 2012[b]
GC	Shorter analysis time Lower detection limits	Suitable only for volatile and thermo-instable compounds Require derivatization for certain compounds	Tistaert et al., 2011
HPLC	Easy to operate and automatable technique with high resolution, selectivity and sensitivity Able to work with different detectors	Costly instrument Large volumes of environmental friendly solvents	Tistaert et al., 2011

Superscript (a and b) represents the corresponding reference.

2.7 SPECTROSCOPIC TECHNIQUES

Spectroscopic techniques are mainly for the identification of the pure constituent. The purity of the constituents plays major role in producing accurate results in identification.

2.7.1 Mass Spectrometry (MS)

Mass Spectrometry (MS) is an analysis to calculate and to determine the mass-to-charge ratio (*m/z*) of ions presents in a specific compound and thus gives an idea of the structure of the compound. The sample of a pure compound was introduced into the ionization source and high energy source were used to bombard the parent compound into gas phase ions. It pasessess through the electron streams and breaks into several fragments based on *m/z* and were counted in the detector. The signal is recorded and processed by the data system. This calculation was done from the parent compound through total molecular weight and fragmentation pattern. The detected ion was then resulted in spectrum according to *m/z* in a magnetic field (Lampman et al., 2010 and Ngan et al., 2008).

2.7.2 Ultraviolet (UV) Spectroscopy

The Ultraviolet spectroscopy (UV) is used to identify the conjugated structure of a compound. The concept of energy was used to conduct transitions on the outer core electron. The electron that absorbs energy is promoted from an occupied orbital to an unoccupied orbital of greater potential energy. The class of the compounds was identified via change of wavelength from maxima to higher or lower λ max whereby the value provides prove on identifying the unknown constituent of the compound (Lampman et al., 2010 and Ngan et al., 2008). The advantage and disadvantage of the spectroscopy technique of IR, MS, NMR and UV are as tabulated in Table 2.8.

Table 2.8: Pros and cons of various spectroscopy techniques

Chromatography Technique	Advantage	Disadvantage	Reference
IR	High speed and sensitivity	Require sample preparation	Ngan et al., 2008
MS	Measurement via qualitative and quantitative High sensitivity High analysis speed Simultaneous detection of multiple analytes Promise of isotope analysis Elucidating chemical structures with fragmentation	High vacuum and power consuming High precision requires often calibration Matrix effect Requiring sample pretreatment	Zhu and Fang, 2013
NMR	Capability of elucidating chemical structures	Poor sensitivity Poor analysis speed Require sample pre-treatment	Zhu and Fang, 2013
UV	Non-destructive of samples Most organic molecules absorbs	Require frequent calibration to maintain accuracy and precision High detection limit Absorption depends on solution conditions	Chester and Winefordner, 1977

2.7.3 Infrared (IR) Spectroscopy

One of the applications of Infrared spectroscopy (IR) is in Fourier Transform Infrared (FTIR) instrument. This method, specially detects the functional groups that are present through vibrations and bending of bonds in a compound. The molecules are excited to higher energy state when absorbs infrared radiation at selected frequencies (Lampman et al., 2010). The results were presented in spectra that consist of a bond range from 4000-1600 cm^{-1}. Another region (1550 - 660 cm^{-1}) that was also present is the fingerprint region that is usually gives less information on the functional group (Ngan et al., 2008).

2.7.4 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance is known for the purpose of structure elucidation through identification of the detailed chemical structures. There are several sub-methods that should be adapted in producing a complete result in the structure identification. Some of the methods are the one dimensional technique (1D-NMR) and two dimensional techniques (2D-NMR) (Ngan et al., 2008).

The 1D-NMR includes several analyses that are the proton NMR (^{1}H-NMR) and carbon NMR (^{13}C-NMR). The ^{1}H-NMR data provide information regarding the environment of protons present in a specific structure. It gives a value of chemical shift (usually 0-12 ppm) via various splitting patterns. The splitting pattern relies on the environment of the neighboring proton that could be either vicinal or germinal protons. The overall spectra show the chemical shift (δ) values of the different type of protons and the intensities of the signals. Whereas the ^{13}C-NMR provides information on the types of carbons present and were also represented in terms of chemical shift (usually 0-230 ppm). The carbon types include the primary, secondary, tertiary and quaternary carbons. In carbon analysis, Distortionless Enhancement by Polarization Transfer (DEPT) analysis provides the information on the number of hydrogen attached to a specific carbon (Lampman et al., 2010 and Ngan et al., 2008).

The 2D-NMR involves the Correlated Spectroscopy (COSY) analysis that identify mutually coupled protons involving coupling networks. Besides that, the Heteronuclear Correlation Spectroscopy (HETCOR), Heteronuclear Multiple Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) are also listed in 2D-NMR. The HETCOR analysis gives information relating the specific proton to a specific carbon through direct bonding whereas the HMQC relates the protons with the heteronuclei in terms of heteronuclear scalar coupling. This technique is used to ignore the proton signals that is not coupled to the heteronuclei by providing a spectrum that indicates which protons is attached to which carbon and its nature. The analysis does also provide a spectrum consisting of different spots representing the proton and carbon signals in two different axes respectively. The HMBC identified the coupling of proton and carbon through two or three bonds away and finally elucidates the overall skeleton of the compound (Lampman et al., 2010 and Ngan et al., 2008).

2.8 PHYSICOCHEMICAL PROPERTIES OF SEED OILS

2.8.1 Physical Properties of Seed Oils

The physicochemical properties are related to the physical state and chemistry of a substance or a medium. Specifically for lipids (fats and oils), each of them might vary according to different physicochemical properties and various factors might be influencing them. The examples of physical properties are the physical state, color, odor, density, refractive index (RI) and UV-Vis transmission, whereas the chemical properties were the acid value (AV), free fatty acid (FFA), iodine value (IV), peroxide value (PV), unsaponifiable matter and pH. Some of the methods of the analysis are explained in the following subtopics.

(i) Colour

The various colors for example the oil are attributed by the genetics of the plant, according to the family and volatile matter. At times, the moisture level has also played

the role in the color pigmentation (Ziyada and Elhussien, 2008). The method determines the colour of oils by comparison with Lovibond glasses of known colour characteristics. The colour is expressed as the sum total of the yellow and red slides used to match the colour of the oil in a cell of the specified size in the Lovibond Tintometer (FSSAI, 2015).

(ii) Specific Gravity

The SG is an index used to measure the density of a liquid. Liquids with a density lower than water, which includes most crude oil grades and petroleum products, will have a specific gravity between 0.0 and 1.0. In the oil industry, specific gravity is a common quality specification for finished products. (www.mckinseyenergyinsights.com)

(iii) Refractive Index

The RI gives a value of the intensity of a light passing through a medium and is also represented by the degree of unsaturation or conjugation (Arya et al., 1969). Or the ratio of velocity of light in vaccum to the velocity of light in the oil or fat; more generally, it expresses the ratio between the sine of angle of incidence to the sine of angle of refraction when a ray of light of known wave length (usually 589.3 nm, the mean of D lines of Sodium) passes from air into the oil or fat. Refractive index varies with temperature and wavelength. Measurement of the refractive index of the sample is done by means of a suitable refractometer (FSSAI, 2015).

2.8.2 Chemical Properties of Seed Oils

Various chemical properties such as Acid Value (AV), Free Fatty Acid (FFA), Iodine Value (IV), Peroxide Value (PV), fatty acids composition and volatile matter were using to determine the characteristic of seed oils. Some of these methods of the analysis are explained in the following subtopics

(i) Acid Value

The AV is the number of FFA in a sample as a result of enzymatic activities. The enzymatic activities cause hydrolysis through the removal of the water molecule and the values are expressed in the unit of milligrams of base required to neutralize the acidic constituents per g of oil (Erakhrumen, 2011).

The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid. The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution. The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lypolytic enzyme lipase (FSSAI, 2015).

(ii) Iodine Value

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100 g of the oil/fat, when determined by using Wijs solution. The oil/fat sample taken in carbon-tetrachloride is treated with a known excess of iodine monochloride solution in glacial acetic (Wijs solution). The excess of iodine monochloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulfate solution. The iodine value is a measure of the amount of unsaturation (number of double bonds) in a fat (FSSAI, 2015).

(iii) Peroxide Value

Peroxide value (PV) states the milliequivalents of peroxide oxygen combined in a kilogram of oil and able, under testing, to liberate iodine from potassium iodide; the iodine is next estimated using a standard sodium–thiosulfate solution (Patterson, 2011). The peroxide value is a useful indicator of the extent of oxidation of lipids, fats, and oils. The oxidation of food lipids is undesirable due to off-flavors, toxins, and loss of fat-

soluble vitamins. Some fixed oils like walnut, hazelnut, wheat germ, sesame, nettle seed, grape seed, and St. John's oil are used in food, cosmetics, and medicine. In addition, the analysis of the peroxide content of oil samples is a very analytical task because high peroxide levels in oils have been a threat to human health. In this investigation, the determination of the peroxide values was performed after opening bottles of different commercial oil samples and after 1 month of storage at room temperature. The peroxide values of fixed oils were analyzed by the improved ferrous oxidation–xylenol orange (mFOX) method (Dermis et al., 2012).

(*iv*) *Free Fatty Acid (FFA) Analysis*

The FFA measures the deterioration due to exposure towards moisture or water. Fatty acids are the carboxylic acids attached with long hydrocarbon chains that could be as long as 10-30 carbons and this fatty acid determine the polarity of the oil. The fatty acid has two different classes that are the saturated and non-saturated and the physicochemical properties are mainly dependent on this (Bachheti et al., 2012). The fatty acid composition of oil is usually being analyzed for the purpose of product formulation for different purposes, for example, in the aspect of nutrition, industrial usage and pharmaceutical. The various fatty acids had been identified and been proven for many activities in terms of biological and chemical studies (Goja, 2013).

(v) Moisture Content

Moisture content of oils and fats is the loss in mass of the sample on heating at 105±10C under operating conditions specified (FSSAI, 2015).

(vi) Saponification Value

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat. The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric

acid. The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa (FSSAI, 2015).

(vii) Unsaponifiable Matter

Unsaponifiable matter represents the matter that could not be saponifiable with the alkali hydroxides but could dissolve in ordinary fat solvents. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter nonvolatile at 100 °C e.g (mineral oil) which may be present. Light Petroleum or diethyl ether is used as a solvent but in most cases results will differ according to the solvent selected and generally the use of diethyl ether will give a higher result.(Ref:- F.A.O Manual of Food quality control 14/8, page 261) (FSSAI, 2015).

2.9 COCKROACH

Cockroaches are considered to be among nature's most adaptable creatures and have been living on the planet for at least 250 million years. They have strong survival skill, they are resistant to radiation than any other living things included animals and human being. However, their inclination for destruction and spreading pathogenic organism and disease has earned man's loathing (Buchanan, 2007). Furthermore, cockroaches are one of the common household pests, which could pose a serious health issues in many countries. There are many types of the species of cockroach, including the American cockroach, German cockroach, Smooth cockroach, Australian cockroach, lobster cockroach and the Brown cockroach. The common species that found in homes, hostels, and restaurants are mostly the German cockroaches (*Blatella Germanica*) (Environmy_adm, 2012). The American cockroach, *P. americana* L.: are an obnoxious and filthy domestic pest found in tropical countries around the world. Cockroaches are a high priority among urban pests because they are aesthetically unappealing, transmit diseases, and damage stored products and household goods. Although cockroaches are

not usually the most significant transmitter of disease, but play a supplementary role in some allergy related diseases (Sittichok et al., 2013). American, *P. americana* (L.), and German, *B. germanica* (L.), cockroaches remain two of the most important insect species to homeowners and in food-handling facilities. Even though wide varieties of insecticidal products are available for cockroach control, most contain synthetic organic insecticides. With homeowner's increased awareness and concern about traditional insecticides, there is a greater potential for use of less toxic materials for cockroach control (Appel et al., 2001).

Natural contamination of cockroaches with wide range of pathogenic organisms including about 40 species of bacteria, nearly 12 species of pathogenic helminthes, the second largest group of vertebrate pathogens, and also viruses, protozoa and fungi affecting man and other vertebrate animals have been reported by numerous studies. Often their movement between waste and food materials led to acquire, carry, and mechanically transfer of these pathogens (Sharififard et al., 2016).

Cockroaches have the potential to mechanically carry and transmit many pathogens, such as bacteria, viruses, fungi, protozoa and helminthes. They also serve as potential carriers of the causes of bacterial diarrhea and nosocomial infections in hospitals. There is ample evidence that substances produced by cockroaches are involved in producing allergic symptoms (Thavara et al., 2007).

Environmentally compatible stored-product control agents are surely needs to be replacing synthetic pesticides that are harmful to environment and not effective due to the increasing difficulty of managing pesticide resistance (Donald et al., 2009). Normally, insect repellents function by releasing a vapour barrier discouraging the insect or arthropod from coming into contact with the surface. Researches showed that essential oils obtained by steam distillation which containing volatile aromatic compounds from various part of plants such as rhizomes, flowers, roots, fruit and trees, have several

properties against various haematophagous hexapods, thus some essential oil was being the basis of commercial and environmental friendly repellent formulations (Nerio et al., 2010; Rajesh and Joshi, 2013). For centuries, naturally occurring insecticides have been used in pest control. Many of these compounds, including alkaloids, quinones, essential oils (including terpenoids), glycosides, and flavonoids, are secondary plant substances. Monoterpenoids are present in cedar, citrus, eucalyptus, mints, and a variety of spices. Many monoterpenoids are used as cosmetic, food, and pharmacological additives where they provide flavors and fragrances. These compounds also induce a variety of responses in insects. For example, several monoterpenoids and cedar oils are repellent to German cockroaches, affect insect growth and development, or are acutely toxic to insects. Monoterpenoids are considered neurotoxic because of their speed of action and their effects on neurotransmission (Appel et al., 2001). Cockroaches undergo an incomplete metamorphosis that consists of three stages: egg, nymph (immature) and adult stages. Table 2.9 shows characteristics of common domestic cockroach species.

Table 2.9: Characteristics of common domestic cockroach species

Roach Species	**Length**	**Color and Markings**	**Eggs[1]**	**Egg to Adult**	**Reproductive Characteristics**
German	9/16 in. (14 mm)	Light brown with two dark stripes on the pronotum	37	55–68 days	Female carries egg case until about 24-hours before hatching then drops it in a secluded place.
Brown banded	9/16 in. (14 mm)	Tan-golden with faint V-shaped lighter bands on wings	16	95–276 days	Egg case glued undersurface of objects, shelves, furniture in crevices.
Oriental	1 –1-1/4 in. (32 mm)	Dark red-brown-black	14	300–800 days	Egg case deposited in debris or food in a sheltered place.
American	1-1/2 in. (38 mm)	Reddish brown throughout with a pale band on the edge of the pronotum. A very large roach.	14	285–616 days	Egg case carried up to six days before depositing in a sheltered area.

[1]Average number per egg case. The number actually hatched can be fewer

Source: Ogg et al. (2006).

2.9.1 American cockroach (Periplaneta americana)

(ix) ***P. americana classification***

American cockroach belongs to Kingdom: Animalia, Phylum: Arthropoda, Class: Insecta, order: Dictyoptera, Suborder: Blattodea, Family: Blattidae, Genus: Periplaneta, Species: *P. americana. P. americana* is the largest of the common peridomestic. This cockroach is readily found in commercial and large buildings such as restaurants, grocery stores, bakeries and anywhere food is prepared and stored. They can produce enormously. Mass migrations of P.americana are common (Mahyoub et al., 2018). Figure 2.15 shows American cockroach *P. Americana.*

Figure 2. 15: American cockroach *P. Americana.*
Source: https://www.ecolab.com/pages/types-of-cockroaches

(x) ***Size of P. americana***

P. americana is the largest species of cockroaches found in Iran. They originated from South America, measures 30–40 mm in length (Ramazani et al., 2018). The American cockroach is a large cockroach. The adult body length is approximately 1-1/2 inches long (38 mm). The antennae extending from the head are equally as long as the body if they are intact (Perrott and Miller, 2010).

(xi) ***Color of P. americana***

Ramazani et al. (2018); Eggleston and Arruda (2001) were reported that *P.* Americana are reddish brown. The adult is a shiny reddish brown to dark brown and has a yellow margin on the pronotum (region directly behind the head). Immature American cockroaches (nymphs) are also reddish brown to dark brown in color. Large nymphs often have yellow markings on the abdomen (Perrott and Miller, 2010).

(xii) ***Description of P. americana***

Adult American cockroaches have wings and will occasionally fly. However, they are awkward fliers and prefer to run when disturbed. Male and female American cockroaches are about the same size and look very similar. Both have a pair of cerci, finger-like appendages, at the tips of their abdomens (Perrott and Miller, 2010). Over 4500 species of cockroaches have been identified, of which 40 species are associated with human habitants while four species are well known as pests (Ramazani et al., 2018). There are over 3,500 cockroach species existing in the world. Only four species live and breed in the northern United States! These four species: the German, brown banded, oriental (a.k.a., waterbugs) (Ogg et al., 2006; Eggleston and Arruda, 2001).

The American cockroach is one of the most important cockroach species (3500) existing in the world; they are world travelers, living with humans in almost everywhere (Omara et al., 2013). American cockroach are more common in restaurant, grocery stores and bakeries as well as other sites where food is prepared and they can develop to enormous numbers. They may be become pests in homes after being introduced in cartons, grocery bags and containers. Although, many pathogens have been isolated from American cockroach, *P. americana* (L.) and it acts as a reservoir for infection pathogens. There are no documented cases directly linking cockroaches as the definitive of any human and animal diseases. On the other hand, being a carrier of germs they could transfer them. Cockroaches transfer germs mechanically by crawling over bacteria–laden substances and later walking over dishes and eating utensils. Cockroaches represent a real hazard for human health and

must be controlled in order to maintain acceptable hygiene standards (Omara et al., 2013).

(xiii) ***Habitat of P. americana***

American cockroaches are a "peridomestic species." This means that they generally live outdoors. However, populations can also move indoors and live in human structures. American cockroaches usually live in warm, moist, humid environments but can survive in drier areas if they have access to water. The cockroaches prefer temperatures between 70°F and 85°F and will not survive if temperatures drop below 15°F. In structures, American cockroaches are common in areas where food is prepared or stored and moisture is plentiful (Perrott and Miller, 2010). The American cockroach lives in hot areas of buildings like the kitchens, heating rooms, warehouses and sewage systems. They usually come out of their hiding places at night for feeding and other activities. The adult cockroaches are long-lived and can live for as long as one year or more producing large number of egg capsules during this period, depending on food availability (Ramazani et al., 2018).

P. americana make their ways to houses from drainages pipes via the plumbing, and from trees and shrubs located alongside buildings or with branches overhanging roofs. During the day, the P.americana, which response negatively to light, rests in harborages close to water pipes, sinks, baths, and toilets where the microclimate is suitable for survival (Mahyoub et al., 2018). American and German cockroaches (Dictyoptera: Blattidae and Blattellidae) are pests that can threat human health. The American cockroach, *P. americana* (Blattidae), is the largest of the house-infesting roaches, while the German cockroach, *B. germanica* (Blattellidae), is smaller. Both cockroaches have been spread throughout the world by commerce Both cockroaches can contaminate food with bacterial diseases that result in food poisoning, dysentery, and diarrhea, and both can cause childhood asthma for the control of cockroaches, boric acid and chemical insecticides have been studied extensively. However, cockroach resistance has been reported to some compounds

such as bendiocarb, cypermethrin, permethrin, propoxur, and chlorpyrifos (Maketon et al., 2010).

(xiv) ***Life Cycle of P. Americana***

The American cockroach egg case contains 14-16 eggs. Nymphs emerge in about six weeks and undergo 13 molts over the next 18 months, before reaching the sexually mature adult stage. During warm conditions, adult females produce an egg case in about one week and can live more than a year. American cockroaches seem to have a tremendous potential for producing offspring. But because of cold winters in northern states, American cockroaches develop at a slower rate and produce fewer offspring than in southern states (Ogg et al., 2006).

American cockroaches will complete their development and become reproductive in six to 12 months. Adult American cockroaches can live approximately a year to a year and a half. An adult female American cockroach will produce a new egg capsule about every 9 days, resulting in the production of between 25-30 egg cases during her adult life (Perrott and Miller, 2010).

Cockroaches undergo an incomplete metamorphosis that consists of three stages: egg, nymph (immature) and adult stages. *P. americana* was identified according to their morphological characteristics using standard taxonomic keys. Identification keys are provided for male, female and nymphal stages for *P. americana*. Following are some illustration photographs with brief comments that help fast identification and make it an interested reader (Mahyoub et al., 2018). Resistance against pesticides is a phenomenon, mainly dependant on genetic basis. Exposure of a population of pest to a certain pesticide results in development of resistance against that chemical (pesticide). Not necessarily all insects need to be killed during this phenomenon. Hemingway et al. (1993) have reported that cockroaches have developed resistance against many groups of insecticides especially pyrethroids (Syed et al., 2014).

(xv) ***Type of Damage of P. Americana***

American cockroaches feed on a wide variety of materials, including cosmetics, beer, potted plants, wallpaper paste, soap, postage stamps, fermenting fruit, pet food, and human food. They contaminate human food, clothing, paper goods, and surfaces with their feces and body parts. American cockroaches also produce a strong unpleasant odor. This characteristic odor is not only detectable in infested buildings but is also transferred to items that the cockroaches crawl across when foraging (Perrott and Miller, 2010). Their aesthetically unappealing dam- age to household materials and stored prod- ucts, and transmission of diseases makes them a high priority pest (Syed *et al*., 2014).

(xvi) ***Health Risks of P. Americana***

When American cockroaches aggregate, their presence is primarily an aesthetic nuisance. However, members of this species are also known to carry infectious bacteria on their bodies and in their gut. These bacteria may be transferred to food and other items that the cockroaches contact. Several bacteria commonly associated with American cockroaches are known to cause food poisoning, dysentery, and diarrhea in humans (Perrott and Miller, 2010).

2.9. 2 Germany cockroach, Blattella germanica **(L.)**

The German cockroach *B.* germanica (L.), is a pest because most people find it is disgusting, and assumed to be associated with unsanitary conditions since ancient time. The fear of others knowing and observing the infestation may cause one to experience psychological problems and social anxiety. Another reason the German cockroach is a pest is because of its rapid population growth, which can be attributed to the German cockroach's short generation time, and result in difficult to control. Normally, the German cockroaches will have a relatively short life (about 200 days). Females cockroaches can produce 5-10 egg cases during their life, and each egg case contains about 35-40 eggs. Its short generation time increases its chance of becoming resistant to the insecticides used to manage its population; therefore, chemical rotation and different products and strategies should be used to reduce the chance of resistance developing in the population (Phillips et al., 2009).

There is ample evidence that proved substances produced by German cockroach (common species) producing allergic symptoms and others negative impact such as adulteration of food with excrement and defensive secretions, and transport of pathogenic organisms make this species one of the most troublesome annoyance pests of the world. Unfortunately, the number of cockroach does not appear to be reducing in a due time, currently no efficient and proverbially applicable strategy that could actively eliminate or reduce the populations of this species (Thavara et al., 2007). Figure 2.16 shows German cockroach *Blattella* germanica.

Figure 2.16: German cockroach *Blattella* germanica.
Source: https://www.ecolab.com/pages/types-of-cockroaches

Germany cockroach, *B.* germanica (L.), control has relied heavily on the use of broad-spectrum insecticides. Recent concerns with human and environmental safety, and widespread prevalence of resistance to insecticides have prompted research on safer, reduced-risk, and environmentally compatible methods of insect control. However, the advent and intense promotion of integrated pest management

(IPM) approaches have not reduced reliance on chemical insecticides as the preferred technology for cockroach control, in large part because the effectiveness of alternatives has been poor and inconsistent (Nalyanya et al., 2000).

2.9.3 Brownbanded Cockroach

Brown banded cockroaches are not as common as German cockroaches and prefer warmer areas (over 80 F, 27 C) and not necessarily humid areas. Higher areas of a room tend to warmer, so checking ceiling and wall molding, pictures, lighting fixtures and switches, closets, and furniture is necessary. Signs of infestation warmer areas are preferred which can be dry. So common living areas need to be inspected as well as food and moisture areas where German cockroaches tend to live (i.e. kitchens and bathrooms). Inspect for fecal deposits, cast skins, and adults in areas mentioned in the general information (https://museumpests.net/) Figure 2.17 shows brownbanded cockroaches.

Figure 2.17: Brownbanded cockroaches.
Source: https://www.ecolab.com/pages/types-of-cockroaches

*2.9.4 Oriental Cockroach (***Blatta orientalis)**

The origin of the oriental cockroach, Blatta orientalis Linnaeus, is uncertain, but it is thought to be from Africa or south Russia. It is a major household pest in

parts of the northwest, mid-west, and southern United States. It is also sometimes referred to as the “black beetle” or a “water bug” because of its dark black appearance and tendency to harbor in damp locations (Kim, 2017). Figure 2.18 shows the oriental cockroach.

Figure 2.18: The oriental cockroach, Blatta orientalis Linnaeus
Source: https://www.ecolab.com/pages/types-of-cockroaches

2.10 INSECT REPELLENT

Insect repellent is defined as the chemical or natural substances that able to repel insect in a distance and block their attack on human and animal when applied to the skin. Also, insect repellents are substances that act at a distance or locally, discouraging insects or an hexapod from flying around, landing on, climbing on a surface and biting human or animal skin. Normally, insect repellents function by releasing a vapour barrier discouraging the insect or arthropod from coming into contact with the surface. The repellent was effective to many insects such as ants, mosquitos, beetles, flies and also cockroaches. Synthetic repellent may be toxic when applied onto the skin as it might absorb and cause cutaneous. The synthetic repellent contain substance such as *N; N*- diethyl-*m*-toluamide (DEET) is toxic to both human and environment if applied for a long period. Hence it is necessary to concern them with their possible risk when in uses.

On the other hand, natural origin repellents are well known as the essential oil that extracted from different plants and directly applied onto the skin, have less or no toxicity to human or animal. Unfortunately, unlike the synthetic insect repellent, the natural repellent have poorly investigated (Motta and Monti, 2003). The perfect insect repellent would repel different species of insect and remain effective for more than 8 hours, resistance to wash off, and nontoxic or no irritation to the skin. However there is no available insect repellent that meets all of the criteria as mentioned. On the other hand, there are many types of insect repellents in presents. One of the repellents is the taste based repellents. They are typically applied using a spray to apply the product directly on the insects. Some of the insects are sensitive to these types of products. Insect that are sensitive to those products may repel using repellents of this kind or necessitate that their application be suspended during different stages of the growing season. Plant-Based Repellents are derived from plants such as citronella, geranium, basil, and peppermint generally provide protection against insect. Studies with products containing a mixture of plant have shown a limited effect in the best of circumstances, repelling mosquitoes for about two hours. Citronella essential oils' products are commonly sold as repellent candles, but these have little repellence against mosquitoes and other pests (Steltenkamp et al., 1992).

The commonly known natural cockroaches repellent are the essential oil of catnip. It contained active compound called nepetalactone. Nepetalactone is non-toxic to both human and animals. It can be simmered into small amount of water and make it in liquid form, then spry around the area of cockroaches activity. Beside this, recent study has investigated the repellent properties of the essential oil of catnip plant and the Osage orange fruit against the cockroaches. Both oils showed relative high percentage of repellency as the standard repellent (DEET) (Schultz et al., 2006).

Research shows that essential oils, steam distilled concentrates which containing volatile aromatic compounds from various part of plants such as rhizomes, flowers, roots, fruit and trees, have several properties against various hematophagous

hexapods, thus some essential oil was being the basis of commercial and environmental friendly repellent formulations (Nerio et al., 2010).

2.11 TYPES OF REPELLENT TESTS

2.11.1 Ebeling Choice-Box Test

This method is the modification of the method of Ebeling et al. (1966). The repellency of the essential oils to the German cockroaches is described by Appel and Tanley (2000) where the 15 cm x 15 cm square box is the choice box which will then be divided into two equal size compartments by partition. The cockroaches can move freely between two compartments by making a hole in the partition. Food and water is available in the centre of both compartments and use a safety glass to cover the top of the compartment to avoid escape. One of the compartments is considered as the untreated zone which the light side, representing a kitchen counter or bedroom.

The hole on the partition is then blocked by plugging with a cork so that the cockroaches will not move to the treated side before the assay. In repellent assay, ten adult German cockroaches were then released into the untreated side which is the light compartment and were allowed to be adopted for 1 hour. Then, the essential oils are spread on the food and the area around the dark compartment. After the cockroaches are adopted in the light compartment for 1 hour, the cork is removed and the cockroaches are allowed to move freely between two compartments (light side and dark side). The dark side or the treated compartment representing the void under the bedroom and where the cockroaches will be distributed on this side. Then, the choice boxes (light and dark compartment is exposed to a photoperiod of 12 hour at 28 ºC. The number of cockroaches that located in the treated compartments and untreated compartment was counted carefully and recorded at about 3-6 hours for 14 days. (Phillips et al., 2009). Treatments of 3 ml of a 5 ppm essential oil solution uniformly spread on the food and the dark area of the treated side. Each treatment had 3 replicates. It is treated with maximum 1 ml of DMSO as the negative control of the assay. Figure 2.19 shows the example of the Ebeling Choice Box Test. The percentage of repellent is calculated as follow (Phillips et al., 2009):

$$\text{Repellency \%} = 100\ \% - (\frac{\text{T}}{\text{N}}\ \text{x}\ 100\ \%)$$

Where: T = Number of cockroaches in the treated side, N=Total number cockroaches been used.

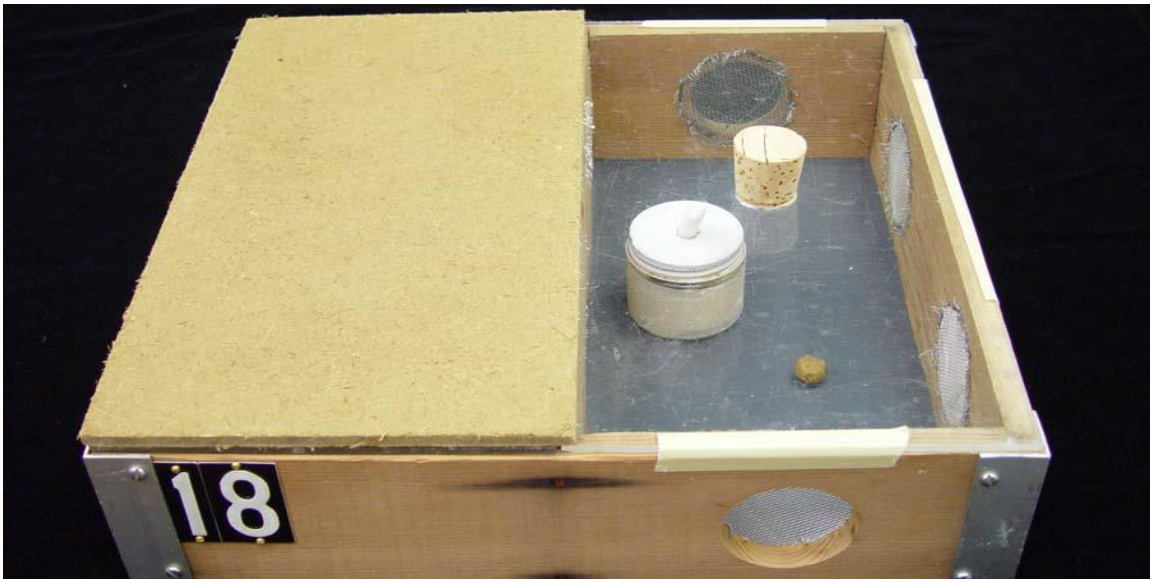

Figure 2.19: Ebeling Choice Box Test
Source: Phillips et al. (2009)

2.11.2 The Ferrero Test

In Ferrero Test, the white filter paper with 15 cm diameter was circulated and cut into two halve whereby one of the half side is treated with 1 mL of the essential oil solution and the other half is treated with 1 mL of the n-hexane which is the solvent for treatment of the essential oil. The filter papers were allowed to dry for 1 hour for the extract solution and 10 minutes for the essential oil solution in order to allowed the solvent to completely evaporate. Then, both halve of the filter paper were match or fitted together and then placed as the single layer on the bottom of the 15 cm Petri dish. Another control experiment was conducted in order to avoid any effect which resulting from the residual solvent where one of the half of filter paper was treated with n-hexane solvent and the another part was considered as the untreated part. Three concentrations were used to test for each of the essential oil solution, which are 0.025 ppm, 0.050 ppm and 0.075 ppm. According to Chopa et al. (2006) and Ferrero et al. (2006), the extraction concentration of the essential oils can be

determined. The positive control is the DEET which will be dissolved in the solvent and fifteen cockroaches were released to the centre of the Pedri disc. The cockroaches were kept in a humid and dark environment to avoid the influence from the surrounding. After 12 hours, their distribution on the treated and untreated area was observed carefully and recorded. The equation below shows the formula for calculating the percentage of repellency of cockroaches (Manzoor et al., 2011):

$$\text{Repellency } \% = 100\ \% - (\frac{\text{T}}{\text{N}} \text{ x } 100\ \%)$$

Where: T= Number of cockroaches in the treated side, N= Total number cockroaches been used.

2.11.3 *Harborage-Choice Method*

This method was used to determine the repellency of essential oils to the German cockroach. All of the 20 male German cockroaches were allowed to spread to the plastic test container with the Harborage-Choice Method of Goodhue and Tissol (1952) as shown in Figure 2.20 and has been used to determine the repellency of the chemical component to the German cockroach over time. Before the assay, 20 male German cockroaches were located in each 20 qt. plastic test container and allowed to adapt with some food and water available in the canter under humid condition. The top of each container was sealed with transparent wrapping plastic to prevent the cockroaches escape form it. Three 2.0 cm holes were cut around the lip of unwaxed paper carton so that the cockroaches can move inside. The essential oil solution with different concentration (5%, 10%, 30% and 100%) was uniformly spread outside of each treated carton. The control cartons were spread with acetone. The carton with acetone is allowed to dry under less humid area. The one that untreated and treated carton were inverted and located in each container. The number of cockroaches on the outside and inside of each treated and untreated carton was recorded at 3-6 hour into the photo phase daily for 5 day. The percentage of repellency is defined as the percentage of cockroaches that repel from the treated carton during the photo phase time (Steltenkamp et al., 1992).

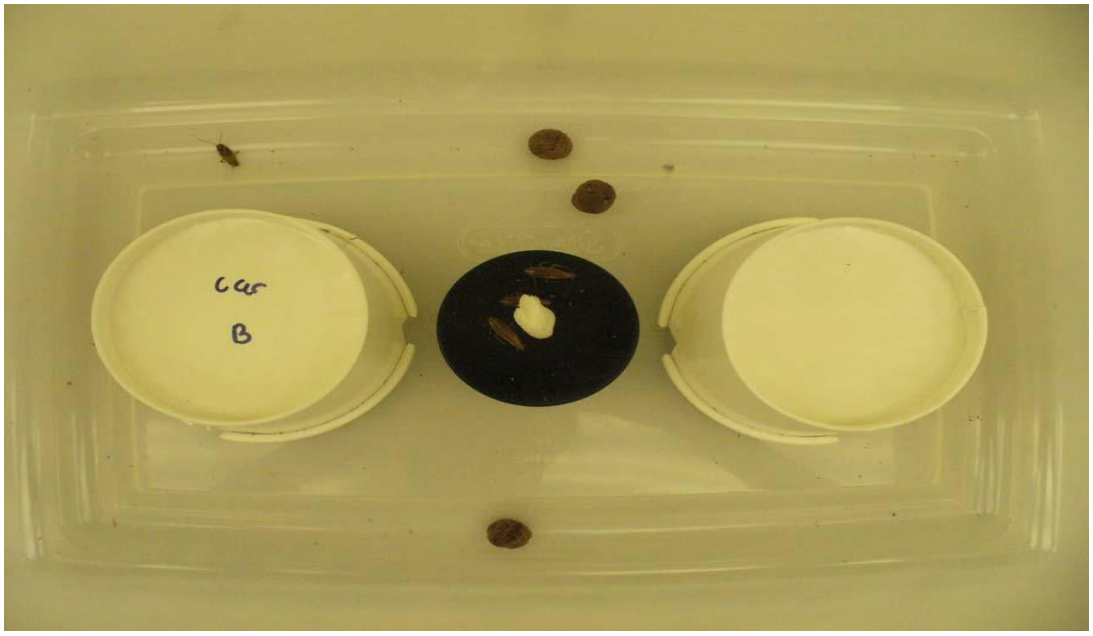

Figure 2.20: Harborage –Choice Method

Source: Phillips et al. (2009)

In summary of this chapter, medicinal plants such as *O. basilicum*, *C. rotundus*, *M. oleifera* and *A. digitata* were widely used in many uses like cooking, medicine, herbs and many others purposes. Meanwhile, essential oils have several biological activities for use in pharmaceutical industry such as antimicrobial activity and repellent activity. The essential oils of *O. basilicum*, and *C. rotundus* can be extracted by using steam distillation extraction. While, the fixed or seed-oil could be obtain from seed of *O. basilicum*, *M. oleifera* and *A. digitata* by Soxhlet using hexane.

The chemical composition of essential oils and physichochemical properties of seed oils were used to determine by several methods. Therefore, we had reviewed and selected standard methods to achieve the objectives of this study. The essential and fixed oils of the mentioned plants as major or minor compositions possesses repellent and insecticidal activities. The methods for repellence test included Ebeling choice-box test, Ferrero test and Harborage-choice method. Among these methods, Ebeling choice-box test is the most suitable repellence test method used in this study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 OVERVIEW

The overall of this chapter discussed the process and procedures that involved in this current study. The chapter was divided into different sub-sections it starts with materials and equipment's using to complete the study and the second parts discuss the experimental procedures and process practice. The second part mainly focused on the methods of extraction of the oils (essential and seed oils), analysis, physicochemical properties, biological test and formulation of repellent product.

3.2 MATERIALS

The raw plant materials involving in this study were three different parts (leaves and rhizome for essential oils and seeds for fixed oils). The seeds were from *Ocimum basilicum* (sample A), *Adansonia digitata* (sample B) and *Moringa olifera* (sample C). Whereas, the essential oil were from *O. basilicum* (sample D) and *Cyperus rotundus* (sample E). All reagents and solvents used were analytical and chromatography grade, unless specified. They were obtained from Lab of Faculty of Pure and Applied Sciences, IUA or from trusted sources.

3.3 EQUIPMENTS

All the instruments and equipment used in this research provided by the laboratory of Faculty of Pure and Applied Sciences, International University of Africa, University of Medicine and Technology or Industrial Research and Consultancy Centre or National Institute of Research, Khartoum, Sudan or others labs in Sudan. Equipment's that were used such as rotary evaporator (Butchi, Switzerland), drying oven (Binder, Germany), analytical balance (Mettler Toledo, Switzerland), moisture content analyzer (AND MS-70, Japan), heating mantle (Mtops, Korea),

electrical blender (Panasonic, Malaysia), glass reagent sprayer for TLC (Analtech, United States of America), capillary tube (Iso Lab, Germany), TLC plates (Analtech, United States of America and Merck, Germany), filter paper (Whatman No.41, United States of America), soxhlet (Favorit, Malaysia) and etc. were of standard laboratory types. Glassware's that used in all experiments were cleaned with chromic acid followed by distilled water before drying in an oven.

3.4 SAMPLE PREPARATION AND PROCEDURES

This sample preparation and procedures include the steps in the plant material source, oils extraction, identification of volatile compounds, determination of physicochemical properties of the seed oil and repellent test.

3.4.1 Plant Materials

The dried seeds and leaves of *O. basilicum* (sample A and sample D) were collected on October 2017, from Ministry of Agriculture and Forestry, General Directorate of Horticultural Production, Department of Medicinal and Aromatic Plants, Khartoum, Sudan. While The *M. oleifera* (sample C) and *A. digitata* (sample B) seeds were obtained on 15 October 2017 from College of Forestry and Range Science, Sudan University of Science and Technology, Khartoum, Sudan. Whereas, the *C. rotundus* (sample E) was collected from Aldaba (River Nile State). Taxonomic identification of seeds was performed by Dr. Yahya of the National Centre for Research, department of biology, Khartoum, Sudan. Specimens of seeds and herbaceous parts of the plant were deposited. The seeds were cleaned, removed the dirt, ground by mortar and crushed by electric blender to reduce the particle size.

3.4.2 Extraction of Essential Oils

The fresh leaves of *O. basilicum* (sample D) and dry rhizomes of *C. rotundus* (sample E) were used to obtain their essential oils. The extraction was conducted in laboratory scale by Steam Distillation (SD) unit as described by Michele et al. (2013) with slight modification. In brief, one hundred grams (100 g) of fresh (*O. basilicum* leaves) and (100 g) of dried (*C. rotundus* rhizomes) were stem distilled for 4 h, (Anonymous, 1996). The distilled essential oils were dried over anhydrous

sodium sulphate, filtered and stored in sealed vials at 4 °C. The obtained oils were stored in hermetically closed dark bottles and keep at -4 °C for further studies. The percentage of oils (v/w %) from SD method was calculated according to the following formula:

$$The\ Essential\ Oil\ (\%) = \frac{\text{volum of } oil\ (v)}{Weight\ of\ sample\ (g)} \times 100\%$$

3.4.3 Lipid Content Determination by Solvent Semi-Continuous (Soxhlet) Extraction Method

The seeds of each plant of *O. basilicum* (sample A), *A. digitata* (sample B) and *M. oleifera* (sample C) were taken for initial moisture content via Moisture Analyzer (AND MS-70, Japan). Then the seeds were crushed and placed in the drying oven at 40 °C for 30 min prior extraction. The crushed seeds (50 g) were loaded in the thimble of Soxhlet apparatus and the bottom part was fitted to 500 mL round bottom flask. Sufficient amount of absolute n-hexane was added into the flask and the top part of the Soxhlet was fitted with a condenser. Constant heat was applied through the heating mantle and the extraction was conducted for a minimum extraction time of 6 h to make sure the maximum oil was extracted (Nour at al., 2009, Kittiphoom and Sutasinee, 2013). After complete extraction and cooling, the obtained oils were filtered through filter paper (Whatman No.2, 125 mm). The solvent was evaporated via rotary evaporator, further dried under open air in a dark area. The lipid content of the seed oils were calculated based on dry seeds weight that were used in the extraction. The yield of the oils was calculated (w/w %) according to the following equation and stored in hermetically closed dark bottles and kept in a refrigerator for further physicochemical study.

$$\% \text{ Lipid} = \frac{\text{Weight of oil (g) x 100\%}}{\text{Weight of sample (g)}}$$

3.5 PHYSICOCHEMICAL CHARACTERISTICS OF SEED OILS

3.5.1 Physical Properties of Seed Oils

The physical characters of the *O. basilicum, A. digitata* and *M. oleifera* seed oils were studied according to eight different aspects as physical state, color, odor, density, freezing-, melting-, boiling points and refractive Index (RI). The methods of the analysis are explained in detail accordingly in the following subtopics.

(i) ***Physical State, Color, Odor, Freezing-, Melting- and Boiling Points Determination***

Physical state at room temperature of 25 °C and color of the oils were determined visually whereby odor was determined by means of sensation through volatilized smell. For freezing point, the oil was filled in a clear glass vial, a thermometer was immersed into the oil and the oil was solidified through the usage of ice blocks. The solidification temperature was recorded as freezing point. The solidified oil was melted over a water bath at a temperature of 29 °C and the melting point was recorded. Again, around 10 mL of oil was filled in a clear glass vial and a thermometer was inserted. The vial was exposed to heat on a heating mantle and the oil was observed, whereby it starts circulating leading to boiling. The temperature at this point was recorded at the boiling point (Ramaiya et al., 2019).

(ii) ***Density, Refractive Index (RI) and Specific GravityDetermination***

The weight of a small empty vial was weighed and was filled with known amount of oil up to the brim. The vial was weighed again and the density was calculated as:

$$\text{Density, } \rho = \frac{[\text{Weight of vial + oil (g)}] - [\text{Weight of empty vial (g)}]}{\text{Volume of oil}}$$

While the RI of the oil was determined by using standard method described by Jessinta et al. (2014) with slight modification. This index was measured at 25 °C via pen Refractometer (Atago, Japan) with resolution and accuracy value of 0.1% and ± 0.2% in 10-60 °C. The pen tip was dipped into the sample and the start key was pressed to obtain the reading. The measurement was repeated in triplicate and the average value was reported. For the Specific Gravity melt sample if necessary and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry. Cool the sample to 30 ^{0}C or ambient temperature desired for determination (FSSAI, 2015).

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3.5.2 Chemical Properties of Seed Oils

Various chemical properties such as Acid Value (AV), Free Fatty Acid (FFA), Iodine Value (IV), Peroxide Value (PV), fatty acids composition and volatile matter were evaluated as follows:

(i) ***Acid Value (AV) Analysis***

The AV was determined through direct titration method of oil in an alcoholic medium against standard potassium hydroxide via method described by Jessinta et al. (2014) with some modifications. A mass of 0.5 g of oil was weighed into a 250 mL conical flask and 50 mL of freshly neutralized hot ethyl alcohol and 1 mL of phenolphthalein indicator solution were added. The mixtures were boiled around 5 min and were titrated against standardized potassium hydroxide (0.24 M). The AV was then calculated according to the following equation.

$$AV = \frac{[56.1]\,[\text{Titration of standard (mL)}]\,[\text{Molarity of standard (M)}]}{\text{Weight of sample (g)}}$$

(ii) ***Fatty Acids Composition, Percentage of Saturated and Unsaturated Fatty Acid Analysis***

The crude oils were analyzed as methyl ester to determine the fatty acid composition. The oils were converted into fatty acid methyl ester through transesterification reaction. A solution of 2M of KOH (Methanolic potassium hydroxide) was prepared. An amount of 2 mL of each oil sample separately was dissolved in 10 mL of hexane in a test tube. An amount of 1 mL of KOH was added into the same test tube and was vortex. The hexane phase was collected and washed twice with 4 mL of water after 15 min and was further dried over anhydrous sodium sulphate. The fatty acids composition analysis was performed on Agilent Technologies 7890A GC Systems coupled with MS detector. The details of chromatography equipment and settings are tabulated in Table 3.1. The individual fatty acids composition were expressed as a percentage. The percentages of saturated and unsaturated fatty acids were calculated by totaling the percentage of fatty acids detected via the analysis of fatty acid composition. The sum percentage of saturated fatty acids was represented as total saturated fatty acids, whereas the sum of all

unsaturated (mono- and polyunsaturated) was represented as total unsaturated fatty acids (Jessinta et al., 2014).

Table 3.1: Chromatographic settings for the analysis of *O. basilicum A. digitata* and *M. oleifera* seed oils methyl ester

Parameters	Settings
Chromatograph	Agilent Technologies 7890A GC Systems coupled with MS detector
Auto-sampler	GC autosampler
Column	Nonpolar capillary DB-1 of 100% dimethyl-polysiloxane (30 m, 0.25 mm i.d, film thickness 0.25 µm)
Carrier gas	Helium
Gas flow rate	1 mL/min
Injector mode	Splitless mode
Injector temp.	250 °C
Injection volume	1µL/L
Temp. program	60 °C for 3 min, 240 °C at the rate of 3 °C/min and held for 10 min
Runtime	93 min
Lab data system	NIST Library Chem Station software

(iii) Free Fatty Acid (FFA) Analysis

Method described by Ouilly et al. (2017) with some modifications was adapted to determine the free fatty AV. An amount of 0.2 g of sample was weighted in 250 mL Erlenmeyer flask with the addition of 50 mL of hot neutralized alcohol and 2 mL of phenolphthalein indicator. The solution was swirled to dissolve and titrated with standard sodium hydroxide (0.24 M) until the first permanent pink color that persists for 30 s. The volume of titration required for the changes was recorded and the FFA percentage was calculated as follows:

$$\text{FFA as Oleic (\%)} = \frac{[\text{Titration volume of standard (mL)}]\,[28.2]}{\text{Weight of sample (g)}}$$

(iv) Iodine Value (IV) Analysis

The Iodine Value (IV) was determined through method described by Jessinta et al. (2014) with slight modification via Wijs reagent. An amount sample was filtered through a dry filter paper and 0.35 g of sample was transferred into a clean, dry, 500

mL glass-stoppered flask containing 20 mL of carbon tetrachloride, and 25 mL of Wijs solution was pipetted into the flask. The mixture was swirled and allowed to stand in the dark for 30 min. Potassium iodide, 20 mL and recently boiled and cooled water, 100 mL was added and the mixture was titrated with sodium thiosulfate (0.11 M) until the yellow color almost disappears. Starch was added and the titration was continued until the blue color disappears entirely. Toward the end of the titration, the stoppered container was shaken violently so that any iodine remaining in solution in the carbon tetrachloride may be taken up by the potassium iodide. Blank determination was conducted in the same manner and condition and the IV was calculated by following equation.

$$IV = \frac{[\text{Titration of blank} - \text{sample (mL)}]\,[\text{Molarity of standard (M)}]\,(12.69)}{\text{Weight of sample (g)}}$$

(v) Peroxide Value (PV) Analysis

Method described by Jessinta et al. (2014) with some modification was applied to determine the Peroxide Value (PV). Amounts of 0.50 g of samples were weight into 250 mL of stoppered conical flask together with 30 mL of acetic acid-chloroform mixture and swirl to dissolve. The mixture was then added to 0.5 mL saturated potassium iodide and allowed to stand in dark with occasional shaking for 1 min and 30 mL of water were added. The liberated iodine in the mixture was titrated with sodium thiosulphate (0.11 M) with vigorous shaking until yellow color is almost gone. Then, 0.5 mL of starch indicator was added and titration was continued until the blue color disappears. The PV was expressed as milliequivalent of peroxide oxygen per kg of sample (meq/kg) via the following equation.

$$PV = \frac{[\text{Titration of standard (mL)}]\,[\text{Molarity of standard (M)}]\,[100]}{\text{Weight of sample (g)}}$$

(vi) Moisture and Volatile Matter Analysis

Moisture and volatile matter were analyzed according to air-oven method of AOCS and method described by Jessinta et al. (2014). About 5 g of oil (each) was weighed on a previously dried and tiered dish. The dish was covered with loose lid

and was heated in the oven at 105±1 °C for 1 h. The dish was removed from the oven, cooled in a desiccator and weighed. The plate was re-heated for the period of 1 h and the cooling and weighing process was repeated. The process was repeated until weight change between two observations does not exceed 1 mg. The following equation used to calculate the observations.

$$\% \text{ Moisture and volatile matter} = \frac{[\text{Loss of material on drying (g)}]\,[100]}{\text{Weight of material taken for test (g)}}$$

(vii) Saponification Value Analysis

The saponification value of the oil samples were estimated using Official Method AOCS (Jessinta et al., 2014). Accurately, 2 g of the oil sample (for each) was weighed into a 250 mL conical flask. An amount of 25 mL of potassium hydroxide KOH (N) added, then the flask and content was refluxed for one h. simultaneously, another conical flask contained only 25 mL of potassium hydroxide KOH (N) was prepared, served as a blank. The condenser connected and the content heated gently, but steadily for one h. After the condenser and the flask has cooled, but not sufficiently to forming gel, the content washed with a small amount of water and the condenser was removed. Then a few drops of phenolphthalein solution added to the flask and the sample titrated with hydrogen chloride, HCl (0.5N) until the pink color disappeared. The volume of the hydrogen chloride was recorded and the saponification value expressed as following:

$$\text{Saponification value (SV)} = \frac{56.1(\text{B} - \text{S})\text{x C x N of HCl}}{\text{weight of sample}}$$

Where, B and S are the volume of hydrogen chloride required by blank and sample, respectively, and C is the concentration of hydrogen chloride.

(viii) Unsaponifiable Matter Analysis

The unsaponifiable matter analysis was performed according to method described by Jessinta et al. (2014) with some modification. An amount of 50 mL of alcoholic potassium hydroxide was added into a conical flask containing 5 g of oil

sample and were boiled under reflux conditions for one h until a transparent medium is formed. The medium was then transferred into a separating funnel and were washed with petroleum ether allowing the layer to separate. The lower layer was collected and the top layer was continued washing for another 3 times with around 50 mL of solvent per wash. The ether extracts were combined and further washed with alcohol and water, 25 mL each. The ether solution was concentrated to 5 mL; then 2 mL of acetone was added with some heat under the water bath to remove the solvent and further dried at 100 °C for 30 min until a constant weight is obtained. Then the residue was dissolved in 50 mL of warm neutralized ethanol with phenolphthalein indicator and titrated with sodium hydroxide (0.02M). The weight of FFA and unsaponifiable matter values were calculated according to the following equations.

Weight of FFA in the extract =

[0.282 Titration of standard (mL)] [Molarity of standard (M)]

Unsaponifiable matter =

$$\frac{100\ [(\text{Weight of the residue}) - (\text{Weight of free fatty acids in the extract})]}{\text{Weight of sample}}$$

3.6 COCKROACHES COLLECTION AND REPELLENT TEST

About 600 adult, male and female of American cockroaches were collected from University of Khartoum, Sudan. The cockroaches kept in boxes and tested. Only the healthy nymphs and adults (male & female) cockroaches were used in this repellence test. The cockroaches reared in the laboratory by feeding on water and biscuits. The temperature maintained at 28 ± 5 °C. The Ebeling et al. (1966) choice-box method with slight modification used in this experiment. The repellency activity of oils against American cockroaches was described by Appel and Tanley (2000), where one square box (choice box) was divided into two equal size compartments by partition.

However in this experiment, three number of choice-boxes were joined together to form a set of test box. The medium size transparent microwave tableware

was used as the choice box for repellency test. The right-side choice box is considered as the untreated zone where 25 g of milled food (Jacob's biscuit) were filled into the central of the box and about 1.5 mL of DMSO was carefully dropped around the food as shown in Figure 3.1. Same treatment were applied to the others test box. The DMSO and distilled water are the negative control of the assay which have no effect of the repellence activity against cockroaches. The left-side choice-box is considered as the treated zone where the test samples (oils) was carefully dropped around the food at the central of the box as shown in Figure 3.2. Naphthalene, the positive control standard. One piece (1 g) of naphthalene was grinded into coarse powder form and placed together as the treatment in the treated zone.

Various concentrations of mixture essential oil (1.25, 2.5, 5.0, 10.0 and 20.0) v/v% were applied to the others choice-box. The oil was prepared in various concentrations by dissolving the essential oil in 1% of DMSO. Ten adult and nymph cockroaches (male and female) were then released into central choice-box (untreated zone). The movement of the cockroaches is blocked at the meddle area of both treated and untreated choice-box as shown in Figure 3.3 before the experiment start. The stop-watches started immediately when the pins were removed and the cockroaches were allowed to move freely between three choice boxes. Then, the choice boxes (treated and untreated) zones were exposed to a photoperiod of 72 hrs at 27 ºC.

The cockroaches located at the treated and untreated zone were carefully observed and counted for every 0, 2, 4, 6, 8, 24, 48 and 72 hrs of treatment. Each treatment with different concentration was conducted in duplicates. The percentage of repellency was calculated as follow:

$$\text{Repellency}\,\% = 100 - (\frac{\text{T}}{\text{N}} \text{x}\ 100\ \%)$$

Where, T stands for the number of cockroaches located at the treated zone and N stands for the total number (ten heads) of cockroaches been used in the repellency test. The mean percentage of the repellence was then calculated from the values obtained in three replicates. Figure 3.4 shows the cockroach test method.

Figure 3.1: Equal amounts of Jacob's biscuit were filled into the central of the treated and untreated choice-box

Figure 3.2: The oil was carefully dropped around the filter paper at the central of treated choice-box

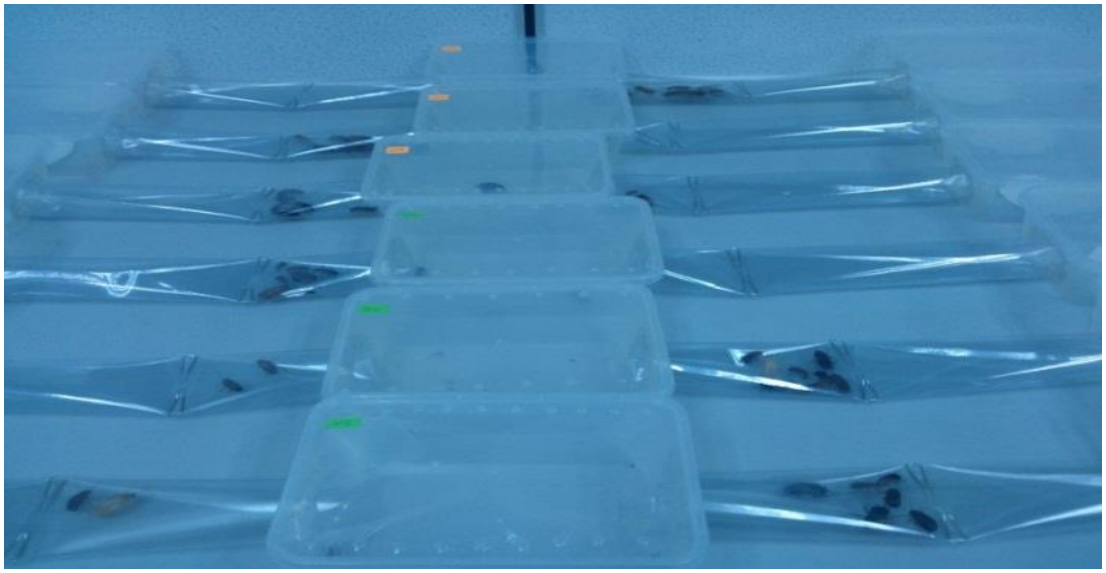

Figure 3.3: The movement of cockroaches is blocked at the middle area of both treated and untreated choice-box before the repellency test start

Figure 3.4: Cockroach test method

3.7 FORMULATION OF NATURAL REPELLENT

To formulate the solid repellent from the oils (essential and fixed), we used petroleum gel as carrier. The amount of each essential oils used in the product was the same (3%), fixed oil was 12% and petroleum gel (vaseline) 85%. The fixed oils involve directly in the formulation as carrier to dissolve the essential oils and also as

active ingredient in the solid formulation. Thus the percentage of carrier oils can be concern as the active ingredients. The main active ingredients in the formulation were essential oils. The main concern in making the solid formulation was the percentage of active ingredient (essential oils) in formulated product. The percentage calculated was in (v/w %) because the weight of the solidifying agent, Vaseline was fixed. The formula below implied the determination of percentage of essential oils in solid insect formulation.

$$\text{(V/w)\% of essential oils in solid formulation} = \frac{Volume\ of\ essential\ oils\ used}{Weight\ of\ vaseline\ used} \text{ x } 100\%$$

3.8 STATISTICAL ANALYSIS

One-way ANOVA test applied in this study to compare the means. The statistical analyses performed using Microsoft Excel 2010 software, and the differences considered significant when $p \leq 0.05$. Beside this, the repellence data expressed as mean ±SEM for the analysis of the variance of the duplicate values of repellency percentage against cockroaches (Appel and Tanley, 2000). Figure 3.5 shows the flow chart of research methodology.

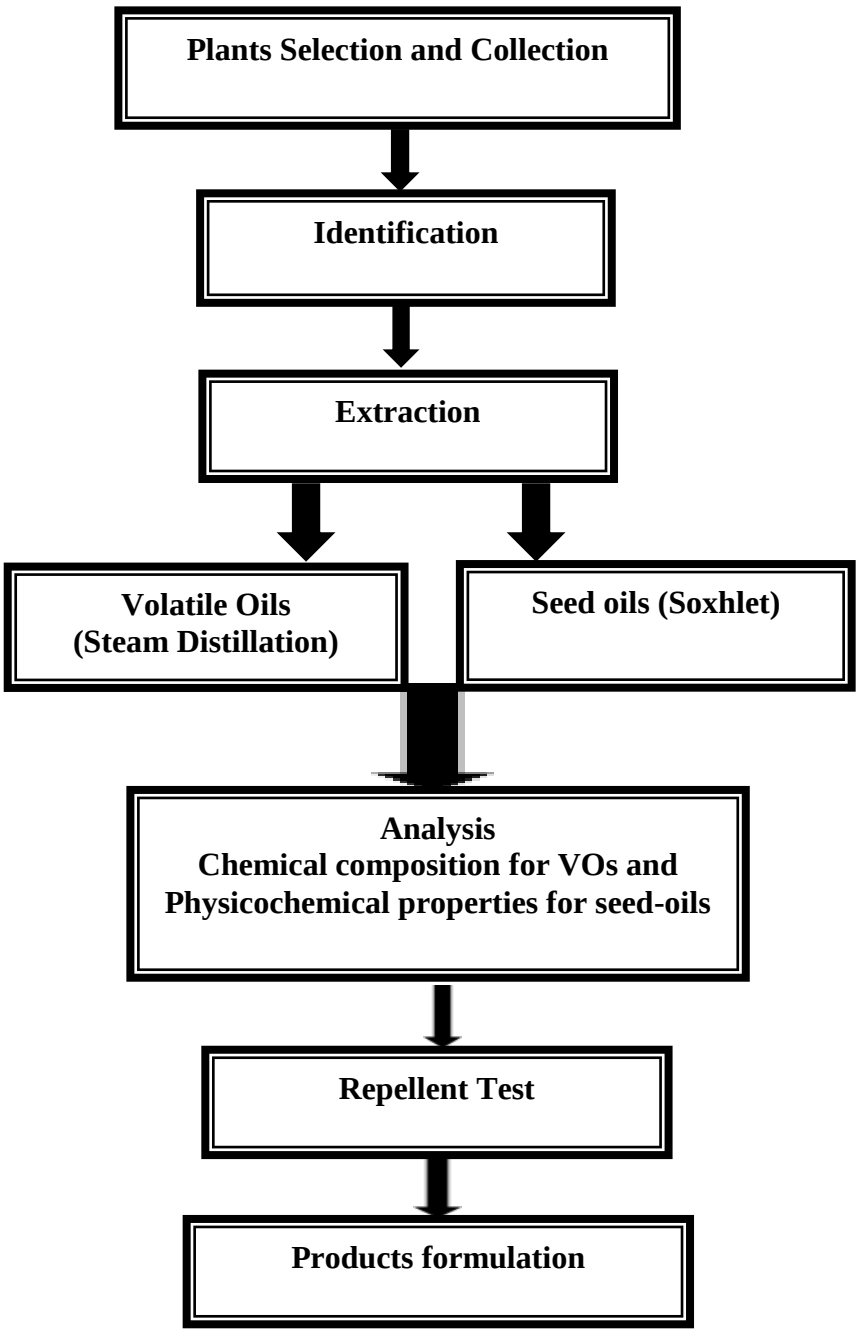

Figure 3.5: Flow Chart of Research Methodology

CHAPTER FUOR

RESULTS AND DISCUSSION

4.1 ESSENTIAL OILS

4.1.1 *O. basilicum* Essential Oil

(i) Yield of O. basilicum Essential Oil

The basil (*O. basilicum*) plant essential oil was separated using steam distillation. The percentage of the oil content was expressed on a fresh leaf weight basis (v/w %). The oil content was 0.78% the colour is light yellow with Camphor smell odor.

Previously, Bilal et al. (2012) reported that *O. basilicum* has 1.56% essential oil with yellowish green colour. While, Ilic et al. (2019) had reported the light yellow colour for essential oils from different types of O. *basilicum* with oil content ranged 0.41- 0.65%. Moreover, the yellow pale extracted from dried leaves with an output of 1.98 ± 0.01% (Khelifa et al., 2012). Different oil contents for *O. bascilicum* essential oils were reported e.g 0.1-0.4% (Ladwani et al. 2018), 0.2%. (Avetisyan et al. 2017), 0.05% to 0.55% (Ismail and Nour, 2011), 0.61% (Pripdeevech et al. 2010), 0.65 to 1.90% (Beatovic et al., 2015), 0.28% (Nawaz et al., 2017), 0.9-1.7% (DW) (Koroch et al., 2017), 0.16%-0.416% (Casanova et al., 2018), 1% (Ivanitskikh and Tarakanov, 2014) and 0.226% (Roshanpour et al., 2014).

Moreover, Abduelrahman et al. (2009) reported that the percentage of oil content for the cultivated-type Sudanese accession ranged from 0.33 to 0.47%. The essential oils yield of the flowers, leaves and stem from *O. basilicum* cultivated in Selcuk, Turkey, were 0.5 %, 1.0% and 0.05% (v/w), respectively, on a dry basis (Chalchat and Őzcan, 2008). Based on the plant material dry weight, the basil oil from Togo has an extraction yield ranging from 1.4 % to 2.2 % (Koba et al., 2009). Hussain et al. (2008) reported that the content of the essential oils was distributed unevenly

among the different seasons. The highest amount of the oil in the *O. basilicum* identified in Pakistani basils was observed during winter (0.8%), and this value decreased to 0.5% during summer. Turkish basils have oil content ranging between 0.4 mL/100 g and 1.5 mL/100 g (Telci et al., 2006).

Lee et al. (2005) reported that the total yields of volatile chemicals from basil leaves (relative to the amount of dried herbs used) purchased from a local market in northern California, USA was 1.24 ± 0.14%. Dambolena et al. (2010) found that semi-dried *O. basilicum* and *O. gratissium* have been different yielded oil depends on the part of plant and origins. For *O. basilicum* collected from a different area in Kenya; it was found yielded 0.4% (Yatta leaves), 1.3 % (Yatta flowering tops), 1.1 % (Sagana flowering tops), 1.9 % (Sagana leaves) and 0.2 % (Kariti leaves) oil.

Amount of yield of basil oil was influenced by the environmental factors such as soil composition, geographical area and climates. The hydro-distilled essential oil's content ranged from 0.5 % to 0.8 %, where the maximum amounts were observed in winter time while a minimum amount produced was in the summer time (Hussain et al., 2008, Rubab et al., 2017). Low essential oil yield in summer might be attributed to the high temperature and partial evaporation of some constituents of oil where it was expected that yield was positively correlated with the nutrients level at the low temperature but negatively at the high temperature (Hussain et al., 2008).

Previous research on *O. basilicum*, *O. basilicum*, *O. kilimandscharicum*, *O. lamiifolium*, *O. suave* and *O. suave* collected wildly in Mbeya region, Tanzania by Runyoro et al. (2010) found oil yield (v/w) was 4.05% , 0.54%, 3.13%, 3.3%, 1.15% and 1.01%, respectively. The essential oil content of fresh leaves reported by Simon et al. (1990) on oil volume per fresh weight basis was generally varied from 0.04% to 0.7% for *Ocimum* species. This result was agreed with Malaysian introduced accessions where the percentage of basil oils is in range as reported by Simon. Basils can be classified according to their geographical origin and growth habitat.

(ii) Chemical Composition of O. basilicum Essential Oil

Essential oil of *O. basilicum,* obtained by steam distillation was subjected to GC-MS. Figure 4.1 shows GC-MS chromatogram of the *O. basilicum* oil of this study with identification of forty one peaks with their retention time as suggested by MS library. The MS Library of *O. basilicum* essential oil shown in Appendix A.

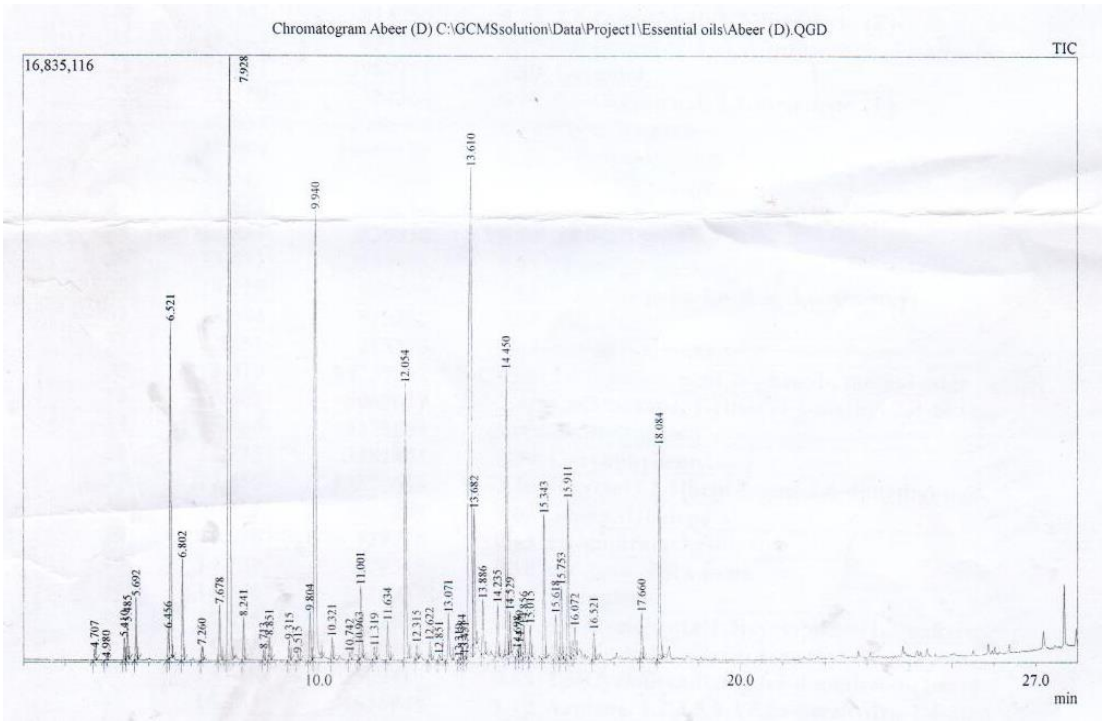

Figure 4.1: GC-MS chromatogram of the *O. basilicum* essential oil

In this study, there are forty one chemical constituents (~97.06%) that obtained from the essential oils from the leaves of *O. basilicum* by using steam distillation. The major constituents in this oil were methyl cinnamate (25.32%), linalool (19.06%) and estragole (12.32%) were the major oxygenated monoterpenes. In sesquiterpenes hydrocarbons, α-bergamoten (5.26%), germacrene (4.58%), γ-cadinene (2.82%) and β-elemene (2.44%) were main compounds. While, in oxygenated sesquiterpenes, Tau-cadinol (4.26%) was important compounds. And ocimene the highest compound in monoterpenes hydrocarbons. Table 4.1 shows the chemical composition of *O.*

basilicum essential oil analyzed by GC-MS. The essential oil isolated from *O. basilicum* was mainly constituted by linalool (35.1%), eugenol (20.7%) and 1,8-cineole (9.9%) (Pirasa et al. 2018). Hussain et al. (2008) and Rubab et al. (2017) were reported that the essential oils consisted of linalool was the most abundant component (56.7-60.6%), followed by epi-α-cadinol (8.6-11.4%), α-bergamotene (7.4-9.2%) and γ-cadinene (3.2-5.4%). In addition, Nawaz et al. (2017) documented that a total 17 compounds were identified with linalool (70.44%) as major compound, followed by estragole (14.43%), tau-cadinol (4.13%) and α-bergamoten (3.71%). In the other hand, Ozcan and Chalchat, (2002) reported that forty-nine constituents were identified and representing (88.1%) in the *O. basilicum* essention oil. Methyl eugenol (78.02%), α-cubebene (6.17%), nerol (0.83%), α-muurolene (0.74%), 3,7-dimethyloct-1,5-dien-3,7-diol (0.33%) and β-cubebene (0.30%) were found as the major compounds. Researcher reported that the major components of *O. basilicum* were linalool (35.99%), estragole (28.56%) and eucalyptol (7.57%) (Mehdizadeh et al., 2016). Pripdeevech et al. (2010) claimed that the dominant components were methyl chavicol (81.82 %), β-(E)-ocimene (2.93 %), α -(E)-bergamotene (2.45 %), α -epi-cadinol (2.08 %), 1,8-cineole (1.62 %), methyl eugenol (1.10 %) and camphor (1.09 %).

Abduelrahman et al. (2009) claimed that the linalool, geraniol, geranial, aldehyde derivative, methyl eugenol, were found in varieties of basil growing in Sudan. Filip et al. (2016) a few compounds were dominant in analyzed extracts: linalool, eugenol, α-bergamtene, germacrene D, γ-cadinene, δ-cadinene and β-selinene. Linalool was major compound in extracts present in range from (12.2 to 141.5g/kg). Ismail and Nour (2011) say that the essential oil contains high percentages of compounds such as estragole, linalool, methyl cinnamate, geranial, geraniol, eugenol, methyl eugenol, eucalyptol, camphor, and limonene. Llana-Ruiz-Cabello et al (2015) the major constituent of EO, is linalool (approximately 70%). Marwat et al. (2011) reported that the major constituents of sweet basil oil were linalool and eugenol. Rahman et al. (2011) mentioned that the leaf contain volatile oil

of eugenol, euginal (also called eugenic acid), urosolic acid, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol (also called estagol).

Bilal et al. (2012) stated that the major oxygenated monoterpenes are: linalool, camphor, cis-geraniol and 1, 8-cineole. While, abergamotene, b-caryophyllene, germacrene D, ccadinene and bicyclogermacrene are the main sesquiterpene hydrocarbons. Whereas, epi-acadinol and viridiflorol are the important oxygenated sesquiterpene. Also, Ismail (2006) found that the major terpenes present are linalool (44.18%), cineole (13.65%), eugenol (8.59%), isocaryophyllene (3.10%), methyl cinnamate (4.26%), and a-cubebene (4.97%). While, the chromatographic analysis of basil oil revealed the presence of 51 compounds. The predominant compounds were: linalolol, 1.8-cineol, geranyl, D germacrene, γ-cadinene, Epi-α-cadinole (Dzida (2010).

Casanova et al. (2018) in the their investigation, reported that basil essential oils contained a lot of estragol and linalool, with low quantities of 1,8-cineol, camphor and eugenol. Marotti et al. (1996) classified three basil essential oils as a high content of linalool, included three chemotypes: “linalool,” “linalool and methylchavicol,” and “linalool and eugenol”. Also Govindarajan et al. (2013) found that the major constituents of basil oil were linalool (52.42%), methyl eugenol (18.74%) and 1, 8-cineol (5.61%). Dambolena et al. (2010) in their investigation for essential oil of *O. basilicum* from Sagana, they claimed that the oil contained mainly linalool (95%), and leaves from Yatta contained mainly camphor (31.0%) and linalool (29.3). The main constituents in the essential oil were Estragole (41.40%), 1,6-Octadien-3-ol, 3,7-dimethyl (29.49%), trans-.alpha.-Bergamotene (5.32%), Eucalyptol (3.51), Citral (3.31%), N-Cyano-3-methylbut-2-enamine (3.08%), cis-.alpha.-Bisabolene (1.92%), Levomenthol (1.81%), and beta.-Myrcene (1.11%) (Falowo et al., 2019).

In addition Ilic et al. (2019) investigated some basil essential oils, they claimed that the main constituent of all basil essential oils was monoterpene alcohol linalool (13.68-40.97%), following constituents were detected: eugenol (10.83-

8.97%), αbergamotene (8.12-9.25%), epibicyclosesquiphellandrene (7.03-8.07%), eucalyptol (5.98-6.20%) and methyl chavicol (4.13-5.26%). Goudoum et al. (2017) reported that twenty nine constituents were identified corresponding to 95.9% of *O. basilicum* essential oil with three major components: Limonene (27.65%), linalool (21.51%) and β-phellandrene (14.86%).

Moreover, Khelifa et al. (2012) found fourty compounds have been identified accounting for 97.4%. The major compounds were: Linalool (32.83%), linalyl acetate (16%), elemol (7.44%), geranyl acetate (6.18%), myrcene (6.12%), allo-ocimene (5.02%), α-terpineol (4.9%), (E)-β-ocimene (3.68%) and neryl acetate (3.45%). Furthermore, Tangpao et al. (2018) says estragole, eugenol, and methyl eugenol were among the major volatiles found in the essential oils of *O. basilicum* types. While, Pandey et al. (2018) recorded three main compounds for *O. basilicum* L. essential oil namely methyl chavicol (70.00), linalool (25.00), eugenol (5.00). Also, Joshi (2014) claimed that the major constituents were identified as methyl eugenol (39.3%) and methyl chavicol (38.3%). Ivanitskikh and Tarakanov, (2014) found essential oil of *O. basilicum* including linalool, pinene, eugenol, camphor and cineole were reported. The main constituents of basil essential oil have been reported as methyl chavicol, citral, linalool, geraniol and eugenol (Roshanpour et al., 2014).

Avetisyan et al. (2017) according to their results, the qualitative and quantitative composition of essential oils was quite different: O. basilicum var. purpureum essential oil contained 57.3% methyl-chavicol (estragol); O. basilicum var. thyrsiflora oil had 68.0% linalool. The main constituents of O. citriodorum oil were nerol (23.0%) and citral (20.7%).

Nour et al. (2009) investigated different accessions of O. basilicum and classified to 3 major classes (chemotypes) namely Linalool chemotype, methyl cinnamate-linalool and geraniaol-geranial according to their major constituents. Poonkodi (2016) reported that the predominant were (1) linalool, (2) methyl cinnamate, (3) methyl cinnamate/linalool, (4) methyl eugenol, (5) citral, (6) methyl

chavicol (estragol), and (7) methyl chavicol/citral for O. basilicum essential oil. The content of methyl chavicol was 74.7%, followed by linalool 14.3% were detected as major compounds in India.

Table 4.1: Chemical Composition of *O. basilicum* Essential Oil

Compound	**Formula**	**Systematic name**	**Area %**
Monoterpene hydrocarbons			
alpha-Pinene	$C_{10}H_{16}$	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hepta-2-ene	0.18
Camphene	$C_{10}H_{16}$	Bicyclo[2.2.1]heptane,2,2-dimethyl-3-methylene	0.03
Sabinene	$C_{10}H_{16}$	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)	0.30
Pseudopinen	$C_{10}H_{16}$	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene	0.53
Myrcene	$C_{10}H_{16}$	1,6-Octadiene,7-methyl-3-methylene	0.88
Limonene	$C_{10}H_{16}$	Cyclohexene,1-methyl-4-(methylethenyl)	0.48
Ocimene	$C_{10}H_{16}$	1,3,6-Octatriene,3,7-dimethyl-(Z)	1.51
Total of monoterpene hydrocarbons			**3.91**
Oxygenated monoterpenes			
Cineole	$C_{10}H_{18}O$	2-oxabicyclo[2.2.2]octane,1,3,3-trimethyl	5.57
Sabinene hydrate	$C_{10}H_{18}O$	Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(methylethyl)-(1.alpha.,2.beta.,5.alpha.)	0.26
Fenchone	$C_{10}H_{16}O$	Bicyclo[2.2.1]heptan-2-one,1,3,3-trimethyl	0.94
Linalool	$C_{10}H_{18}O$	1,6-Octadien-3-ol,3,7-dimethyl	19.06
Fenchol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl	0.62
	$C_{10}H_{14}O_2$	Oxirane,2-(hexyn-1-yl)-3-methoxymethylene	0.17
Camphor	$C_{10}H_{16}O$	Bicyclo[2.2.1]hepta-2-one,1,7,7-trimethyl-,(1R)	0.36
Isoborneol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethl,exo	0.32
Terpinenol-4	$C_{10}H_{18}O$	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	0.11
Terpineol schlethin	$C_{10}H_{18}O$	3-Cyclohexene-1-methanol,alpha.,alpha.4-trimethyl	0.92
Estragole	$C_{10}H_{12}O$	1-ally-4-methoxybenzene	12.32
Beta-Citral	$C_{10}H_{16}O$	2,6-Octadienal,3,7-dimethyl-,(Z)	0.13
Geraniol	$C_{10}H_{18}O$	2,6-Octadien-1-ol,-3,7-dimethyl-,(E)	1.20
Alpha-Citral	$C_{10}H_{16}O$	2,6-Octadienal,3,7-dimethyl-,(E)	0.21
Methyl cinnamate	$C_{10}H_{10}O_2$	2-Propenoic acid,3-phenyl-,methyl ester	25.32
3-Allylguaiacol	$C_{10}H_{12}O_2$	Phenol,2-methoxy-3-(2-propenyl)	0.95
8-Hydroxylinalool	$C_{10}H_{18}O_2$	2,7-Octadiene-1,6-diol,2,6-dimethyl	0.04
Total of oxygenated monoterpenes			**68.50**
Sesquiterpene hydrocarbons			
Elixene	$C_{15}H_{24}$	Cyclohexane,1-ethynyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)	0.30
Alpha-Ylangene	$C_{15}H_{24}$	8-Isopropyl-1,3-dimethyltricyclo[4.4.0.$0^{2,7,}$]dec-3ene	0.10

Table 4.1: continued

Copaene	$C_{15}H_{24}$	Tricyclo[4.4.0.02,7]dec-3-ene,1,3-dimethyl-8-(1-methylethyl)-,stereoisomer	0.16
b-Elemene	$C_{15}H_{24}$	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylenyl)-,[1S-(1.alpha.,2.bata.,4.beta.)]	2.44
Caryophyllene	$C_{15}H_{24}$	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methlene-,[1R-(1R*,4E,9S*)]	0.99
Alpha-Bergamoten	$C_{15}H_{24}$	Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	5.26
Alpha-Guaiene	$C_{15}H_{24}$	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,4.alpha.,7.alpha.)]	0.62
	$C_{15}H_{24}$	Cis-Muurola-3,5-diene	0.13
Beta-Farnesene	$C_{15}H_{24}$	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-,(Z)	0.18
Humulene	$C_{15}H_{24}$	1,4,8-Cycloundecatriene,2,6,6,9-tetramethyl-,(E,E,E)	0.55
Beta-Cubebene	$C_{15}H_{24}$	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-.[3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*	0.55
Germacrene	$C_{15}H_{24}$	1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)	3.58
Alpha-Bulnesene	$C_{15}H_{24}$	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1.alpha.,7.alpha.,8a,beta.)]	1.40
Gamma-Cadinene	$C_{15}H_{24}$	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)	2.82
Total of sesquiterpene hydrocarbons			**19.08**
Oxygenated sesquiterpenes			
Beta-Elemol	$C_{15}H_{26}O$	Cyclohexanemethanol,4-ethyenyl-.alpha.,alpha.,4-trimethyl-3-(1-methylethenyl)-,[1R-(1.alpha.,3.alpha.,4.beta.)]	0.50
Cubedol	$C_{15}H_{26}O$	4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cydopropa[1,2]benzene-3-ol	0.77
Tau-Cadinol	$C_{15}H_{26}O$	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol-,(1S-(1.alpha.,4a.alpha.,4a.alpha.,8a.beta.))	4.26
Total of oxygenated sesquiterpenes			**5.53**
Others			**2.94**

Figure 4.2 shows structures of twelve compounds (>1%) identified in *O. basilicum* essential oil which are ocimene, cineol, linalool, estragole, cinnamate, geraniol, β-elemene, α-bergamotene, germacrene D, α-bulnesene, γ-cadinene and taucacinol.

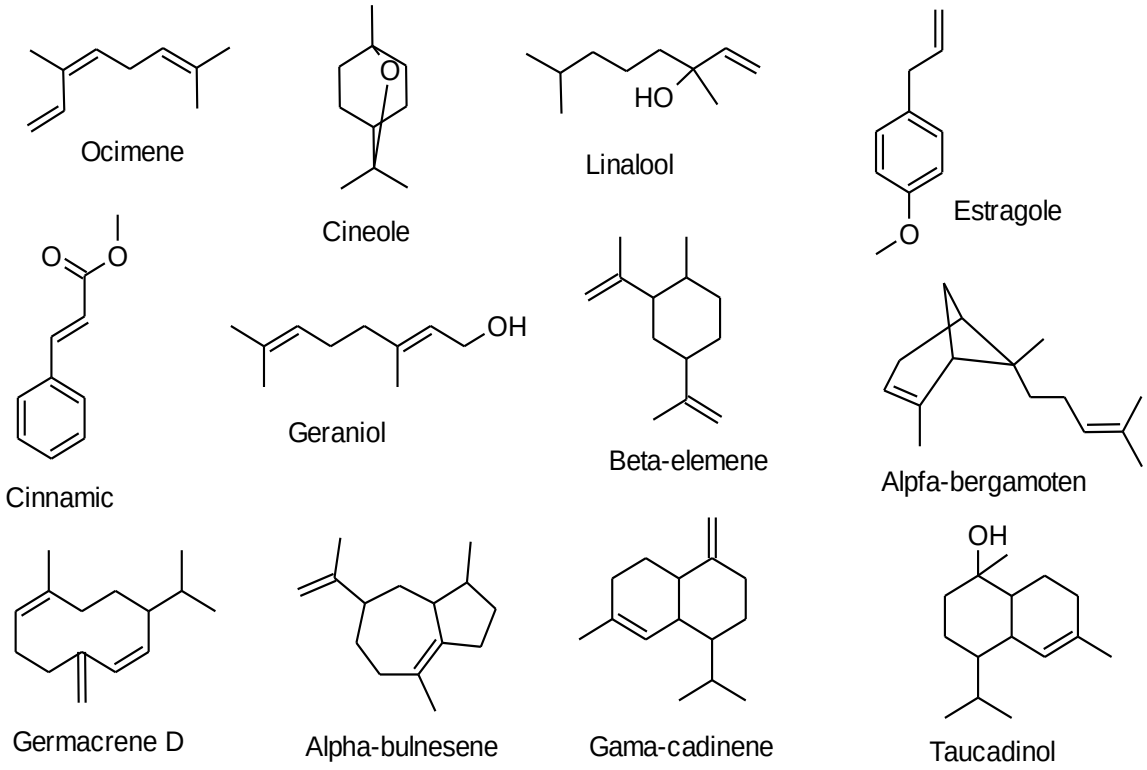

Figure 4.2: Structures of some compounds (>1%) in *O. basilicum* essential oil

The total of monoterepenes represnt 72.41% of the oil the ratio is hydrocarbon (3.91%) and oxygenated monoterpene (68.50%). While the total of sesquiterpenes was 24.61% representing 19.08% sesquiterpene hydrocarbon and 5.53% are oxygenated sesquiterpenes. Figure 4.3 shows the distribution of mono and sesquiterpenes in *O. basilicum* essential oil. Pirasa et al. (2018) reported that the *O. basilicum* essential oil comprise (97.6%) of compounds. While, *O. basilicum* presents high amounts of oxygenated monoterpenes (47.7%), phenylpropanoids (21.1%) and hydrocarbon sesquiterpenes (17.7%). Also, sample collected in winter were found to be richer in oxygenated monoterpenes (68.9%), while those of summer were higher in sesquiterpene hydrocarbons (24.3%) (Hussain et al. 2008, Rubab et al., 2017).

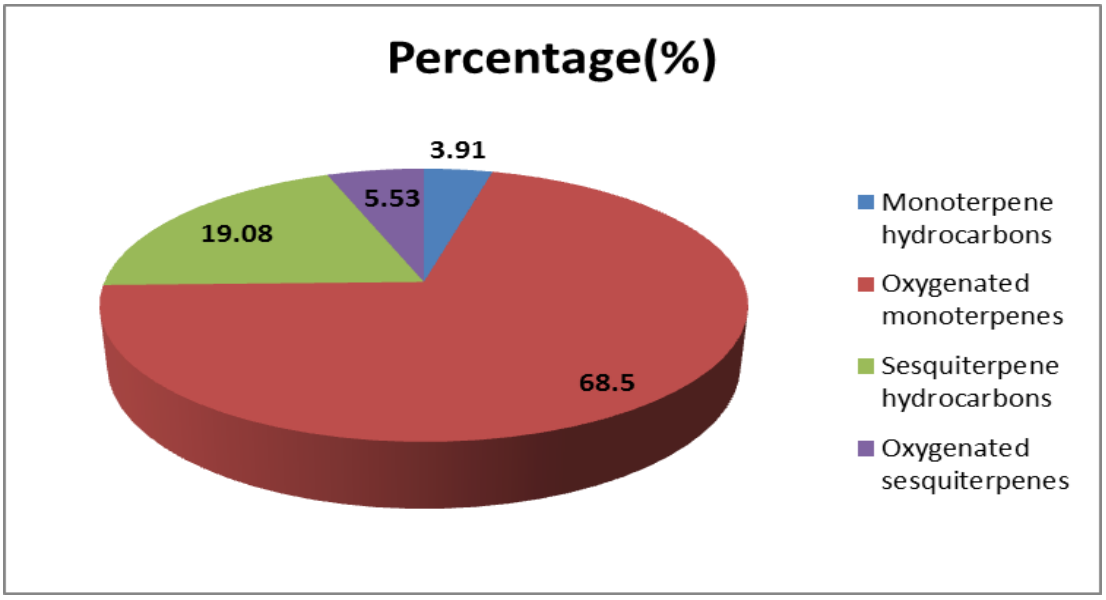

Figure 4.3: Distribution of mono and sesquiterpenes in *O. basilicum* essential oil

4.1.2 *C. rotundus* Essential Oil

(i) Yield of C. rotundus Essential Oil

The *C. rotundus* essential oil was obtained by steam distillation. The percentage of the oil content was expressed on dry rhizome weight basis (v/w) %. The oil content was 0.73 % the colour is radish yellow. Zhang et al. (2017) claimed that the light yellow essential oil was obtained by hydrodistillation of dried *C. rotundus* rhizomes with a yield of 0.83% (v/w).

The light yellow essential oil was obtained by hydrodistillation from dried *C. rotundus* rhizomes with a yield of 0.83% (v/w) (Hu et al., 2017). Lawal and Oyedeji (2009) used hydrodistillation to extract fresh rhizomes of *C. rotundus* collected from the two different locations yielded 0.20% and 0.16% with pale yellowish oils. Eltayeb et al. (2016) reported about three samples of *C. rotundus* essential oils collected from different states in Sudan yielded 1.2% (Dongola) and 0.5% (Khartoum and AlGazera). In anthor study by Eltayeib and Um-Ismaeel (2014) the percentage of the oil in the three samples (A, B and C) was found to be (2.9, 0.6, and 1.8) (w/w) respectively.

Moreover, Bajpay et al (2018) and Al-Snafi (2016) both found that *C. rotundus* rhizomes have 0.19% essential oils. El-Gohary (2004) documented that the percentage yields 0.46 and 0.19% for *C. rotundus* rhizomes oil.

(ii) Chemical Composition of C. rotundus Essential Oil

Figure 4.4 shows the GC spectrum of *C. rotundus* rhizome essential oil analyzed by GC-MS. The constituents were recognized by comparing mass spectra and retention indices with those in literatures and by computer searching followed by matching the mass spectra data with NIST held in a computer library. MS Library of *C. rotundus* rhizome essential oils was shown in Appendix B.

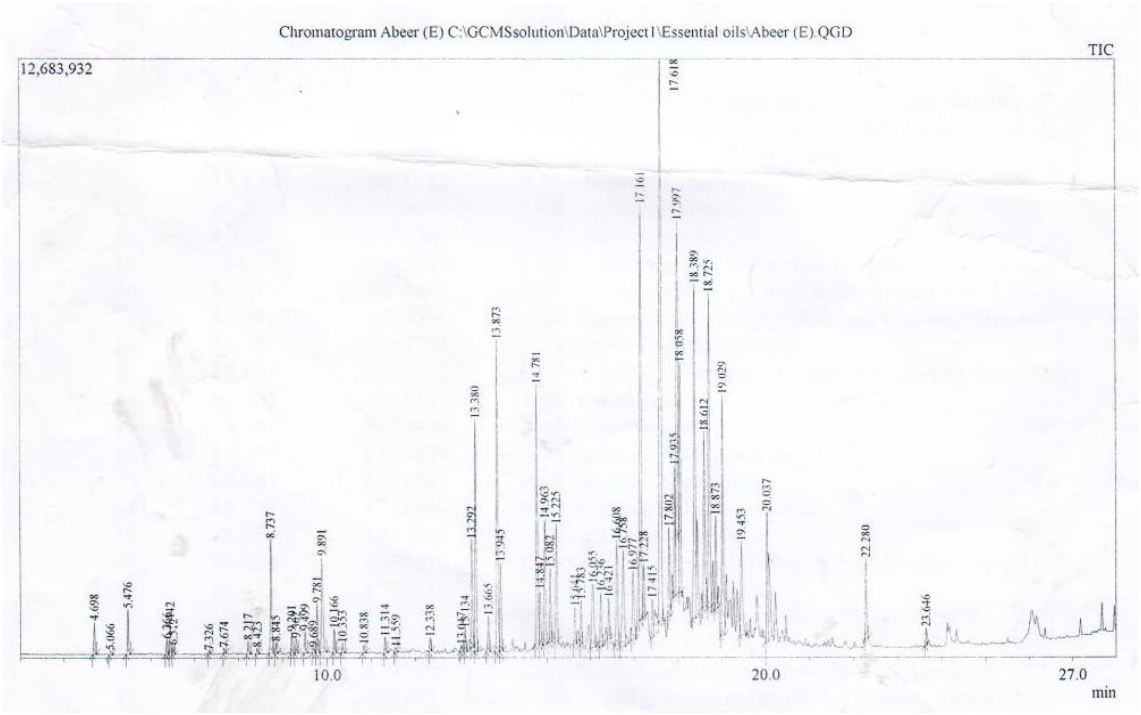

Figure 4.4: GC-MS chromatogram of the *C. rotundus* essential oil

In this study, *C. rotundus* essential oil obtained by steam distillation subjected to GC-MS analysis. From the GC-MS analysis forty-four components representing

(~40%) were identified. Table 4.2 shows chemical composition of *C. rotundus* essential oil. The major compounds were (-)-Isolongifolol (7.63%), longiverbenone (5.61%) and longifolenaldehyde (5.16%) were the major oxygenated sesquiterpen. In sesquiterpenes hydrocarbons, β-cadinene (5.54%), α-copaene (3.72%), γ-muurolene (1.92%) and rotundene (1.91%) were main compounds. While, in oxygenated monoterpen, (IR)-(-)-myrtenal (2.01%) and E-pinocarveol (1.73%) was important compounds. And pseudopinen (0.56%) the highest compound in monoterpenes hydrocarbons. Moreover, elemenone (13.59%), α-cyperone (13.41%) and caryophllene oxide (13.03%) were the most important sesquiterpenes of the oil (Janaki et al., 2018). Again, fifty two secondary metabolites were isolated from *C. rotundus*. (+) oxo-α-ylangene (9.35%), (+) α-cyperone (9.07%) trans-pinocarveol (7.92%) and cyperene (7.83%) were the major constituents in the oil of *C. rotundus*.

Furthermore, a total of 30 components were identified, representing 94.7% of the total amount. The α -cyperone (38.46%), cyperene (12.84%) and α -selinene (11.66%) was found to be the major components in the essential oil of *C. rotundus* rhizomes, followed by β -caryophyllene oxide (4.33%), (d)-limonene (3.62%), α -calacorene (3.14%), and γ -muurolene (3.13%), besides, other components (0.13–1.58%) (Hu et al., 2017, Zhang et al. 2017). Das et al. (2015) reported that the plant contains the following chemical constituents – Cyproterone, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2, 4-diene, g-gurjunene, trans-calamenene, d-cadinene, g-calacorene, epi-a-selinene, a-muurolene, g-muurolene, cadalene, nootkatene by comparison with a spectral library established under identical experimental conditions, cyperotundone, mustakone, cyperol, isocyperol, and a-cyperone.

Sivapalan (2013) mentioned that the major compounds isolated from essential oil and the extracts of *C. rotundus* rhizome are Alpha-cyperone, Alpha-rotunol, Beta-cyperone, Beta-pinene, Beta-rotunol, Beta-selinene, Calcium, Camphene, Copaene, Cyperene, Cyperenone, Cyperol, Cyperolone Cyperotundone Dcopadiene, D-epoxyguaiene, D-fructose, D-glucose, Flavonoids, Gamma-cymene, Isocyperol,

Isokobusone, Kobusone, Limonene, Linoleic-acid, Linolenic-acid, Magnesium, Manganese, C. rotunduskone, Myristic-acid, Oleanolic-acid, Oleanolic-acid-3-oneohesperidoside, Oleic-acid, P-cymol, Patchoulenone, Pectin, Polyphenols, Rotundene, Rotundenol, Rotundone, Selinatriene, Sitosterol, Stearic-acid, Sugeonol, Sugetriol.

In other hand, Singh et al. (2012) clamied that the major compounds isolated from essential oil are: α-cyperone, cyperene, cyperotundone, cyperol, β-selinene, β-caryophyllene, valerenal, sugeonyl acetate, α-copanaaene, patchoulene, trans-pinocarvol, patchoulenenone, aristrol-9-en-3-one, seline-4, 11-diene, aristrol-9-en-8-one, kobusone, sugetriol, isokobusone, isocyperol, sugeonl and sitoerol. While, Eltayeib and Um-Ismaeel (2014) mentioned that three samples (A, B and C) with the following compounds were detected in the three samples: alpha,beta Pinene, D-Limonene, Camphene, Thymol, Copaene, Caryophyllene Isoledene, and cyclo hydrocarbon like Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl), Bicyclo [2.2.1]heptan-2-ol,1,3,3trimethyl, Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1methylethyl), Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene,and aromatic compound like (Benzene, 1-methyl-4-(1-methylethyl), Naphthalene, 1,6-dimethyl-4-(1methylethyl)), and alcohol Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1methylethyl), p-menth-1-en-8-ol, 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl), Methanol, (1,4dihydrophenyl).and ester like -Tetradecynoic acid, methyl ester. and aldhed like Cyclohexanebutanal, 2-methyl-3-oxo. Whereas, Aghassi et al. (2013) reported that the cyperene (37.9 %) was the dominant constituent. Other components present at appreciable content were cyperotundone (11.2 %), isorotundene (9.5 %), cyperol (6.4 %), α-cyperone (4.3 %) and β-pinene (3.9 %).

El-Gohary (2004) mentioned that the GC-MS analysis revealed that 52 and 37 components were identified in the tubers essential oils of *C. rotundus L.* and *C. alopecuroides* Rottb. Representing 99.21% and 93.90% of the total composition of both oils respectively, 25 components of them are common in both oils.

Caryophellene oxide (12.48%) was the major constituent of *C. alopecuroides* Rottb. oil followed by α -cyperone (9.57%), βcalacorene (6.75%) and oxo-α-ylangene (6.39%), while oxoα -ylangene (9.35%), α-cyperone (9.07%) transpinocarveol (7.92%) and cyperene (7.83%) were the major constituents in the oil of *C. rotundus* L.

Himaja et al. (2014) said that the chemical analyses showed the presence of compounds with best-known antimicrobial activity, as well as one, 8-cineole, geranial, germacreneD, limonene, linalool. Kumar et al. (2017) reported that the rotunol, betacyperone, βselinene, camphene, calcium, cyperene, cyperenon, cyperol, cyperolon selinene, cyperotundone, D-copadiene, linolenic acid, linoleic acid, oleic acid, rotundene, rotundenol, rotundone, polyphenols, pectin, stearic acid, camphene, sugeonol, sugetrio, the major chemical constituent in the extract of C. rotundus rhizomes essential oil.

Somwa and Konig (2001) documented that the analysis of the essential oil of *C. rotundus* by GC and GC–MS allowed the identification of cyprotene, cypera-2,4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2,4-diene, g-gurjunene, trans-calamenene, d-cadinene, g-calacorene, epi-a-selinene, a-muurolene, g-muurolene, cadalene, nootkatene.

Morimoto and Komai (2005) mentioned two sesquiterpene ketones, cyperotundone and α-cyperone, were isolated from dried tubers of purple nutsedge (*C. rotundus* L.) as major constituents: ≈0.26% and 0.1% of dried tuber, respectively. In addition, Xiao-shan et al. (2006) and Nalini et al. (2014) claimed that the main components are α-copaene (1.97%), cyperene (15.73%), α-hisaholene (2.14%), α-gurjunene (1.29%), 2-methoxy-8-methyl-1,4-naphthalenedione (4.01%), β-selinene (17.99%), oxo-α-ylangene (3.00%), 4,4α,5,6,7,8,-hexahydro-4α,5-dimethyl-3-(1-methyl ethylidene) -2(3H)-naphthalenone (8.11%), α-cyperone (26.15%), longipinocarvone(1.11%), etc.

Table 4.2: Chemical Composition of *C. rotundus* Essential Oil

Compound	Formula	Systematic name	Area %
Monoterpene hydrocarbons			
alpha-Pinene	$C_{10}H_{16}$	2,6,6-Trimethylbicyclo[3.1.1]hepta-2-ene	0.44
Thujadiene	$C_{10}H_{14}$	Bicyclo[3.1.0]hex-2-ene,4-methylene-1-(1-methylethyl)	0.05
Pseudopinen	$C_{10}H_{16}$	Bicyclo[3.1.1]heptane,6,6-dimethyl-2-methylene	0.56
o-Cymene	$C_{10}H_{14}$	Benzene,1-methyl-2-(1-methylethyl)	0.19
Limonene	$C_{10}H_{16}$	Cyclohexene,1-methyl-4-(methylethenyl)	0.32
p-Cymenene	$C_{10}H_{12}$	Benzene,1-methyl-4-(1-methylethenyl)	0.06
Total of monoterpene hydrocarbons		**1.62**	
Oxygenated monoterpenes			
Cineole	$C_{10}H_{18}O$	2-oxabicyclo[2.2.2]octane,1,3,3-trimethyl	0.11
Fenchol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl	0.19
Camphenol	$C_{10}H_{16}O$	5,5-dimethyl-6-methyenebicyclo[2.2.1]heptan-2-ol	0.09
E-Pinocarveol	$C_{10}H_{16}O$	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene,[IS-(1.alpha.,3.alpha.,5.alpha.)]	1.73
Cis-Verbenol	$C_{10}H_{16}O$	Bicyclo[3.1.1]hept-3-en-2-ol,4,6,6-trimethyl,(1.alpha.,2.beta.,5.alpha.)	0.17
Pinocarvone	$C_{10}H_{14}O$	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptan-3-one	0.29
Isoborneol	$C_{10}H_{18}O$	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethl,exo	0.21
Terpinenol-4	$C_{10}H_{18}O$	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	0.32
Benzenemethanol	$C_{10}H_{14}O$	1-methyl-4-(.alpha-hydoxyisopropyl)benzene	0.11
Terpineol schlethin	$C_{10}H_{18}O$	3-Cyclohexene-1-methanol,alpha.,alpha.4-trimethyl	0.71
(IR)-(-)-Myrtenal	$C_{10}H_{14}O$	6,6-dimethyllbiclo[3.1.1]hepta-2-ene-2-carbaldehyde	2.01
Vebenone	$C_{10}H_{14}O$	Bicyclo[3.1.1]hepta-3-en-2-one,4,6,6-trimethyl	0.33
Cis-Carveol	$C_{10}H_{16}O$	2-Cyclohexane-1-ol,2-methyl-5-(1-methylethenyl)	0.15
(-)-Carvone	$C_{10}H_{14}O$	2-cyclohexane-1-one,2-methyl-5-(1-methylethenyl)	0.12
Royaltac	$C_{10}H_{22}O$	n-decan-1-ol	0.27
Total of oxygenated monoterpenes		**6.81**	
Sesquiterpene hydrocarbons			
Beta-Vatirenene	$C_{15}H_{22}$	8,8a-dimethyl-2-(1-methylethylidene)-1,23,7,8,8a-hexahydronaphthalene	0.35
Alpha-Ylangene	$C_{15}H_{24}$	8-Isopropyl-1,3-dimethyltricyclo[4.4.0.$0^{2,7,}$]dec-3-ene	1.65
Alpha-Copaene	$C_{15}H_{24}$	8-Isopropyl-1,3-dimethyltricyclo[4.4.0.$0^{2,7,}$]dec-3-ene	3.72

Beta-Elemene	$C_{15}H_{24}$	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylenyl)-,[1S-(1.alpha.,2.bata.,4.beta.)]	0.53
	$C_{15}H_{24}$	Naphthalen,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)-(+/-)	5.54
	$C_{15}H_{24}$	1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl-Z,Z,Z	0.87
Rotundene	$C_{15}H_{24}$	1,5,9-Trimethyltricyclo[6.2.2.0^{2,6,}]dedec-9-ene	1.91
Gamma-Muurolene	$C_{15}H_{24}$	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.alpha.,8a.alpha.)	1.92
Alpha-Muurolene	$C_{15}H_{24}$	[1.alpha.,4a.alpha.,8a.alpha.]-,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-[1-methylethyl]naphthalene	0.55
Trans-calamenene	$C_{15}H_{22}$	4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	1.59
Alpha-Calacorene	$C_{15}H_{20}$	1-Isopropyl-4,7-dimethyl-1,2-dihydronaphthalene,(S)	0.60
	$C_{15}H_{24}$	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-.[3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*	0.65
	$C_{15}H_{24}$	Bicyclo[5.2.0]nonane,4-methylene-2,8,8-trimethyl-2-vinyl	0.62
	$C_{15}H_{24}$	Longifolene-(V4)	1.23
Total of sesquiterpene hydrocarbons		**21.73**	
Oxygenated sesquiterpenes			
Caryophyllene oxide	$C_{15}H_{24}O$	5-Oxatricyclo[8.2.0.0(4,6)-]dodecane,4,12,12-trimethyl-9-methylene[1R-(1R*,4R*,6R*,10R*)]	0.61
Spathulenol	$C_{15}H_{24}O$	1H-Cycloprop[e]azulen-7-ol,dechydro-1,1,7-trimethyl-4-methylene-[1ar-(1a.alpha.,4a.alpha.,7.beta.,7b.alpha.)]	0.86
(-)-Isolongifolol	$C_{15}H_{26}O$	1,4-Methanoazulene-9-methanol,dehydro-4,8,8-trimethyl-[1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9R*)]	7.63
	$C_{15}H_{22}O$	Isologifolen-5-one	1.32
	$C_{15}H_{24}O$	Longifolenaldehyde	5.16
	$C_{15}H_{24}O$	Tetracyclo[6.3.2.0(2,5).0(1,8)tridecan-9-ol,4,4-dimethyl	3.33
Longiverbenone	$C_{15}H_{22}O$	2,6,6,11-Tetramethyltrcyclo[5.4.0.02,8]undec-10-en-9-one	5.61
	$C_{15}H_{22}O$	2H-Cyclopropa[a]naphthalene-2-one,1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-	4.26

Table 4.2: continued

		,(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)	
Ledol	$C_{15}H_{26}O$	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-,[1ar-(1a.alpha.,4.alpha.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]	1.94
Total of oxygenated sesquiterpenes			**30.72**
Others			**39.1**

Previously reported that the chemical composition of essential oils of *C. rotundus* from around the world; a-cyperone, cyperene, cyperotundone and (b-selinene) were found to be the major compounds identified in higher concentrations, along with other constituents such as, a-copaene, valerenal, caryophyllene oxide, patchoulenyl acetate and sugeonyl acetate. In addition some reports have had the occurrence of a-pinene, limonene and 1, 8-cineole as minor components of the essential oils of *C. rotundus*. Comparing the present result with those previously reported in the literature on the essential oil composition of *C. rotundus* from different countries (Kilani et al., 2008, Komai and Tang 1989, Jirovetz et al., 2004), it's apparent that, there are many difference regarding the major constituent of the oils of *C. rotundus*, which further suggests the existence of more chemical diversity within the Cyprus rotundus species (Kilani et al., 2008). Figure 4.5 shows structures of some compounds (>1%) in *C. rotundus* essential oil

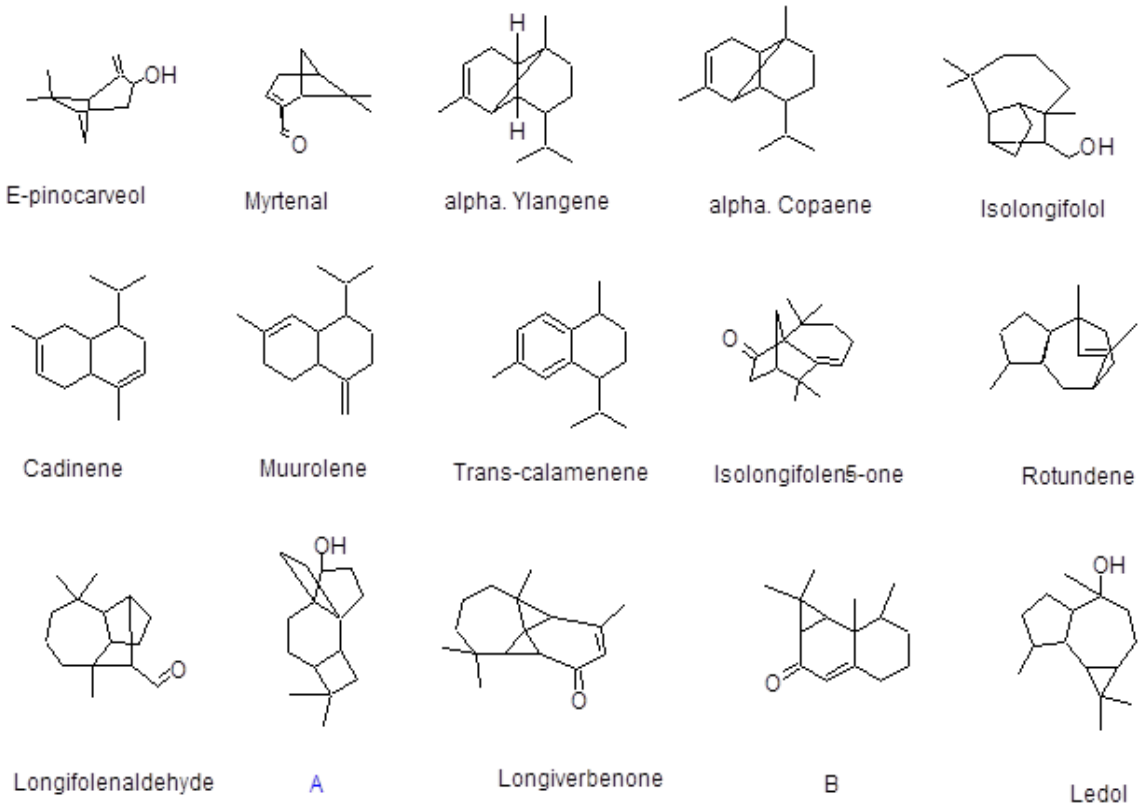

Figure 4.5: Structures of compounds (>1%) in *C. rotundus* essential oil

A= Tetracyclo [6.3.2.0(2,5).0(1,8)tridecan-9-ol,4,4-dimethyl
B= 2H-Cyclopropa[a]naphthalene-2-one,1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)

Essential oil of *C. rotundus* showed the highest oxygenated sesquiterpene (30.72%), followed in sesquiterpene hydrocarbons (21.73%), oxygenated monoterpene (6.81%), monoterpene hydrocarbons (1.62%). Aghassi et al. (2013) reported that more than twenty compounds were identified in the oil of *C. rotundus*, such as 4 monoterpenes, 8 sesquiterpene hydrocarbons and 10 oxygenated sesquiterpenes which represented (96.9 %) of the total composition of the oil. The sesquiterpene hydrocarbons showed the highest mean percentage (54.9 %) followed in decreasing order by oxygenated sesquiterpenes (35.6 %) and monoterpenes (6.4 %). Janaki et al. (2018) claimed that the major compounds, which account for 74.53% of the total composition were sesquiterpenes. Moreover, the volatile oil constituents of C. rotundus were distinguished quantitatively with high amounts of sesquiterpenes (70%), with a low proportion of oxygenated monoterpenes (10%) and monoterpene compounds (5%) (Das et al., 2015).

In the other hand, Lawal and Oyedeji (2009) in their study of two types of *C. rotundus* essential oil from two different locations namely Empangeni (A) and KwaDlangezwa (B) in the Kwa-Zulu Natal province South Africa, they reported that a total 58 components were detected, 41 and 43 of which were identified, accounting for 88.9% and 92.0% of the oil of Empangeni and KwaDlangezwa samples, respectively. The oil of the Empangeni sample was characterized by larger amounts of sesquiterpenes (59.8%) than monoterperenes (29.1%), while the KwaDlangezwa oil sample had a relatively similar content of sesquiterpenes (45.9%) and monoterperenes (46.1%). The sesquiterpenic composition of the oil of Empangeni is dominated by α-cyperone (11.0%), caryophyllene oxide (5.4%) and β-selinene (5.1%), and the compounds: myrtenol (7.9%), β-pinene (5.3%) and trans-pinocarveol (4.0%) were the major representative of monoterpenoids. In the oil of KwaDlangezwa, β-pinene (11.3%), α-pinene (10.8%), α-cyperone (7.9%), myrtenol (7.1%), α-selinene (6.6%), limonene (5.7%) and β-selinene (4.6%) were the main constituents.

However, cyperene and a-cyperone are the two major compounds similar in the essential oils of *C. rotundus* from Africa (Nigeria, Tunisa and South Africa). The observed compositional difference between *C. rotundus* found in South Africa and the

rest of the world could be due to climatic and environmental conditions, chemo type's nutritional states of the planets, other factors, which can influence essential oil composition. The percentages (%) of the terpenes (mono- & sesquiterpenes) of *C. rotundus* essential oil were shown in Figure 4.6.

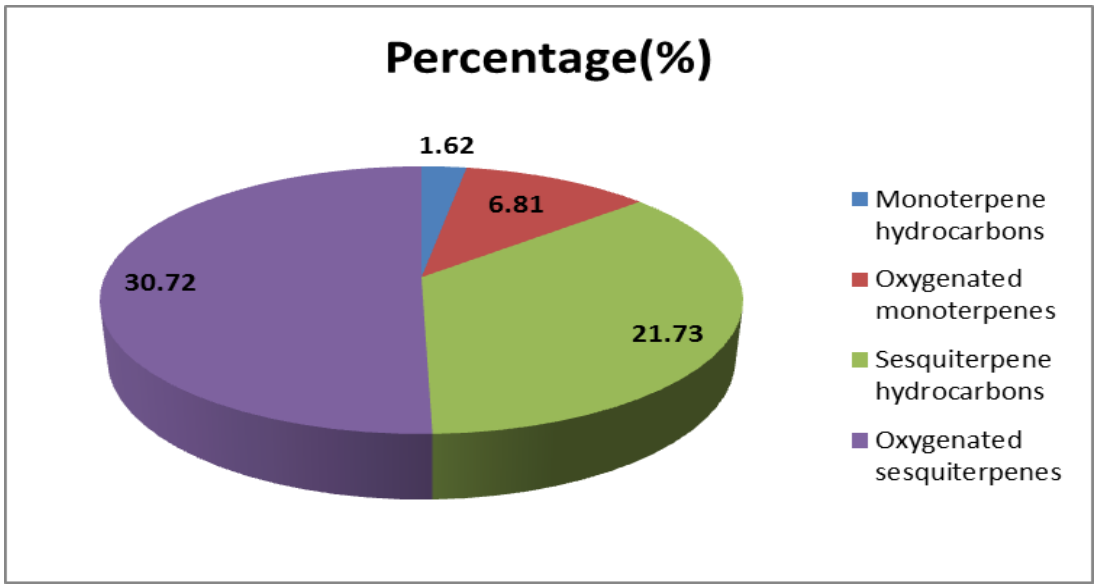

Figure 4.6: Distribution of mono and sesquiterpenes in *C. rotundus* essential oil

4.2 PHYSICOCHEMICAL CHARACTERISTICS OF SEED OILS

4.2.1 Physicochemical Properties of *A. digitata* Seed Oil

A. Physical Properties of *A. digitata* Seed Oil

The physical characters were studied according to eight different aspects as physical state, color, odor, density, freezing-, melting-, boiling points and refractive Index (RI). The methods of the analysis are explained in details accordingly in the following subtopics.

(i) Lipid Content, Physical State, Color and Odor of *A. digitata* Seed Oil

Table 4.3 shows various physicochemical properties of the *A. digitata* seed oil. In general, the results showed that *A. digitata* seed was found to be rich in oil with an average yield of 33.83% (w/w); and this value is represented in terms of lipid content, and the oil was highly unsaturated with a high FFA. The obtained oil is liquid at room temperature of 25 °C, reddish yellow in color with characteristic odor. The freezing-,

melting-, boiling points, specific gravity and viscosity were -14 °C, 8 °C, 227 °C, 0.8741 and 35.03 mm^2/s, respectively. The obtained yield is agreeable with a literature stating that this plant's seed contains 22-45% oil on dry matter basis (Abubakar et al., 2015; AlPashir and Shakak, 2016; Babiker et al., 2017; Chatepa et al., 2019; Cisse et al., 2018; Khalil and Khalil, 2016; Nkafamiya et al., 2007). Previously, reported a golden yellow color for *A. digitata* seed oil and researchers reveals that the difference in the color intensity of oil from the same plant species, but from different location might be attributed due to the presence of various pigments such as the chlorophyll content (Ayadi et al., 2009). The green color of the immature seeds disappears upon maturation resulting in chlorophyll retention. Besides, there is also a report stated that the presence of moisture contents at greater levels impacts the color of the oil, whereby the moisture rises the chlorophyll content and thus contribute in increment of color intensity (Orhevba et al., 2013). The normal and thermal oxidation process of oil can also contribute towards the deterioration of lipids, and thus it might also influence the color changes of the oil (Ayadi et al., 2009; Aleksic and Knezevic 2014).

Table 4.3: Physicochemical properties of *A. digitata* seed oil

Parameters		**Units**	**Experimental Values***
Lipid Content		%	33.83
Physical State at 25 °C		-	Liquid
Freezing point		°C	-14
Melting point		°C	8
Boiling point		°C	227
Color		-	reddish yellow
Odor		-	Characteristic
Specific gravity			0.8741
viscosity		mm^2/s	35.03
Density at 25 °C		g/cm^3	0.867
RI at 25 °C		-	1.436
AV (% FFA as oleic)		mg KOH/g	6.8
FFA	Linoleic	%	30.63
	Oleic	%	23.34
	Palmitic	%	22.87
	Stearic	%	5.89
	Malvalic	%	5.52
	cis-10-nonadecenoic	%	2.67

Sterculic	%	1.61
Arachidic	%	1.43
PV	meq O_2/kg	4.3
Moisture and volatile matter	wt %	14.79
Saponification value	mg KOH/g oil	180.7
Unsaponifiable matter	wt %	1.7
Total Saturated Fatty Acids	wt %	32.34
Total Unsaturated Fatty Acids	wt %	66.57

*Values were recorded as mean average

(ii) **Density, Viscosity and Specific Gravity of *A. digitata Seed Oil***

The density recorded for the oil of this study is 0.867 g/cm^3. Whereas, the literature had reported values ranged from 0.195 to 1.024 g/cm^3, that is, agree to the obtained result (Radha and Manikandan, 2011). The density differs as the concentration of the wall material varies at which more heavy material fits into spaces between the particles and causes an increase in mass and thus contribute towards high density (Fernandes et al., 2013).

From the Table 4.3 it can be seen that the viscosity of *A. digitata* seed oil is 35.03 mm^2/s which is consider high viscosity oil and it slightly lower than crude rubber seed oil (40.86 mm^2/s) and crude palm oil (38.1 mm^2/s). Therefore it is not advisable to use *A. digitata* seed oil directly as a fuel, because viscosity is an essential property that has to be monitor in a vegetable oil in order to meet the gasoline standard.

Generally, vegetable oil is highly viscous. Even though there are suggestions made by some authors reporting the viability of running raw vegetable oil as an alternative fuel in a compression-ignition engines with slight modification and maintenance, but this will create problems related to long-term durability test due to high viscosity and low volatility of such oils. Especially at low temperatures, viscosity increases affecting the fuel fluidity, causing a disturbance in the injection of the fuel operation equipment.

Furthermore high viscosity also promotes soot formation and deposition on the engines due to poor fuel atomization. In contrast high viscous oil has its own advantages also. They provide extra lubrication of the injector and also avoid leakage and exhaustion generated by fuel injection pumps that fits imprecisely resulted by low viscous oil (Singh et al., 2016).

(iii) Refractive Index (RI) of A. digitata Seed Oil

The RI value is acceptable according to the amount of unsaturated fatty acids and long chain hydrocarbon. The RI of the *A. digitata* seed oil is 1.436 and this is attributed by the amount of unsaturated fatty acid, length of the hydrocarbon chain, molecular weight and degree of unsaturation as well as conjugation (Ibeto et al., 2012). Previously, reported that the RI for *A. digitata* seeds oil as 1.459 (Nkafamiya et al., 2007).

B. Chemical Properties of *A. digitata* Seed Oil

Various chemical properties such as Acid Value (AV), Free Fatty Acid (FFA), Iodine Value (IV), Peroxide Value (PV), fatty acids composition and volatile matter were evaluated as following:

(i) Acid Value (AV) of A. digitata Seed Oil

In addition, the AV is the relative measure of rancidity as FFAs that are formed during decomposition or hydrolysis of oil glycerides due to the action of moisture, temperature and/or lipolytic enzyme lipase. The AV obtained in this study is 6.8 mg KOH/g and this is high when compared to the studies recorded by Erwa et al. (2019) and Nkafamiya et al. (2007).Their reported values were 0.33 and 2.5 mg KOH/g, respectively. The oxidation and hydrolysis processes are also a factor that led towards increment in AV as the percentage of unsaturated fatty acids increase (Orhevba et al., 2013).

(ii) Iodine Value (IV) of A. digitata Seed Oil

Among various factors of oil classification, the drying quality of the oil is also being considered, whereby it could be drying, semi-drying or non-drying oil through the analysis of the IV (Talkit et al., 2012). The IV for current study was 98.3 gI_2/100g; and it suggests that it is non-drying oil and it is comparable to the standard IV of less than 100 gI_2/100g in accordance with its physical state of being liquid at room temperature of 25 °C under expose air condition (Warra et al., 2011). The low IV represents the fewer amounts of unsaturated bonds and thus the oil has fewer tendencies to go through oxidative rancidity (Orhevba et al., 2013). Researchers reported IV for *A. digitata* ranged from 56 to 96 gI_2/100g which is almost closed to the result obtained in this study (Nkafamiya et al., 2007).

(iii) Peroxide Value (PV) of A. digitata Seed Oil

On the other hand, the oil had also undergone some chemical decomposition process whereby the obtained PV is 4.3 meq O_2/kg, and this value is lower than that reported by Erwa et al. (2019) which is 6.6 meq O_2/kg. The PV indicates the rancidity process whereby the higher the PV is the higher the oxidation level and the deterioration of lipids. Theoretically, oil that shows a high amount of PV is more prone to undergo rancidity that affects the total quality of the oil (Ibeto et al., 2012).

(iv) Moisture and Volatile Matter of A. digitata Seed Oil

Besides that, the moisture and volatile matter analysis prove that the oil contains a high amount of moisture and volatile matter, whereby the value recorded is 14.79 wt%. The presence of water or moisture contributes towards hydrolysis in breaking up of triglycerides into glycerol and FFA. This process might be accelerated due to the presence of the action of lipase enzyme. Therefore, both oxidation and hydrolysis reduce the amount of unsaturated FFA and thus contributing towards the reducing of IV and average molecular weight and increasing in the AV (Orhevba et al., 2013).

(v) Saponification value and Unsaponifiable Matter of A. digitata Seed Oil

The saponification value (SV) using to know the amount of free fatty acids present in the oil, and amount of free fatty acids will estimate by determining the quantity of alkali that must be added to the fat to render it neutral. The SV for studied oil was 180.7 mg KOH/g of oil and it is agreeable to that reported in literature, which is 133 to 200 mg KOH/g of oil (Nkafamiya et al., 2007; Zahra'u et al., 2014).

Unsaponifiable matter consists of constituents such as sterols, higher molecular weight alcohols, pigments, waxes, and hydrocarbon which do not react with bases during formation of soap. The value for unsaponifiable matter for the present seed oil was 1.7 wt% and it is closed to previously reported values of 2-3.8 wt% (Zahra'u et al., 2014). From the current results, it could be said that the oil had undergone some oxidation and hydrolysis process as indicated by the value of unsaturated fatty acids. This oxidation process might be influenced by storage of the oil, whereby the presence of air in the bottle is in contact with the oil surface.

Thus, the oxidation process converts the triglycerides into peroxides and hydroperoxides. Moreover, researchers reported that the low value of unsaponifiable matter (< 2 wt %), for *A. digitata* seed oil could be suitable in the application of biodiesel production (Tamboli et al., 2013).

(vi) Free Fatty Acids (FFA), Fatty Acid Composition, Percentage of Saturated and Unsaturated Fatty Acid of A. digitata Seed Oil

Figure 4.7 shows the GC chromatogram and Table 4.4 shows the fatty acid composition of the *A. digitata* seed oil. Generally, fatty acid is a compound that contains carboxylic acids with long hydrocarbon chains, is a main constituent of seed oil and known to be a major parameter that differentiates the physicochemical properties of the seed oils. MS Library of the *A. digitata* seed oil shown in Appendix C.

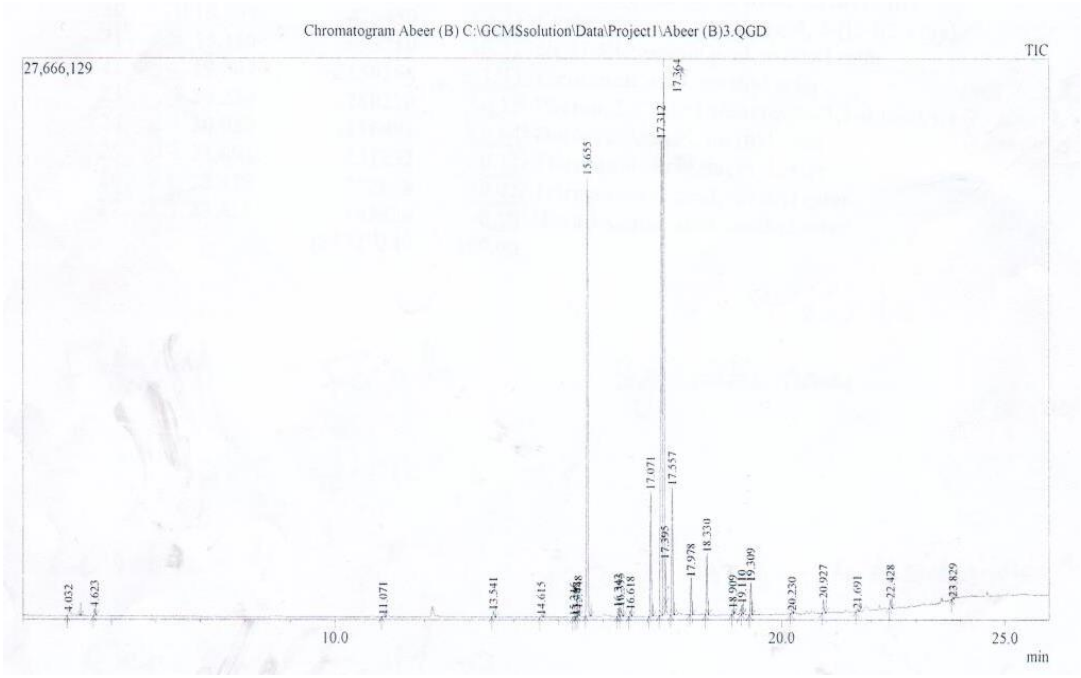

Figure 4.7: GC chromatogram of the *A. digitata* seed oil

In this study, an amount of twenty three different fatty acids were detected and includes both saturated and unsaturated. The sequence arrangement according to the increasing percentage (>1%) of fatty acid is linoleic- (30.63%), oleic- (23.34%), palmitic- (22.87%), stearic- (5.89%), malvalic- (5.52%), cis-10-nonadecenoic- (2.67%), sterculic- (1.61%), arachidic acid (1.43%). Figure 4.8 shows the structures of some acids (>1%) in *A. digitata* seed oil.

Table 4.4: Fatty acid composition of *A. digitata* seed oil

Fatty Acid*	Formula	Systematic name	Structure	Composition (%)
Saturated				
Palmitic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid	C16:0	22.87
Stearic acid	$C_{16}H_{36}O_2$	Octadecanoic acid	C18:0	5.89
Arachidic acid	$C_{20}H_{40}O_2$	Eicosanoic acid	C20:0	1.43
Myristic	$C_{14}H_{28}O_2$	Methyl tetradecanoate	C14:0	0.28
Behenic	$C_{22}H_{44}O_2$	Docosanoic acid	C22:0	0.64
Lignoceric	$C_{24}H_{48}O_2$	Tetracosanoic acid	C24:0	0.42
Margaric	$C_{17}H_{34}O_2$	Heptadecanoic acid	C17:0	0.28

Tricosylic	$C_{23}H_{46}O_2$	Tricosanoic acid	C23:0	0.12
Cyclopropaneoctanoic	$C_{21}H_{36}O_2$	Cyclopropane octanoic,	C21:0	0.23
Cerotic	$C_{26}H_{52}O_2$	Hexacosanoic acid	C26:0	0.10
Pentadecylic	$C_{15}H_{30}O_2$	Pentadecanoic acid	C15:0	0.08
Unsaturated				
Linoleic acid	$C_{18}H_{32}O_2$	9,12-octadecadienoic acid	C18:2	30.63
Oleic acid	$C_{16}H_{34}O_2$	9-octadecenoic acid	C18:1	23.34
Malvalic	$C_{18}H_{32}O_2$	Methyl2-propene-1-butyl-cyclo heptanoic acid	C18:1	5.52
Cis10- nonadecenoic	$C_{19}H_{36}O_2$	Cis-10-Nonadecenoic acid	C19:1	2.67
Sterculic acid	$C_{19}H_{34}O_2$	Methyl2-butyl cyclopropane-1-octanoic acid	C19:1	1.61
Elaidic	$C_{18}H_{34}O_2$	9-Octadecenoic acid(E)	C18:1	0.94
Cis-10-Heptadecenoic	$C_{17}H_{32}O_2$	Cis-10-Heptadecenoic acid	C17:1	0.48
8,11-Octadecadienoic	$C_{18}H_{32}O_2$	8,11-Octadecadienoic acid	C18:2	0.38
Gondoic	$C_{20}H_{38}O_2$	Cis-11-Eicosenoic acid	C20:1	0.37
Palmitoleic	$C_{16}H_{30}O_2$	9-Hexadecenoic acid	C16:1	0.36
Terephthalic	$C_9H_8O_4$	1,4-Benzenedicarboxylic acid	C9:3	0.10
7,10-Hexadecadienoic	$C_{16}H_{28}O_2$	7,10-Hexadecadienoic acid	C16:2	0.02

*The obtained results in terms of fatty acid methyl esters from GC-MS library data system was reviewed and the final results were listed out in the form of fatty acid chains.

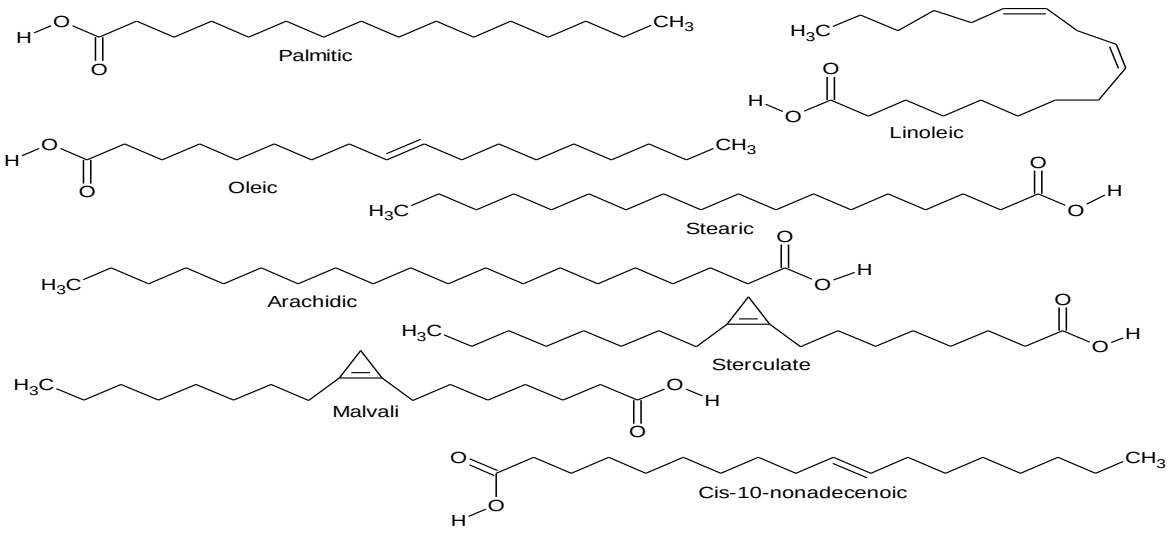

Figure 4.8: Structures of some acids (>1%) in *A. digitata* seed oil

The total percentage of fatty acid chains were 98.76 wt%. All the values are represented as the relative percentage area from the sum of all identified peaks. Figure 4.9 shows different types of fatty acids present in *A. digitata* seed oil. The overall results of this analysis show that the unsaturated fatty acid makes 66.42% of the

compositions, whereby the monounsaturated fatty acids (MUFA) are 35.29 wt% and polyunsaturated fatty acids (PUFA) are 31.13 wt%; and the saturated fatty acids (SFA) were recorded to be the balance at the level of 32.34 wt%. The fatty acid balance of *A. digitata* seeds was 1.04:1.13:1.00 for saturated fatty acid (SFA): MUFA: PUFA which is close to the fat dietary guideline of the current National Cholesterol Education Program (NCEP) and American Heart Association (AHA) compared with other dietary vegetable oils such as palm, soybean oil, sesame, olive, and coconut oils (Janporn et al., 2015).

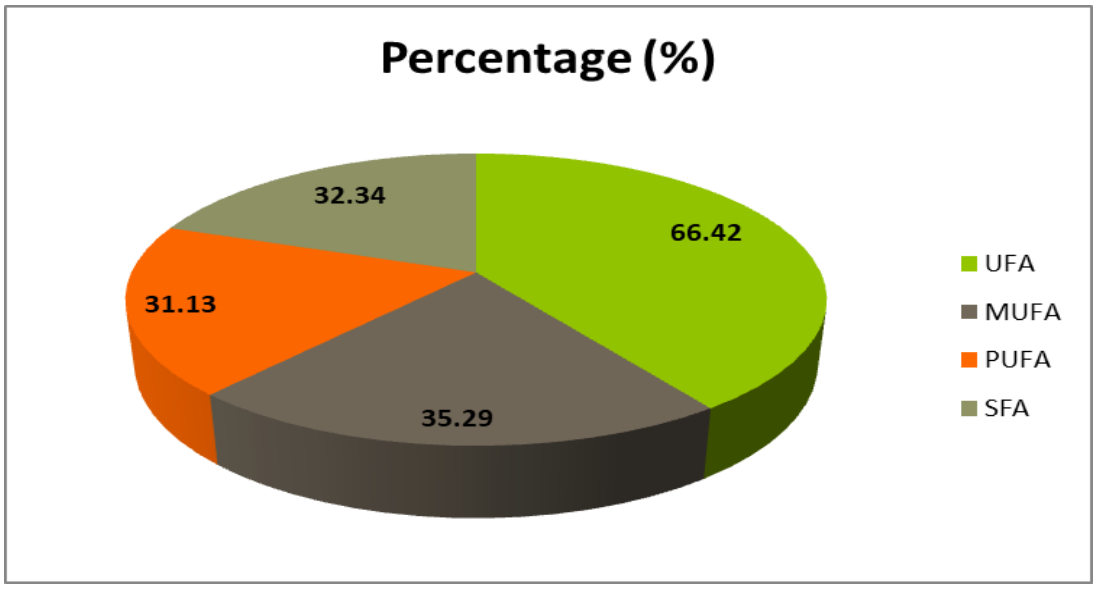

Figure 4.9: Types of fatty acids present in *A. digitata* seed oil

The preponderance chain detected in the oil was the polyunsaturated linoleic acid with the weight percentage of 30.63%. Several studies have reported oleic acid (35.8%) as the dominance of *A. digitata* seed oil followed by linoleic acid (30.7%) and palmitic acid (24.2%). It was reported that *A. digitata* seed oil contained 17-22% saturated fatty acids (SFA), 32-38% monounsaturated fatty acids (MUFA) and 22-26% polyunsaturated fatty acids (PUFA). Palmitic acid (C16:0) was the most abundant SFA, while oleic (C18:1) and linoleic acid (C18:2) were the dominant MUFA and PUFA, respectively (Muthai et al., 2019). However, the obtained results were

supported by other studies, that the major content was linoleic and followed by oleic acid. Komane et al. (2017) reported that the major fatty acids were linoleic- (36.0%), oleic- (25.1%) and palmitic acid (28.8%).

Apraku et al. (2019) reported that the high content of the essential PUFA is noted to meet the requirements in human nutrition. Fatty acids that are needed for a better growth and nutrition and cannot be biosynthesize by the body and are necessary to be incorporated in diets are the essential FAs. The essential FAs are necessary for the skin regeneration, boosting the immune system and membrane cells functioning. It helps in the synthesis of eicosanoids which aids in renal, cardiovascular, reproductive process and also gastrointestinal functions and disease resistance.

Overall, an author had stated that the various fatty acid composition of a same plant from different areas is varied due to its genetic make-up. The fatty acid profile could significantly change due to the storage and climatic conditions whether it could increase with period of storage, air, heat, traces of metal, peroxides, light, or double bonds present in the oil and thus leads towards the deterioration of the quality. *A. digitata* seed oil has reported to be one of the most suitable feedstocks for biodiesel production, according to the fatty acid methyl ester profile that becomes one of the key factors (Ali et al., 2013).

Therefore, most of the obtained results in this study were acceptable and similar to previous studies. In terms of the overall quality of oil, it is said that the quality decreases as the storage lifetime is longer and the factors that influences are the decrease in IV and RI; and also increase in acid number. As comparison with the past studies, there might be a slight difference in the physicochemical properties as few factors might have influenced such as the geographical origin and environmental condition of the plant, climate cultivation, soil composition, time of fruit harvesting and maturity and the drying process (Goja, 2013).

In summary, in this study, the physicochemical properties and fatty acid composition of the of Sudanese baobab (*A. digitata*) seed oil were assessed by standard and established methods. Based on the results of the study, the oil properties are interesting and promising for several applications. The overall results of this analysis show that the oil content was 33.83%; major fatty acid compositions were linoleic acid (30.63%) and followed by oleic acid (23.34%), palmitic acid (22.87%), and stearic acid (5.89%). The unsaturated fatty acid makes 66.42% of the compositions, whereby the MUFA, PUFA and SFA were 35.29, 31.13 and 32.34 wt%. Most of the obtained results in this study were acceptable and similar to previous studies. The worldwide demand for baobab has increased dramatically as raw material for many industrial products. Thus, the Sudanese baobab seed oil which does not contain of linolenic acid could not be suitable for several applications such as paint, varnish and ink industries, but it might suitable for others industrial aspects such as pharmaceutical, cosmetic and food industries, due to its fatty acid content. Therefore, further studies on Sudanese baobabs are needed to investigate their potential as raw materials for new industrial products and applications to increase the economic feasibility of future commercial cultivation of the tree.

4.2.2 Physicochemical Properties of *O. basilicum* Seed Oil

A. Physical Properties of *O. basilicum* Seed Oil

(i) *Lipid Content, Physical State, Color and Odor of O. basilicum Seed Oil*

Table 4.5 represents various physicochemical properties of the *O. basilicum* seed oil. The yield of the obtained oil was 18.01% (w/w) and this value was represented in terms of lipid content, and the oil was highly unsaturated (77.29%). The oil was liquid at room temperature of 25 °C, pale yellow with characteristic odor. The freezing-, melting- boiling and density points were -2 °C, 5 °C, 215 °C and 0.913 g/cm^3, respectively. Kadam et al. (2012) reported that the seed oil of *O. basilicum* is yellowish with characteristic odor. The oil yield of *O. basilicum* in this study was within the range reported by Kakaraparthi et al. (2015) (12.4-21.6%) and Nour et al. (2009) (8.82-30.01%), less than that reported by Angers et al. (1996) (24%),

Ghaleshahi et al. (2019) (22.04%) and Amini et al. (2017) (22%) and slightly higher than amounts reported for Mostafavai et al. (2019) (3.1-17.78%).

Table 4.5: Physicochemical properties of *O. basilicum* seed oil

Parameters		**Units**	**Experimental values***
Lipid Content		%	18.01
Physical State at 25 °C		-	Liquid
Color		-	Pale yellow
Odor		-	Characteristic
Freezing pint		^{0}C	-2
Melting point		^{0}C	5
Boiling point		^{0}C	215
Density at 25 °C		g/cm^3	0.913
Viscosity		mm^2/s	10.29
Specific gravity			0.921
Refractive index at 25 °C			1.485
Acid value (% FFA as oleic)		mg KOH/g	4.0
FFA**	Linolenic	%	43.95
	Linoleic	%	32.18
	Palmitic	%	13.38
	Stearic	%	6.55
Iodine value		g I_2/100g	108.6
Peroxide value		meq O_2/kg	4.6
Moisture and volatile matter		wt %	4.97
Saponification value		mg KOH/g	164.2
Unsaponifiable matter		wt %	1.6
Total Saturated Fatty Acids		wt %	22.28
Total Unsaturated Fatty Acids		wt %	77.29

**Values were recorded as mean average, ** FFA >1%*

(ii) ***Density, Viscosity and Specific Gravity of O. basilicum Seed Oil***

The viscosity of the *O. basilicum* seed oil was 10.29. The viscosity defined as resistance liquid to flow. Viscosity increased with molecular weight, but decreased with increasing unsaturated level and temperature. Viscosity of oil depends upon the density; when density of oil increase, there are chances that its viscosity would be

increase. Previously, Kadam et al. (2012) found the viscosity of the *O. basilicum* seed oil is 11.51.

Specific gravity (SG) of the *O. basilicum* seed oil was 0.921. The SG is considered as a good index of purity of oils. The increase in chain length of fatty acid present in oil tends to increase the specific gravity of oils. The specific gravity of oil samples increases during frying and this may be interpreted due to the generation of dipoles on heating of oils, which interact with each other and increase the specific gravity of oils. In previous study, the SG of *O. basilicum* seed oil found to be 0.9525 (Kadam et al., 2012).

(iii) ***Refractive Index (RI) of O. basilicum Seed Oil***

The refractive index (RI) of the *O. basilicum* seed oil was 1.485. The RI varies with temperature and wavelength. RI of oils increases with the increase in unsaturation and also chain length of fatty acids. Researchers reported the RI for *O. basilicum* seed oil in range of 1.460-1.484 (Kadam et al., 2012).

B. Chemical Properties of *O. basilicum* Seed Oil

(i) ***Acid Value (AV) of O. basilicum Seed Oil***

The acid value (AV) is the relative measure of rancidity as FFAs that are formed during decomposition or hydrolysis of oil glycerides because action of moisture, temperature and/or lipolytic enzyme lipase. Thus, AV is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acids. The AV obtained in this study was 4.0 mg KOH/g and this amount was high when compared to the previously reported 0.9525 mg KOH/g (Kadam et al., 2012). The percentage of unsaturated fatty acids is increasing by some factors, like hydrolysis and oxidation, which leads to rise of AV (Orhevba et al., 2013).

(ii) ***Iodine Value (IV) of O. basilicum Seed Oil***

The drying quality of the oil can be considered as one of factors of oil classification; it could be non-drying, semi-drying or drying oil through the analysis of the IV (Talkit et al., 2012). IV represents true unsaturation of fats only when double bonds are unconjugated and addition of iodine is not interfered by other groups. The higher iodine value, the more unsaturated fatty acid bonds are present in a fat/oil. It is a measure, which indicates the potential of a fat to be oxidized. Previously, researchers reported the IV for some oils as groundnut oil (84 - 99), olive (79 - 90) and castor oil (81 - 91). The oil of the current study in the range, but is higher than some edible oils as reported. The IV for current study was 108.6 g/100 g; and it suggests that it is semi-drying oil and it is comparable to the standard IV of more than 100 gI_2/100 g in accordance with its physical state of being liquid at room temperature of 25°C under expose air condition (Warra et al., 2011). The high IV represents the more amounts of unsaturated bonds and thus the oil has higher tendencies to go through oxidative rancidity (Orhevba et al., 2013). Researchers reported that the IV for *O. basilicum* ranged from 172 to 200 gI_2/100 g (Kadam et al., 2012).

(iii) ***Peroxide Value (PV) of O. basilicum Seed Oil***

The peroxide value (PV) indicates the rancidity process, whereby the higher the PV, the higher is the oxidation level and the deterioration of lipids (Mohammed and Hamza, 2008). Oil that shows a high amount of PV is considered more prone to undergo rancidity, which affects the total quality of the oil (Ibeto et al., 2012). In this study, the obtained PV for *O. basilicum* seed oil is 4.6 mEq/kg and was determined immediately after the extraction of the oil. A low peroxide value increases the suitability of the oil for a long storage due to low level of oxidative and lipolytic activities (Ibeto et al., 2012). In previous study, the PV was found to be 4.5 mEq/kg, which is similar to that in this study.

(iv) ***Moisture and Volatile Matter of O. basilicum Seed Oil***

The moisture and volatile matter recorded for *O. basilicum* seed oil is 4.97%wt%. The presence of water or moisture contributes towards hydrolysis in breaking up of triglycerides into glycerol and FFAs. Orhevba et al. (2013) documented that, both oxidation and hydrolysis reduce the amount of unsaturated FFA and thus contributing towards the reducing of IV and increasing in the AV. In this study, the observed moisture content in *O. basilicum* seed oil was 4.97%, thus, the low moisture content of the oil serves as an indication that, the activities of the micro-organisms would be reduced and thereby increases the shelf life of the oil.

(v) ***Saponification Value and Unsaponifiable Matter of O. basilicum Seed Oil***

The numbers of milligrams of potassium hydroxide require converting one gram of the fat into soap and glycerin known as saponification value (SV). SV gives information concerning the character of the fatty acids of the fat (Orhevba et al., 2013). Moreover, the saponification value (SV) used to know the amount of free fatty acids present in the oil. Determination of the quantity of alkali that must be added to the fat to render it neutral will estimate the amount of free fatty acids (Kadam et al., 2012). Higher SV indicates high proportion of lower fatty acids, since SV is inversely proportional to the average molecular weight or chain length of the fatty acids. The SV for the studied oil was 164.2 mg KOH/g of oil. Previously, Kadam et al. (2012) reported that the SV for *O. basilicum* seed oil as 194.94 mg KOH/g of oil.

Unsaponifiable matter is that fraction of oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents. Unsaponifiable matters such as hydrocarbon, pigments, waxes, higher molecular weight alcohols, and sterols do not react with bases during formation of soap. The unsaponifiable matter value for *O. basilicum* seed oil was 1.6 wt%. Previously, unsaponifiable matter for *O. basilicum* seed oil which is determined by titration with sodium hydroxide solution in alcoholic, found to be 36.6, which is too high (Kadam et al., 2012). The current studied oil showed lower unsaponifiable matter than reported value. Therefore, due to the small

value of unsaponifiable matter (< 2 wt %), *O. basilicum* could be suitable in the application of biodiesel production (Tamboli et al., 2013).

(vi) ***Free Fatty Acids (FFA), Fatty Acid Composition, Percentage of Saturated and Unsaturated Fatty Acid of O. basilicum Seed Oil***

Figure 4.10 shows GC chromatogram and Table 4.6 shows the fatty acids composition of the *O. basilicum* seed oil. In general, fatty acids are main constituents of seed oils and known to be a major parameter that differentiates the physicochemical properties of the seed oils. The MS Library of the *O. basilicum* seed oil shown in Appendix D.

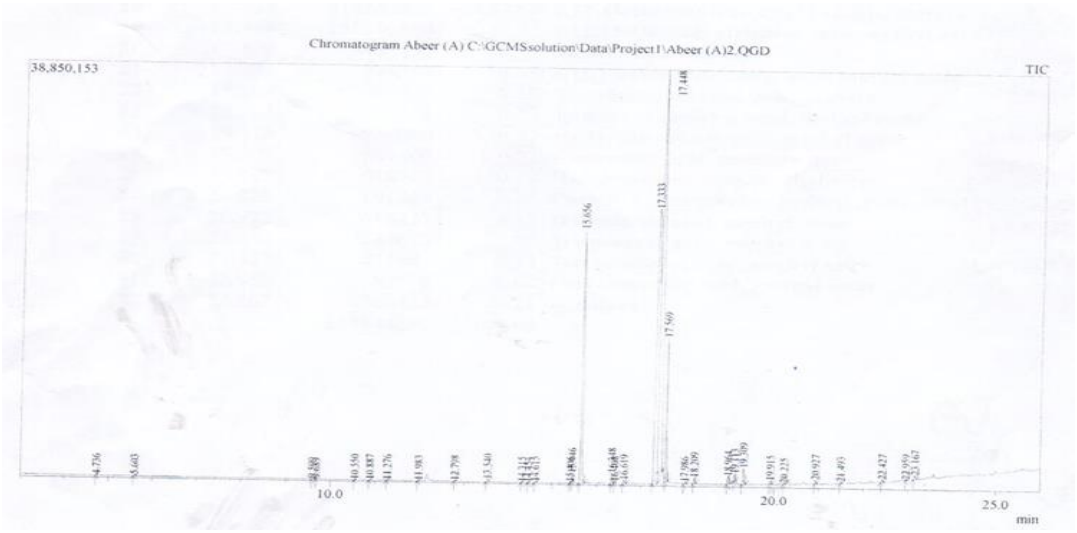

Figure 4.10: GC-MS chromatogram of the *O. basilicum* seed oil

Table 4.6: Fatty acids composition of *O. basilicum* seed oil

Fatty acid	Formula	Systematic name	Structure	Composition (%)
Saturated				
Palmitic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid	C16:0	13.38
Stearic	$C_{18}H_{36}O_2$	Octadecanoic acid	C18:0	6.55
Arachidic	$C_{20}H_{40}O_2$	Eicosanoic acid	C20:0	0.72
Anteisomargaric	$C_{17}H_{34}O_2$	Hexadecanoic acid,	C17:0	0.45
Nonadecylic	$C_{19}H_{38}O_2$	Nonadecanoic acid	C19:0	0.28
Margaric	$C_{17}H_{34}O_2$	Heptadecanoic acid	C17:0	0.20
Behenic	$C_{22}H_{44}O_2$	Docosanoic acid	C22:0	0.17
Heneicosylic	$C_{21}H_{42}O_2$	Heneicosanoic acid	C21:0	0.14
Lignoceric	$C_{24}H_{48}O_2$	Tetracosanoic acid	C24:0	0.13
Myristic	$C_{14}H_{28}O_2$	Tetradecanoic acid	C14:0	0.11
Tricosylic	$C_{23}H_{46}O_2$	Tricosanoic acid	C23:0	0.07
Pentadecylic	$C_{15}H_{30}O_2$	Pentadecanoic acid	C15:0	0.04
Tridecylic acid	$C_{13}H_{26}O_2$	Tridecanoic acid	C13:0	0.02
Pentacosylic	$C_{25}H_{50}O_2$	Pentacosanoic acid	C25:0	0.02
Unsaturated				
Linolenic acid	$C_{18}H_{30}O_2$	9,12,15-octadecatrienoic acid(Z,Z,Z)	C18:3	43.92
Linoleic acid	$C_{18}H_{32}O_2$	9,12-octadecadienoic acid	C18:2	32.18
Palmitoleic	$C_{16}H_{30}O_2$	9-Hexadecenoic acid	C16:1	0.78
Gondoic	$C_{20}H_{38}O_2$	Cis-11-Eicosenoic acid	C20:1	0.27
Docosatrienoic	$C_{22}H_{38}O_2$	8,11,14-Docosatrienoic acid	C22:3	0.08
Cinnamic	$C_9H_8O_2$	2-Propenoic acid, 3-phenyl	C9:5	0.05
5-Octadecenoic	$C_{18}H_{34}O_2$	5-Octadecenoic acid	C18:1	0.01

In this study, twenty two different fatty acids were identified and they include both saturated and unsaturated acids. The dominant fatty acid was linolenic acid (43.92%), followed by linoleic (32.18%), palmitic (13.38%) and stearic (6.55%). The structures of acids (>1%) in *O. basilicum* seed oil are shown in Figure 4.11. The total percentage of fatty acids chains were 99.57 wt%. All the values are represented as the relative percentage area from the sum of all identified peaks. The overall results of this analysis showed that the unsaturated fatty acid (UFA) makes 77.29 wt% of the compositions, whereby the monounsaturated fatty acids (MUFA) are 1.06 wt%, polyunsaturated fatty acids (PUFA) are 76.23 wt%; and the saturated fatty acids (SFA) were 22.28 wt% as shown in Figure 4.12. It is reported that, *O. basilicum* seed oil

contained up to 91.6% UFA; the major constituents were alpha-linolenic (57.4 to 62.5%), linoleic (18.3 to 21.7%), and oleic (8.7 to 11.6%) (Angers et al., 1996). Moreover, Mostafavai et al. (2019) claimed that α-linolenic- (69%), followed by palmitic- (16.2%) and linoleic acid (9.7%) were the major fatty acids for *O. basilicum* seed oil grown in Iran.

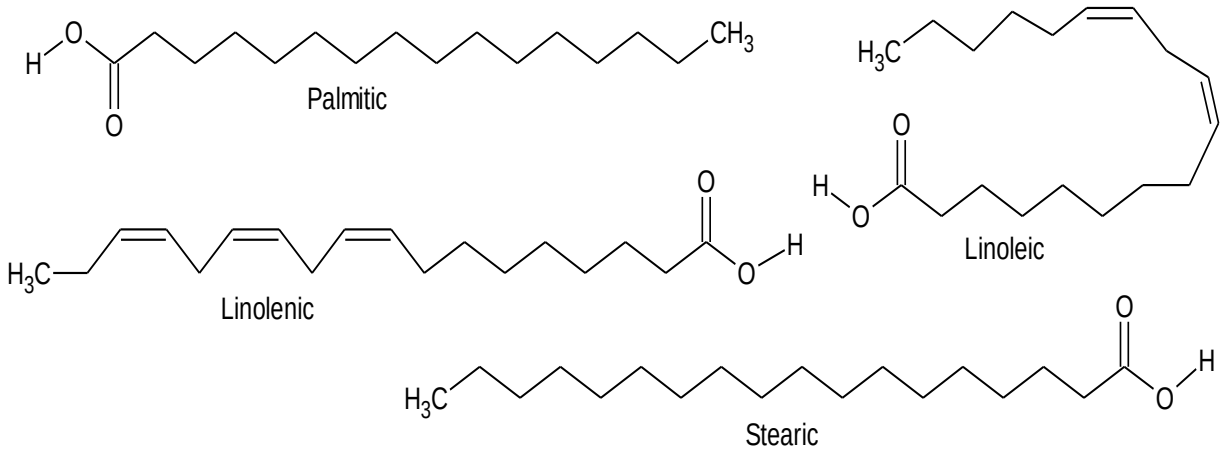

Figure 4.11: Structures of some acids (>1%) in *O. basilicum* seed oil

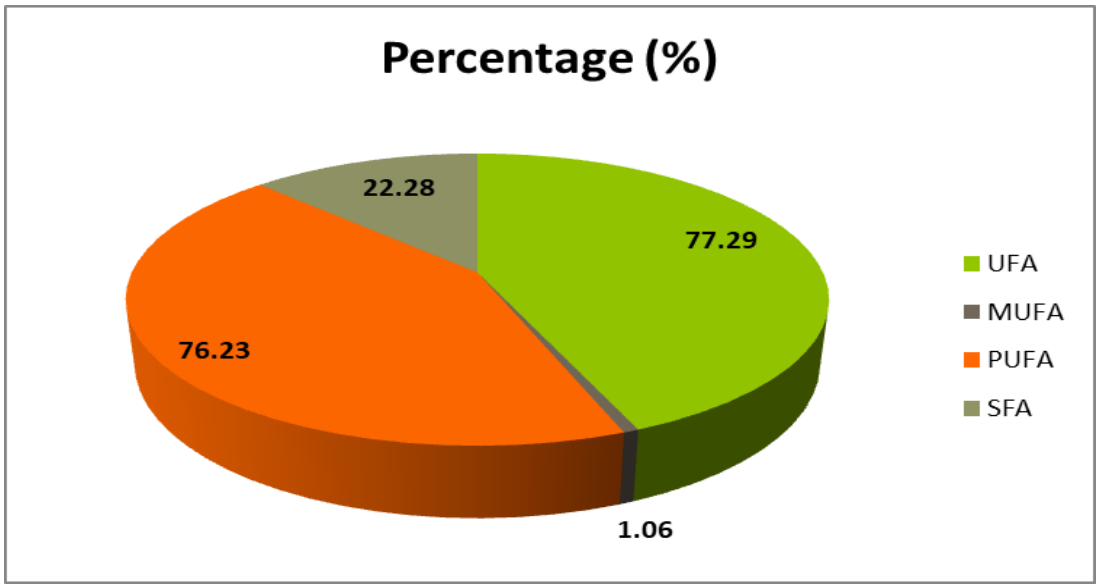

Figure 4.12: Types of fatty acids present in *O. basilicum* seed oil

In addition, in this study the dominant SFA were palmitic (13.38%) and stearic (6.55%). Similar results reported previously, the most abundant SFA were palmitic (6.1% to 11.0%) and stearic (2.0% to 4.0%). *O. basilicum* seeds from Pakistan contain lauric- (0.85%), myristic- (0.36%), palmitic- (9.70%), stearic- (5.45%), oleic- (13.33%), linoleic- (32.18%) and linolenic acid (48.50%) (Mostafavai et al., 2019). On the other hand, in our previous study in 2005, we found that the major fatty acids were palmitic- (5-13%), stearic- (2-3%), oleic- (6-10%), linoleic- (12-32%) and linolenic acid (44-75%) for seed oils of *O. basilicum* grown in Sudan. Linolenic acid which is a desirable for industrial uses as a drying oil is high as (75%) in seeds of the Sudanese wild-type basil (Nour et al., 2009). A high linolenic acid oil, such as that found in *O. basilicum* and *O. canum*, could be used in the paint, varnish and ink industries, and as a source of linolenic acid, while oils with lower linolenic acid content, such as those of *O. gratissimum* and *O. sanctum*, might be used by the food industry. The differences in the results obtaining by the researchers can be attribute to many factors, like environmental conditions, geographical origin, soil,

cultivation climate, harvesting time, maturity and the drying process (Jessinta et al., 2014; Goja, 2013).

Our results agreed with many previously reported results. However, there are some differences in some physicochemical properties and fatty acids composition, e.g. Mostafavai et al. (2019) investigated 18 basil (*O. basilicum*) populations and reported that the populations were significantly different in terms of saturated FA ranging from 10.73% to 13.51%, but unsaturated FA (expect linoleic acid) were not significantly different from each other (average=87.27%).The differences may be because of some factors, such as environmental conditions and extraction methods. Seed oils vary to a great or significant extent in fatty acid composition across different taxonomic levels. Many studies have reported strong phylogenetic patterns in the fatty acid profiles of the seed oils. The relative abundance of common fatty acids in seed oils is more strongly explained by taxonomic affiliation than by climate.

The importance of genetics over plastic response to environment as determinants of fatty acid composition (Zhang et al., 2015). Moreover, Mostafavai et al. (2019) claimed that the composition and quantity of fatty acids (phytovariability) in the *O. basilicum* can be affect by certain parameters such as environmental and the climate; but the most important parameter was plant genotype. The large variation in fatty acid composition indicates a large potential to select ideal plants for specific health, nutritional and industrial usages (Zhang et al., 2015). In general, oils rich in certain acids like linoleic, linolenic, lauric, myristic and stearic acids are used in many applications such as treatment of acne and skin permeation enhancement effects (Vermaak et al., 2011). Therefore, according to literature and results obtained in this study, we suggest that the current studied *O. basilicum* seed oil would be suitable for industrial purposes such as cosmetic uses.

In summary, this study provides an insight on the physicochemical characteristics and fatty acids composition of *O. basilicum* seed oil. This seed oil demonstrates promising properties that could be a potential source for unlimited applications. The results of fatty acid composition analysis revealed that linolenic acid (43.92%) and linoleic acid (32.18%) are present in significant quantities. This oil could be a good natural source for linolenic and linoleic acids. Linolenic acid is desirable for industrial uses as a drying oil could be used in the paint, varnish, ink and cosmetic industries. Therefore, this study may suggest that the *O. basilicum* seed oil may be an alternative good source for several industrial applications, especially for applications that require acids such as linolenic and linoleic.

4.2.3 Properties of *M. oleifera* Seed Oils

A. Physical Properties of *M. oleifera* Seed Oils

(i) *Lipid Content, Physical State, Color and Odor of M. oleifera Seed Oil*

The physiochemical properties of *M. oleifera* seed oil were determined and the obtained results shown in Table 4.7. Generally, the results showed that *M. oleifera* seed was found to be rich in oil with an average yield of 42.87% (*w*/*w*); and this value is represented in terms of lipid content, and the oil was highly unsaturated with a high FFA. The obtained oil is liquid at room temperature of 25 °C, golden yellow in color with nutty flavor odor. The freezing-, melting-, and boiling points were 0 °C, 21 °C, and 225 °C, respectively. The obtained yield is agreeable with a literature stating that this plant seed contains 26.2-40.90% oil on dry matter basis (Adegbe et al., 2016; Adejumo et al., 2013; Akleshwar, 2014; Chatepa et al., 2019; Díaz-Dominguez et al., 2017; Janaki, 2015; Leone et al., 2016; Ogbunugafor et al., 2011; Ogunsina et al., 2014; Orhevba et al., 2013; Pereira et al., 2016). Moreover, our results were closed to result obtained by Ogbunugafor et al. (2011) their result 41.47%, although it is slightly higher than the result obtained by Adegbe et al.

(2016) where their result was 38.00%. However, it was higher than result obtained by Pereira et al. (2016) their result 35.48%.

Again, Ogbunugafor et al. (2011) reported that the melting point for *M. oleifera* seeds oil was 28±0.00 ^{o}C which is higher than our obtained results. Previously, reported a yellow color for *M. oleifera* seeds oil; researchers reveals that the difference in the color intensity of oil from the same plant species, but from different location might be attributed due the presence of various pigments such as the chlorophyll content (Ayadi et al., 2009). The green color of the immature seeds disappears upon maturation resulting in chlorophyll retention. Besides, there is also a report stated that the presence of moisture contents at greater levels impacts the color of the oil whereby the moisture rises the chlorophyll content and thus contribute in increment of color intensity (Orhevba et al., 2013). The normal and thermal oxidation process of oil can also contribute towards the deterioration of lipids, and thus it might also influence the color changes of the oil (Aleksic and Knezevic, 2014; Ayadi et al., 2009).

Table 4.7: Physicochemical Properties of *M. oleifera* Seed Oil

Components	Units	Experimental Values*
Yield	%	42.87
Color	-	Golden yellow
Odor	-	Nutty smell
Freezing point	°C	0
Melting point	°C	21
Boiling point	°C	225
Density point at 25 °C	g/cm^3	0.90052
Viscosity	mm^2/s	60.99
Specific gravity		0.9070
Refractive index at 25 °C	-	1.44732
Acid value (% FFA as oleic)	mg KOH/g of oil	1.4
FFA		0.07
Oleic	%	50.74

Behenic	%	10.54
Palmitic	%	9.20
Stearic	%	8.46
Arachidic	%	6.41
Gondic	%	4.88
Iodine value		96.6
Peroxide value	meq.O_2/kg of oil	7.6
Unsaponifiable matter	wt %	3.2
Saponification value	mg KOH/g of oil	185.2
Moisture and volatile matter	wt %	4.91

(ii) Density, Viscosity and Specific Gravity of *M. oleifera* Seed Oil

The density recorded for the oil of this study is 0.90052 g/cm^3. Whereas, the literature had reported a value g/cm^3 that is agree to the obtained result. The density differs as the concentration of the wall material varies at which more heavy material fits into spaces between the particles and causes an increase in mass and thus contribute towards high density (Fernandes et al. 2013). Previously, reported, (Adejumo et al. (2013); Anwar et al. (2005); Leone et al. (2016)) found the density of *M. oleifera* seed oil was range 0.90-0.99.

The viscosity and specific gravity of the *M. oleifera* seed oil were 60.99 and 0.907, respectively. The viscosity defined as resistance liquid to flow. Viscosity increased with molecular weight, but decreased with increasing unsaturated level and temperature. Viscosity of oil depends upon the density; when density of oil increase, there are chances that its viscosity would be increase. Previously, found the viscosity of the *M. oleifera seed* oil was range 41.7-84.4.43 (Adejumo et al. 2013; Janaki, 2015; Lalas and Tsaknis, 2002; Leone et al. 2016; Pereira et al. 2018; Pereira et al. 2016). Adegbe et al. (2016) reported that the specific gravity was 0.9050, where their result was closed to our obtained results.

(iii) Refractive Index (RI) of *M. oleifera* Seed Oil

The RI value is acceptable according to the amount of unsaturated fatty acids and long chain hydrocarbon. The RI of the oil is 1.447 and this is attributed by the amount of unsaturated fatty acid, length of the hydrocarbon chain, molecular weight and degree of unsaturation as well as conjugation (Ibeto et al. 2012). Previously, Abiodun et al. (2012); Adegbe et al. (2016); Anwar et al. (2005); Barakat & Ghazal, (2016); Chatepa et al. (2019); Lalas & Tsaknis, (2002); Leone et al. (2016); Ogbunugafor et al. (2011) reported the RI for *M. oleifera* seed oil in range of 0.60-1.47 which is closed to our result.

B. Chemical Properties of *M. oleifera* Seed Oil

(i) Acid Value (AV) of *M. oleifera* Seed Oil

The acid value (AV) is the relative measure of rancidity as FFAs that are formed during decomposition or hydrolysis of oil glycerides due to the action of moisture, temperature and/or lipolytic enzyme lipase. Thus, AV is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acid. The AV obtained in this study is 1.4 mg KOH/g. Adegbe et al. (2016) reported that the AV was 6.73 mgKOH/g; our obtained results for AV was found lower than their results. Moreover, although our result was within many of the previously reported results range 0.29-9.46 mg KOH/g (Adejumo et al., 2013; Barakat and Ghazal, 2016; Chatepa et al., 2019; Leone et al., 2016; Ogbunugafor et al., 2011). The oxidation and hydrolysis processes are also a factor that led towards increment in AV as the percentage of unsaturated fatty acids increase (Orhevba et al., 2013).

(ii) Iodine Value (IV) of *M. oleifera* Seed Oil

The drying quality of the oil can be consideration as one of factors of oil classification; it could be non-drying, semi-drying or drying oil through the

analysis of the IV (Talkit et al., 2012). The IV for the current study was 96.6 g/100g; and it suggests that it is non-drying oil and it is comparable to the standard IV of more than 100 gI_2/100g (Warra et al., 2011) in accordance with its physical state of being liquid at room temperature of 25 °C under expose air condition. The high IV represents the more amounts of unsaturated bonds and thus the oil has higher tendencies to go through oxidative rancidity (Orhevba et al., 2013). Babatunde et al. (2014) reported that the iodine values for cold pressed and hexane extracted moringa seed oils (CPMSO and HEMSO) were found to be 67.8 and 68.5 gI_2 / 100 g oil. Moreover, Adegbe et al. (2016) reported that the IV was 68.65g100/g; our obtained results for IV were found higher than their results. Furthermore, in general researchers reported IV for *M. oleifera* ranged from 34.11-252.34 gI_2/100 g (Abiodun et al., 2012; Adejumo et al., 2013; Barakat & Ghazal, 2016; Chatepa et al., 2019; Lalas and Tsaknis, 2002; Leone et al., 2016; Ogbunugafor et al. 2011; Orhevba et al. 2013; Pereira et al. 2018) which is agreeable to our obtained result. Also previously, researchers reported the IV for some oils as groundnut oil (84 - 99), olive (79 - 90), castor oil (81 - 91), and *O. basilicum* (172-200, Hanus method). The oil of the current study in the range, but is higher than some edible oils as reported.

(iii) Peroxide Value (PV) of *M. oleifera* Seed Oil

The oil had also undergone some chemical decomposition process whereby the obtained PV is 7.6 meq O_2/kg; and was determined immediately after the extraction of the oil. PV indicates the rancidity process whereby the higher the PV, the higher is the oxidation level and the deterioration of lipids (Mohammed and Hamza, 2008). Theoretically, oil that shows a high amount of PV is more prone to undergo rancidity that affects the total quality of the oil (Ibeto et al., 2012). A low peroxide value increases the suitability of the oil for a long storage due to low level of oxidative and lipolytic activities. Adegbe et al. (2016) reported that the PV was 2.60 meq/Kg for this oil which is lower than our result. Generally, from many previous studies reported that the PV

was found to between range from 0.65-85.30 mEq/kg (Abiodun et al., 2012; Adejumo et al., 2013; Barakat & Ghazal, 2016; Chatepa et al., 2019; Ogbunugafor et al., 2011; Pereira et al., 2018; Pereira et al., 2016).

(iv) Moisture and Volatile Matter of *M. oleifera* Seed Oil

The moisture and volatile matter analysis prove that the oil contains low amount of moisture and volatile matter, whereby the value recorded is 4.91wt% thus, the low moisture content of the oil serves as an indication that, the activities of the micro-organisms would be reduced and thereby increases the shelf life of the oil. The presence of water or moisture contributes towards hydrolysis in breaking up of triglycerides into glycerol and FFAs. Therefore, both oxidation and hydrolysis reduce the amount of unsaturated FFA and thus contributing towards the reducing of IV and average molecular weight and increasing in the AV (Orhevba et al., 2013). The moisture content reported previously, Abiodun et al. (2012); Adejumo et al. (2013); Leone et al.. (2016); Orhevba et al. (2013) reported that the moisture has range 0.60-20%

(v) Saponification value and Unsaponifiable Matter of *M. oleifera* Seed Oil

The saponification value (SV) used to know the amount of free fatty acid present in the oil, and amount of free fatty acid is estimated by determining the quantity of alkali that must be added to the fat to render it neutral. The SV for studied oil was 185.2 mg KOH/g. Adegbe et al. (2016) reported that the saponification number was 180.92 mgKOH/g; our obtained results for saponification number was found higher than their results. In general our obtained result is agreeable to that reported previously which is range 91.16-230.81 (Abiodun et al., 2012; Adejumo et al., 2013; Anwar et al., 2005; Banerji et al., 2003; Barakat and Ghazal, 2016; Chatepa et al., 2019; Lalas andTsaknis, 2002; Leone et al., 2016; Ogbunugafor et al., 2011; Orhevba et al., 2013; Pereira et al., 2018).

Unsaponifiable matter is that fraction of oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents. Unsaponifiable matter do not react with bases during formation of soap such as hydrocarbon, pigments, waxes, higher molecular weight alcohols, and sterols (AOCS Ca 6a-40, 1998). The unsaponifiable matter value for *M. oleifera* seed oil was 3.2 wt%. Previously reported unsaponifiable matter *M. oleifera* seed oil which is determined by titration with sodium hydroxide solution in alcoholic, found to bettwen range 0.60-083 of (Anwar et al., (2005); Barakat and Ghazal, (2016)). The current studied oil showed higher unsaponifiable matter than reported value. Therefore, due to the small value of unsaponifiable matter (< 2 wt %), *M. oleifera* could be suitable in the application of biodiesel production (Tamboli et al., 2013). From the current study, it could be said that the oil had undergone some oxidation and hydrolysis process as indicated by the value of unsaturated fatty acids. This oxidation process might be influenced by storage of the oil whereby the presence of air in the bottle is in contact with the oil surface. Thus, the oxidation process converts the triglycerides into peroxides and hydro peroxides.

(vi) Free Fatty Acids (FFA), Fatty Acid Composition, Percentage of Saturated and Unsaturated Fatty Acid of *M. oleifera* Seed Oil

The fatty acid compositions of extracted seed oil were determined using GC-MS. The GC chromatogram of the oil shown in Figure 4.13, while Table 4.8 shows the fatty acid compositions of the *M. oleifera* seed oil. The MS Library of *M. oleifera* seed oil shown in Appendix E.

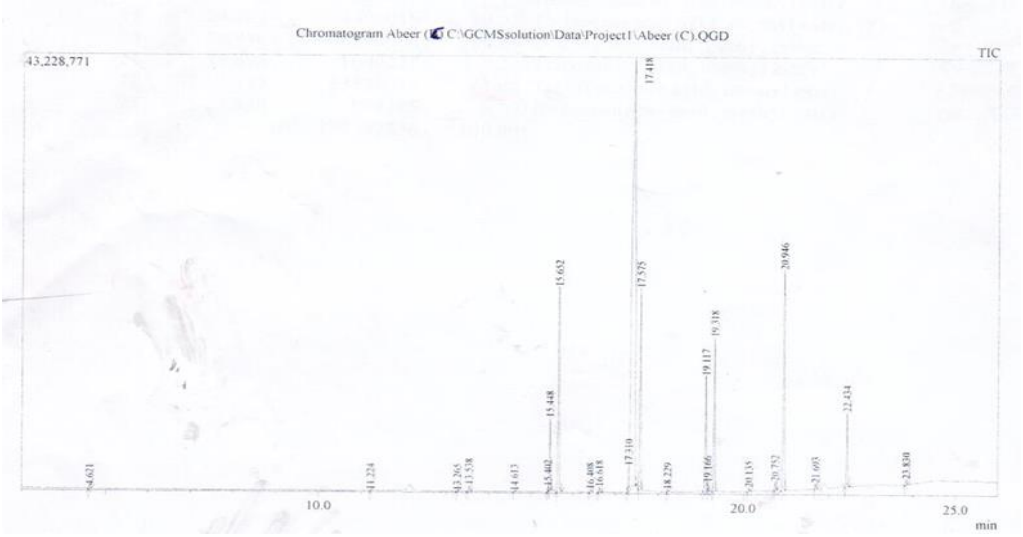

Figure 4.13: GC chromatogram of the *M. oleifera* seed oil

Table 4.8: Fatty acid composition of *M. oleifera* seed oil

Fatty acid	Formula	Structure	%
Myristic	$C_{14}H_{28}O_2$	$C_{14:0}$	0.23
5-Octadecenoic	$C_{18}H_{34}O_2$	$C_{18:1}$	0.02
Pentadecylic	$C_{15}H_{30}O_2$	$C_{15:0}$	0.02
Palmitoleic	$C_{16}H_{30}O_2$	$C_{16:1}$	2.85
Palmitic	$C_{16}H_{32}O_2$	$C_{16:0}$	9.20
Margaric	$C_{17}H_{34}O_2$	$C_{17:0}$	0.19
Linoleic	$C_{18}H_{32}O_2$	$C_{18:2}$	0.86
Oleic	$C_{18}H_{34}O_2$	$C_{18:1}$	51.74
Stearic	$C_{18}H_{36}O_2$	$C_{18:0}$	8.46
Arachidic	$C_{20}H_{40}O_2$	$C_{20:0}$	6.41
Heneicosylic	$C_{21}H_{42}O_2$	$C_{21:0}$	0.15
Behenic	$C_{22}H_{44}O_2$	$C_{22:0}$	10.54
Tricosylic	$C_{23}H_{46}O_2$	$C_{23:0}$	0.23
Cerotic	$C_{26}H_{52}O_2$	$C_{26:0}$	0.23
Lignoceric	$C_{24}H_{48}O_2$	$C_{24:0}$	3.08
Lauric	$C_{12}H_{24}O_2$	$C_{12:0}$	0.02
Cis-10-Heptadecenoic	$C_{17}H_{32}O_2$	$C_{17:1}$	0.12
Cis-10-Nonadecenoic	$C_{19}H_{36}O_2$	$C_{19:1}$	0.09
Erucic acid	$C_{22}H_{42}O_2$	$C_{22:1}$	0.32
Paullinic	$C_{20}H_{38}O_2$	$C_{20:1}$	0.29
Gondic	$C_{20}H_{38}O_2$	$C_{20:1}$	4.88

The obtained results indicted the present of twenty-one components; where the predominant components were oleic-, behenic-, palmitic-, stearic-, arachidic-, gondic-, lignoceric- and palmitoleic acid; their percentage were 51.74, 10.54, 9.20, 8.46, 6.41, 4.88, 3.08 and 2.85%, respectively. Their structures of the compounds are shown in Figure 4.14. The total percentage of fatty acids chains were 99.93 wt%. All the values are represented as the relative percentage area from the sum of all identified peaks. The overall results of this analysis showed that the unsaturated fatty acid (UFA) makes 61.17wt% of the compositions, whereby the monounsaturated fatty acids (MUFA) are 60.31wt%, polyunsaturated fatty acids (PUFA) are 0.86 wt%; and the saturated fatty acids (SFA) were 38.76 wt% as shown in Figure 4.15.

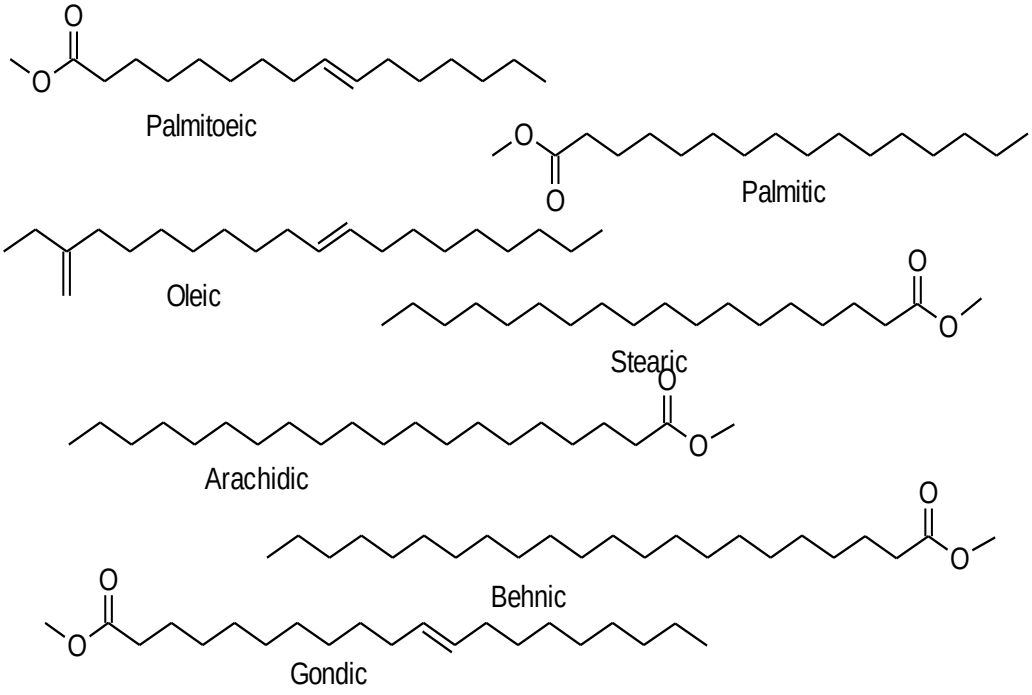

Figure 4.14: Structures of some acids (>1%) in *M. oleifera* seed oil

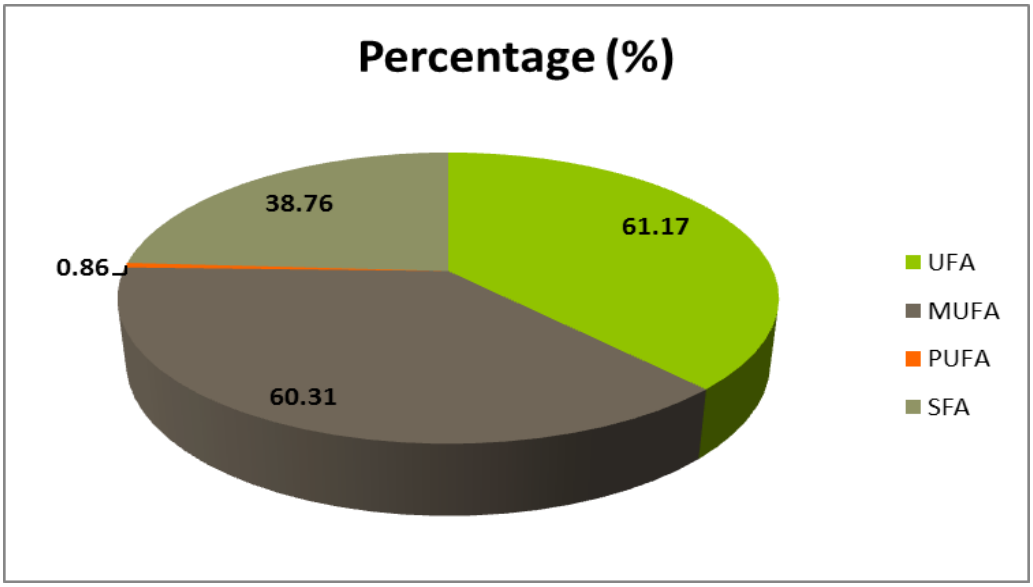

Figure 4.15: Types of fatty acids present in *M. oleifera* seed oil

In general, our obtained results were agreed to the results obtained by Lalas and Tsaknis (2002), they claimed that oleic acid (71.60%) was major component of *M. oleifera* seed oil, in addition to present of palmitic and behenic acid both up to 6.4%. Also the results obtained by Adegbe et al. (2016); mainly oleic acid (22.51%) and erucic acid (1.98%), palmitic (10.64%), stearic acid (6.07%), arachidic acid (2.21%) and docosanoic (behenic acid) (1.03%); confirmed the presences of the same acid, but the percentage of acids were slightly different from our obtained results.

Pereira et al. (2016) results were also agreed our obtained results as oleic acid was the major component in the extracted oil; but there was slight different in the percentage of these acids with our obtained results. Janaki (2015), obtained results oleic acid, palmitic acid, stearic acid, linoleic acid, margaric acid and α linolenic acid were 77.40 ± 0.40, 12.97 ± 0.15, 2.95 ± 0.04, 1.40 ± 0.01, 1.40 ± 0.15 and 1.39 ± 0.01, respectively; where results agreed our obtained results for oleic acid as major components, palmitic was closed to and stearic acid was lower than our results, but for margaric acid and

linoleic acid results were very low, while linolenic acid was not detected in our obtained results. These differences may be due to environmental issues.

4.3 COCKROACHES REPELLENT ACTIVITY

4.3.1 Repellent Activities of Oils against American Cockroaches

The positive control of this experiment is the naphthalene which has 100% repellence against American cockroaches while the negative control is the 1%DMSO and distilled water which shows zero per cent of repellence against cockroaches. When the repellence tests start, the number of cockroaches in first, second and third choice box for each concentrations have been recorded, where the first box in a treated side while the second and third box are the untreated side. It revealed that none of the cockroaches went into the treated choice-box and the amounts of food were remained the same. Then the number of cockroaches in the untreated choice-box were also recorded and showed in Figures 4.16, 4.17 and 4.18 respectively. It revealed that all of the cockroaches were located at untreated zone and none of the cockroaches locate in treated zone.

Figure 4.16: The observation in the left-side treated choice-box with 100 % concentration after 72 hour of exposure

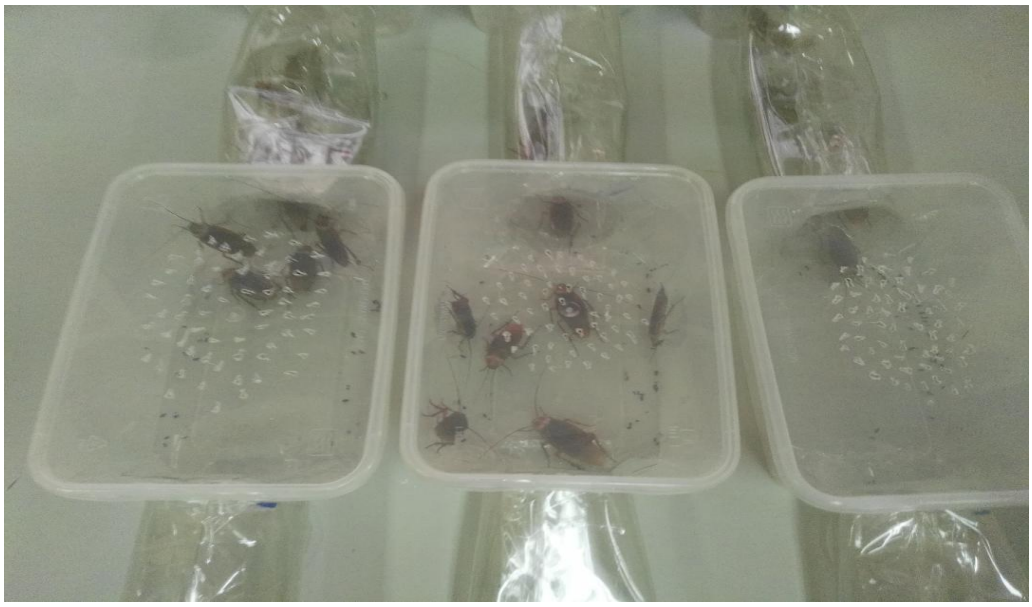

Figure 4.17: The observation of the number of cockroaches in the central untreated choice-box after 72 hours of exposure

Figure 4.18: The observation of the number of cockroaches in the right-side untreated choice-box after 72 hours of exposure

For 100% concentrations of the all oils (A,B, C, D and E) , the number of cockroaches at the treated choice-box after 72 hour of exposure was recorded and shown in Figures 4.19 to 4.23. It is noted that there was about 12.6% of cockroaches died in this repellence test. This revealed that the chemical components of the oils (essential and fixed) have toxicity to the American cockroaches and this observation agreed with reports in previous studies. According to Alicia (2009), the chemical components such as alpha-pinene which have been proved to poses the ability to repel the cockroaches, were only slightly toxic to adult male and non-toxic to adult female American cockroaches. While geraniol is non-toxic to both male and female cockroaches. When refer to the GC-MS analysis data, alpha-pinene consisted in both (D and E) essential oils while geraniol in oil of D. In addition caused 80% after 72 hrs after treatment.

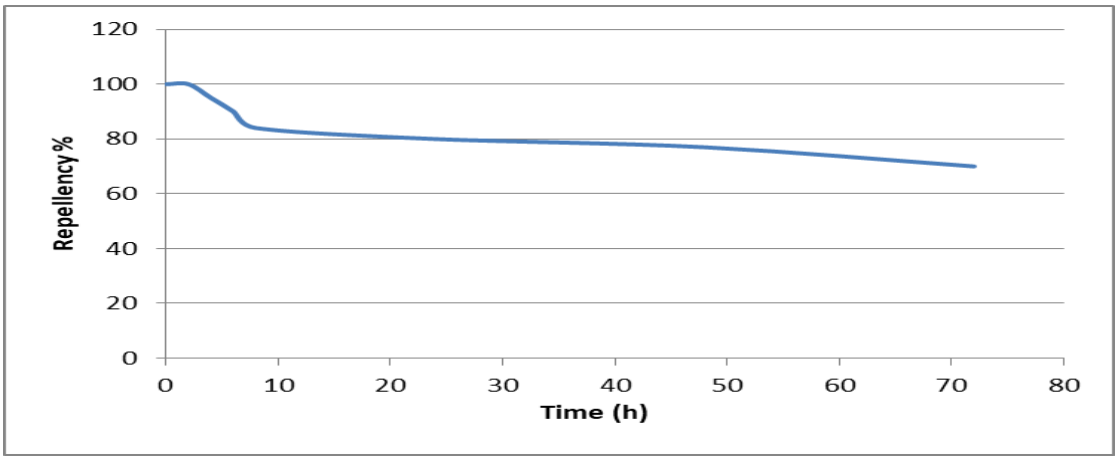

Figure 4.19: Percentage repellences of American cockroaches of the A for 72 hour treatment

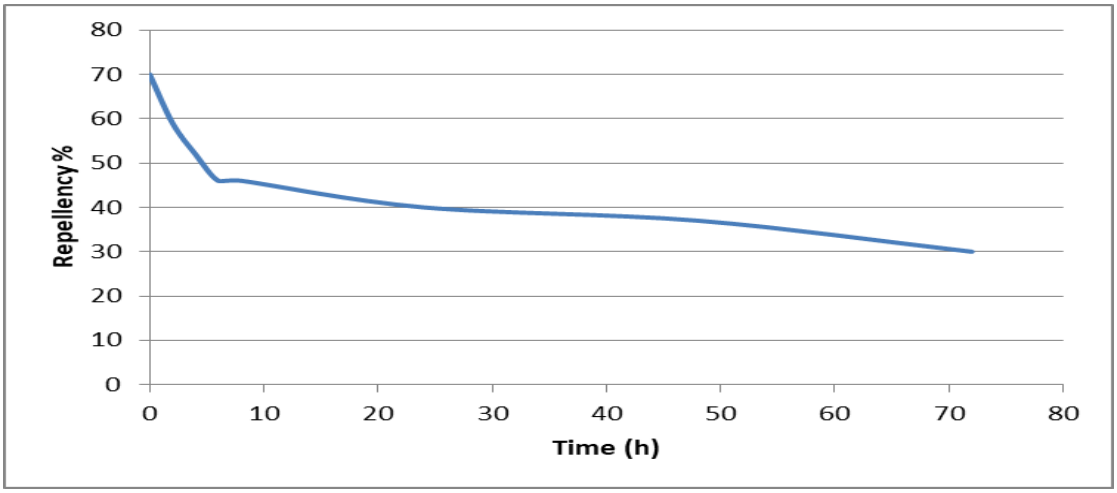

Figure 4.20: Percentage repellences of American cockroaches of the B for 72 hour treatment

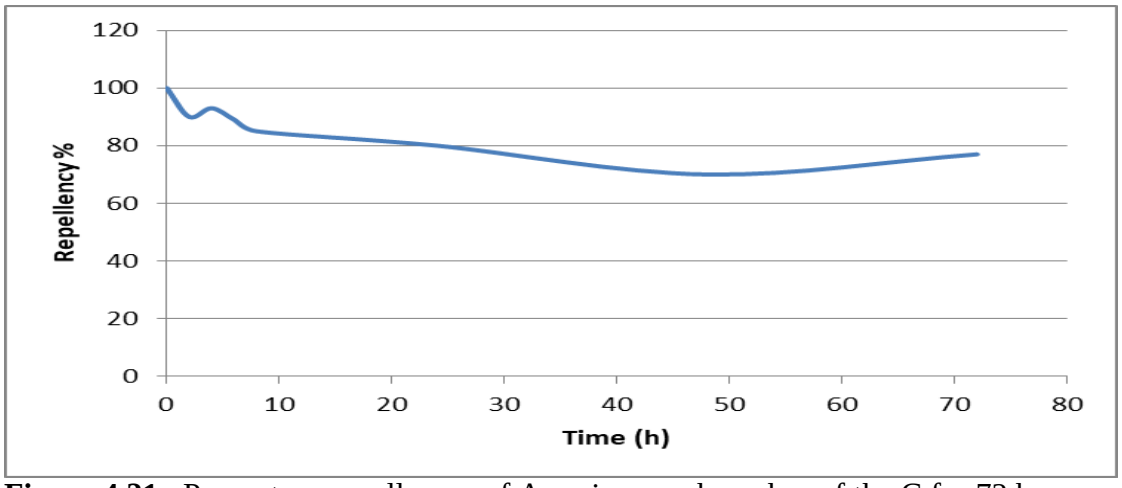

Figure 4.21: Percentage repellences of American cockroaches of the C for 72 hour treatment

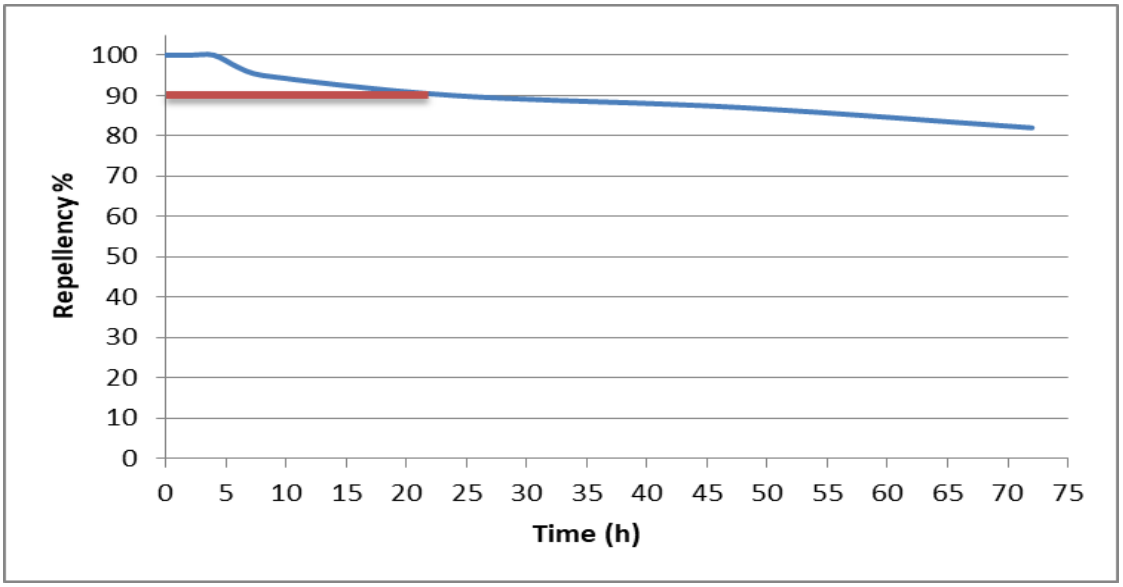

Figure 4.22: Percentage repellences of American cockroaches of the D at 72 hour treatment

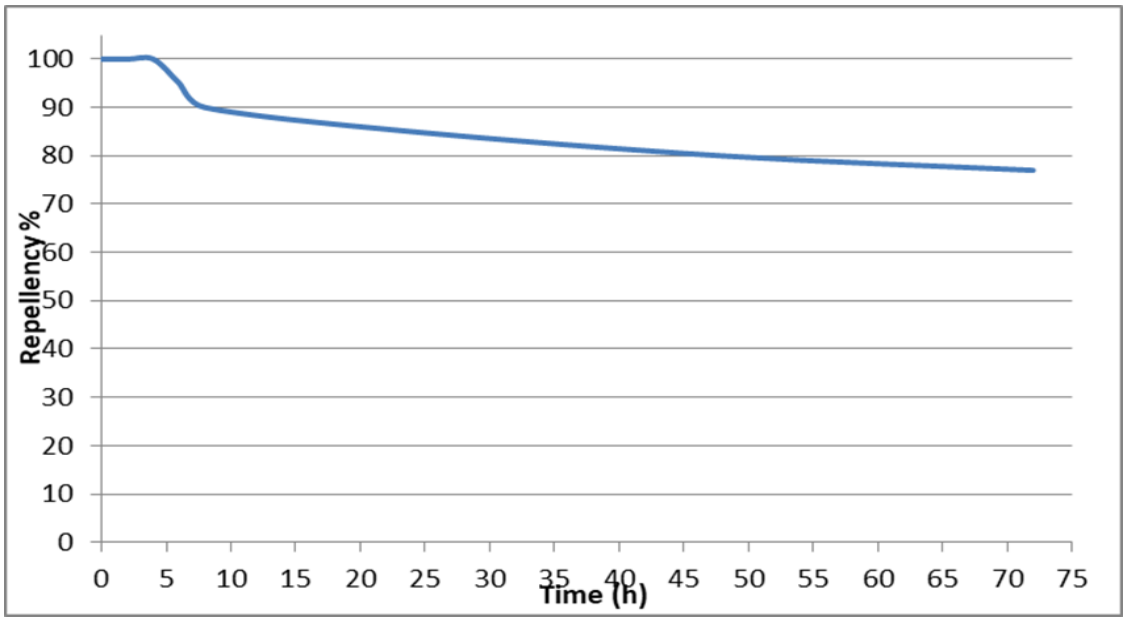

Figure 4.23: Percentage repellences of American cockroaches of the E at 72 hour treatment

The repellent efficacies of certain components were tested and the results showed varied with different doses and the cockroach species, and the major components responsible for the repellent activity of the essential oils were limonene, β-pinene and γ-terpinene (Yoon et al., 2009). El-Seedi et al. (2012) claimed that the *O. basilicum* oil with major compounds of 1,8-cineole, camphor, linalool, 4-terpineol, borneol, and carvone was the most repellent oil among many oils tested against cockroach. Moreover, Liu et al. (2011) essential oil of *C. rotundus* contains α-pinene (28.74%), 1,8-cineole (27.18%),

spathulenol (6.63%), globulol (6.53%) and ρ-menth-1-en-8-ol, showed strong repellency against nymphs of the German cockroaches.

Paranagama and Ekanag (2004) reported freshly rhizomes of *Alpinia calcarata* showed higher repellent properties in *P. Americana* than in the control because it's have component α-pinene, camphene, 1,8-cineole, champhor. The fumigant toxicity of 12 essential oil components including: carvacrol, 1,8-cineole, trans-cinnamaldehyde, citronellic acid, eugenol, geraniol, S-(-)-limonene, (-)-linalool, (-)-menthone, (+)-alpha-pinene, (-)-beta-pinene, and thymol was determined against adult male, adult female, gravid female, and large, medium, and small nymphs of the German cockroach, *B. germanica*. 1,8-Cineole was the most toxic essential oil component to adult males and females, gravid females, and large nymphs, and (monoterpene oil component) was the most toxic compound (Sharififard et al., 2016).

Seven commercial essential oils extracted from the plant species including *Boesenbergia rotunda* (L.) were evaluated for repellent activity against three cockroach species *P. americana* (L.), *Blattella germanica* (L.) and *Neostylopyga rhombifolia* (Stoll) (Thavara et al., 2007).

On the other hand, too many single and isolated compounds of studied plants are used and approved to have activity against insects repellent such as: Cinnamic acid (Sharma, 2011), Linalool (Beier et al., 2014; Campos et al., 2018), Estragole (Bedini et al., 2016; Guo et al., 2016; de-Paula et al., 2007), Ocimene (Chen et al., 1984; Dube et al., 2011; Dekker et al., 2011; Nchu et al., 2012), Cineole (Fu et al., 2015; Cansian et al., 2015), -α-pinene (Carroll et al., 2011; Mahmoudvand, 2018; Nour et al., 2017), Camphene (100%) against German cockroaches (Athuman et al., 2016; Carroll et al., 2011; Mahmoudvand, 2018; Nour et al., 2017), camphor (Cansian et al., 2015; Carroll et al., 2011; Fu et al., 2015; Maia and Moore, 2011; Nour et al., 2017;

Ponomarev and Mettee, 2016), Limonene (Athuman et al., 2016; Benelli et al., 2012; Carroll et al., 2011; Guo et al., 2016; Maia and Moore, 2011; Ranasinghe et al., 2016), Sabinene (Adjalian et al., 2015; Benelli et al., 2012; Carroll et al., 2011; Vongsombath et al., 2012), terpinene 4-ol (Benelli et al., 2012; Carroll et al., 2011; Hwang et al., 1985), Myrcene (Carroll et al., 2011; Hwang et al., 1985; Maia and Moore, 2011).

Geraniol was toxic to the cockroaches by contact or injection and repellency (Baldacchino et al., 2013; Maia and Moore, 2011; Nour et al., 2017), Caryophyllene (Maia and Moore, 2011), Fenchone (Bedini et al., 2016; Carroll et al., 2011; Pal et al., 2011), β-Citral (Baldacchino et al., 2013; Oyedele et al., 2002), α-Citral (Baldacchino et al., 2013; Nour et al., 2017), β-Elemene (Adjalian et al., 2015; Blythe et al., 2016; Gokulakrishnan et al., 2013; Nour et al., 2017), β-Cubebene (Nour et al., 2017), Fenchol (Nour et al., 2017), α-Bergamoten (Nour et al., 2017), α-Guaiene (Nour et al., 2017), β-Farnesene (Nour et al., 2017), α-Ylangene (Carroll et al., 2011; Nour et al., 2017), β-Elemol (Carroll et al., 2011; Nour et al., 2017), γ-Cadinene (Carroll et al., 2011; Guo et al., 2016; Pal et al., 2011), Germacrene (Guo et al., 2016), Humulene (Carroll et al., 2011), β-Bulnesene (Gokulakrishnan et al., 2013), β-Cubebene (Nour et al., 2017), Fenchol (Nour et al., 2017), α-Ylangene (Carroll et al., 2011; Nour et al., 2017), Verbenone (Athuman et al., 2016; Carroll et al., 2011), Caryophyllene oxide (Carroll et al., 2011; Pal et al., 2011; Suleiman et al., 2015), o-Cymene (Nour et al., 2017), α-Copaene (Carroll et al., 2011; Nour et al., 2017; Pal et al., 2011), γ-Muurolene (Carroll et al., 2011; Nour et al., 2017), p-Cymene (Carroll et al., 2011; Guo et al., 2016), Carvone (Carroll et al., 2011; Guo et al., 2016), Longifolene (Carroll et al., 2011), Myrtenal (Carroll et al., 2011), Pinocavreol (Carroll et al., 2011), α-Muurolene (Carroll et al., 2011), α-Calacorene (Carroll et al., 2011)

4.3.2 Formulation of the Repellent Product

(i) Concentration-Repellence Response

Figure 4.24 shows the oils (mixed from A, C, D and E) provided complete 100% repellence against American cockroaches with highest concentration. All oils and concentrations showed repellent activity against American cockroaches up to 72 hrs of exposures. The results showed that the percentage of repellent against cockroaches was increased when the test concentration is increased from 1.25 to 20% (v/v). This indicated that the repellency of oils against cockroaches were concentration dependents. At 1.25% of concentration, repellence was 10% for 72 hrs after treatment. Concentration of 2.5% caused 20% repellency for 72 hrs after the treatment, while the repellency was 40% for 5% concentration. Whereas, at 10% concentration the repellent achieved 80%. Moreover, at 20% concentration of oil the complete repellent of 100% was achieved. All concentrations showed repellency in varying degrees against American cockroaches. The oils were found to be effective as naphthalene repellent in higher concentration. The pure (100 v/v %) oils especially the essential oils showed excellent repellency (100%) against American cockroaches.

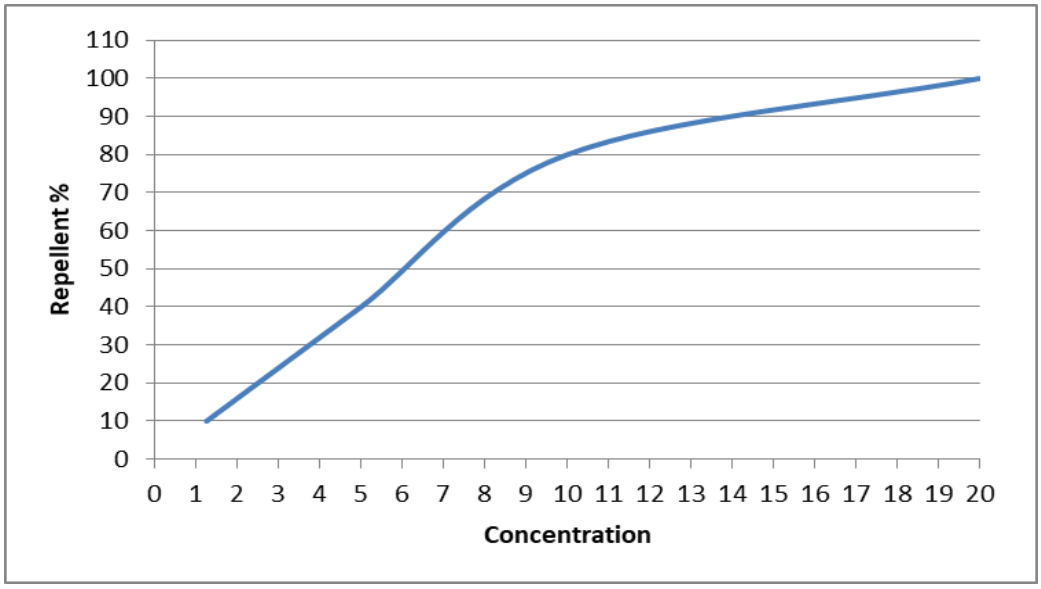

Figure 4.24: The percentage of repellency versus test concentration within 72 hours

(ii) The Effect of Exposure Time of the product on Repellency against American cockroaches

Figure 4.25 showed the effect of exposure time of the formulation on repellence against American cockroaches caused up to 100% repellency for 72 hrs of treatment.

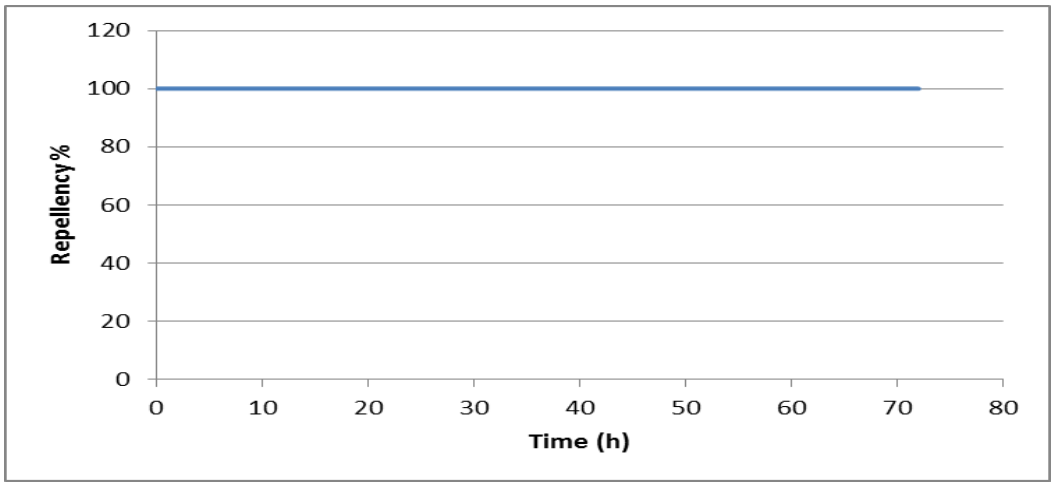

Figure 4.25: Percentage repellences of American cockroaches of the formulation at 72 hour after treatment

(iii) IC_{50} and IC_{90} Values in the Repellency Test

Figure 4.26 showed the IC_{50} and IC_{90} values (percentage concentration) of the insect product formulation against American cockroaches, indicated after 72 hr treatments. The IC_{50} and IC_{90} are the measurement of the concentration of the inhibitor which required inhibiting a given biological function by half and 90% respectively. In contact repellency test, American cockroaches were exposed to four different test concentrations (1.25, 2.5, 5, 10 and 20 v/v %) of oils (essential and fixed). The result revealed the IC_{50} and IC_{90} values of the oil after 72 hrs treatments, has the highest repellency rate, which were 6.0% and 14.2% respectively. Thus it would practical for use as

cockroaches repellent as it requires very low doses (6.0% or 14.2%) to be effective for 100% repellency.

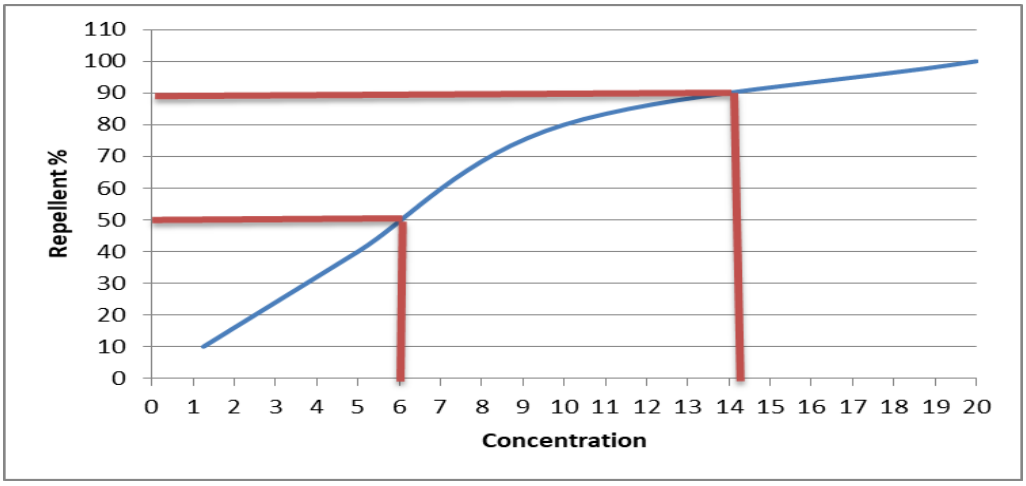

Figure 4.26: IC_{50} and IC_{90} values of oil formulation against American cockroaches 72 hour after treatment

However the essential oils were volatile and their effectiveness will reduce when applied for long period. Therefore a new product was formulated used natural sources which are oils (essential and fixed) cooperated with Vaseline. The formulation formulated from four oils (A, C, D and E) excluded oil sample B, beceause of low activity. Oils (essential and fixed) are readily degradable and less hazardous to human and animals health by having significantly lower toxicity level. Figure 4.27 displayed the formulated product from two different essential oils and two fixed oils from four medicinal plants

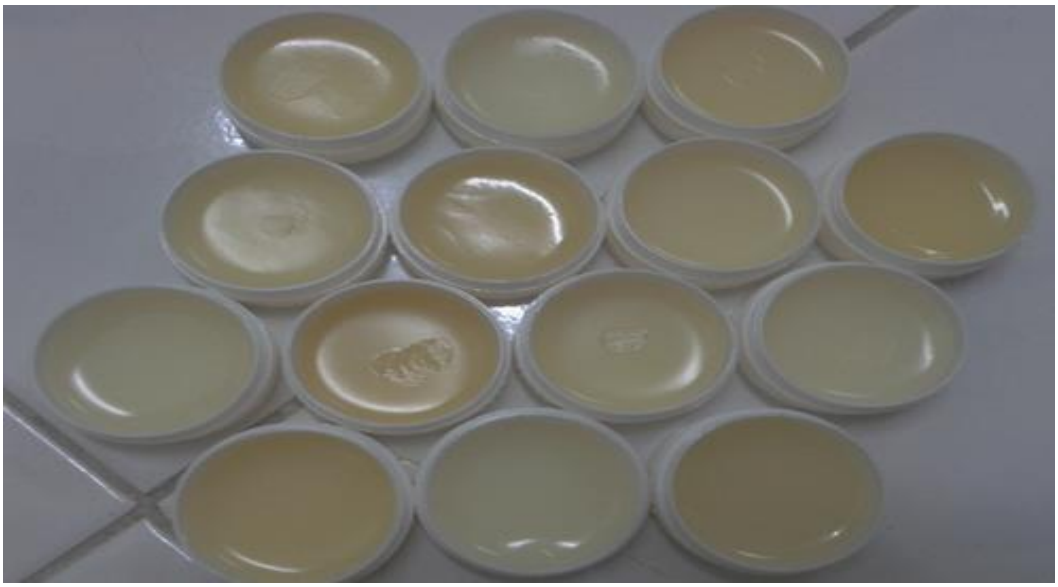

Figure 4.27: Formulated product from oils (essential and fixed) and Vaseline

4.4 POTENTIAL INDUSTRIAL APPLICATIONS OF THE OILS

C. rotundus is commonly growing weed all over the world. It was used as food, fodder, medicine, insect repellant, molluscicide, etc. It exhibits lot of chromosomal and morphological variations. Recent studies highlight its allelopathic effect, pharmaceutical or therapeutic uses, insecticidal potential, bioethanol yield, etc. It can be cheaper substitute for endangered medicinal plant Aconitum heterophyllum. Its nanoparticles are helpful in reducing toxicity to the environment (Cheema, 2015). It was reported that the mixtures formulated from the active constituents of the *C. rotundus* rhizome which is rich of 1, 8-cineole, (+)-dihydrocarvone or (R)-(+)-limonene could be useful as potential repellents for controlling *B. germanica* (Chang et al., 2017).

The essential oil of *S. riederi* var. japonica and its isolates showed potential as fumigants, and for their contact toxicity against grain storage insects because it is have major compounds in the essential oil were acetanisole (15.43%), anisole (9.43%), 1,8-cineole (8.07%), geraniol (7.89%), eugenol (4.54%), caryophyllene oxide (4.47%), caryophyllene (4.21%) and linalool (4.07%). Five active constituents (acetanisole, anisole, 1,8-cineole, eugenol

and geraniol) (Quan et al., 2018). In addition, Chang et al. (2012) evaluated the essential oil of *C. rotundus* rhizome obtained by steam distillation and they determined that the insecticidal principles were to be the monoterpenoids such as 1,8-cineole, p-cymene, pinene, myrtenal, and terpinen-4-ol. In addition, strong toxicity was also produced by (1S)-camphor, (S)-citronellal, m-cymene, o-cymene, linalool, nerol, and terpinolene. Geranial and neral (namely trans-citral, citral A and cis-citral, citral B, respectively) are two isomeric acyclic monoterpene aldehydes from citral (3, 7-dimethyl-2,6-Octadienal). Citral, which possesses a strong lemon aroma, has been used widely in food, cosmetics, detergents, perfumery and pharmaceutical industries as flavouring or scenting agents (Pihlasalo et al., 2007; Lalko and Api 2008). It is also commercially used in the production of vitamin A, ionones and methylionones (Pihlasalo et al., 2007). However, it has been identified as a potential contact allergen as it may react with skin proteins to form an immunogen because of its capability to penetrate through both animal and human skin (Lalko and Api 2008). The existence of elemol in this essential oil has been suggested to be formed as an artifact to Ceylon-type (Akhila, 2010).

The compound methyl cinnamate which in exist in basil (*O. basilicum*) essential oil at 25.32% is very important compound and could be used in wide area of industrial applications as reported previously. Repellent activity (Dekker et al., 2011), larvicidal activit (Fujiwara et al., 2017), oxidative damage diseases, such as coronary heart disease, stroke and cancers. However, their potential interest for the human health goes far beyond their protective antioxidant behavior (Ernawati et al., 2014; Lone et al., 2014), antibacterial activity, antifungal activity (Padalia et al., 2017), cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic pro such as household cleaners and detergents (Sharma and Kanwar, 2012).

The compound linalool which in exist in basil (*O. basilicum*) essential oil at 19.06% is very important compound and could be used in wide area of industrial applications as reported previously. The insecticidal activities against adult German cockroaches and found that it is slightly toxic (Campos et al., 2018; Nour et al., 2017), anti-inflammatory (Lee et al., 2018). Moreover, linalool is used in perfumes, cosmetics, flavoring agents and the compound has proven as antimicrobial (Aprotosoaie et al., 2014; Beier et al., 2014; Kim et al., 2015; Mughal, 2018; Siani et al., 2002), antioxidant (Peana and Moretti, 2008). The manufacture of vitamin E and vitamin A (Bauer and Garbe, 1985; Steffani et al., 2006). Anticancer (Iwasaki et al., 2016). Pharmacological properties (Alinejad et al., 2013; Woronuk et al., 2011).

The compound Estragole which in exist in basil (*O. basilicum*) essential oil at 12.32% is very important compound used and could be used in wide area of industrial applications as reported previously, to have neurotropic, antimicrobial, antispasmodic, and immunostimulant properties (Alves et al., 2013; Andrade et al., 2015; Cardoso et al., 2004). Anti-diarrheic, antispasmodic, analgesic and anti-inflammatory (de-Paula et al., 2007). Used in food products, perfumes, soaps and detergents (Ismaiel et al., 2016; Ponte et al., 2012; Punt et al., 2009). The compound ocimene which in exist in basil (*O. basilicum*) essential oil at 1.51% is very important compound used in wide area of industrial applications as reported previously, mosquito repellent activity (Chen et al., 1984; Dube et al., 2011), manufacture of a number of perfume chemicals (Zviely et al., 2013). Used as antioxidant and antibacterial (Gundidza et al., 2009; Mahdian et al., 2017; Mighri et al., 2015).

The 1,8-cineole compound which is present in the two essential oils of the studied plants in ratio of 5.57% (*O. basilicum*) and 0.11% (*C. rotundus*) are very important compound used and could be used in wide area of industrial applications as reported previously. The insecticidal activity against red

imported fire ant (RIFA) (Cansian et al., 2015; Fu et al., 2015; Lee et al., 2003; Liska et al., 2010). Anti-inflammatory, anti-microbial, antifungal, antiaflatoxigenic, analgesic and antioxidant activity (Brown et al., 2017; Kim et al., 2018; Sadlon and Lamson, 2010; Santos and Rao, 2000; Takaishi et al., 2012).

α-pinene, this compound which is present in the two essential oils of the studied plants in ratio of 0.18% (*O. basilicum*) and 0.44% (*C. rotundus*) is very important compound used and could be used in wide area of industrial applications as reported previously, insecticidal activities (Nour et al., 2017; Silva et al., 2012). Used in flavorings, fragrances, antimicrobial, hypertensive, antinociceptive, and anti-inflammatory (Him et al., 2008; Yang et al., 2013; Yang et al., 2016).

The compound Camphene which in exist in basil (*O. basilicum*) essential oil at 0.03% is very important compound used and could be used in wide area of industrial applications as reported previously, Used as drug (Vallianou et al., 2011; Vallianou and Hadzopoulou-Cladaras, 2016). Insecticidal (Chen et al., 2018; Sharaby and EL-Dosary, 2015). The compound camphor which in exist in basil (*O. basilicum*) essential oil at 0.36% is very important compound used and could be used in wide area of industrial applications as reported previously, cosmetics, as a food flavourant, as a. insecticidal, antimicrobial, antiviral, anticoccidial, anti-nociceptive, anticancer and antitussive activities, (Aguirre et al., 2008; Cansian et al., 2015; Chen et al., 2013; Chen et al., 2018; Fu et al., 2015; Liska et al., 2010; Ponomarev and Mettee, 2016).

Limonene was compound which is present in the two essential oils of the studied plants in ratio of 0.48% (*O. basilicum*) and 0.32% (*C. rotundus*) is very important compound used and could be used in wide area of industrial

applications as reported previously, flavor and fragrance industries, biosynthetic, ecological and pharmacological (Erasto and.Viljoen, 2008). Showed strong insecticidal, pesticide, antifungal (Chen et al., 2018; Guo et al., 2016; Hollingsworth, 2005; Ibrahim et al., 2008; Ranasinghe et al., 2016; Verza et al., 2011).

Sabinene compound was exist in basil (*O. basilicum*) essential oil at 0.30% is very important compound used and could be used in wide area of industrial applications as reported previously, pesticides, insecticidal, phytotoxic, anti-bacterial, anti-fungal and antimicrobial activities (Adjalian et al., 2015; Arunkumar et al., 2014; Sieniawska et al., 2016; Zhou et al., 2019).

Terpinene 4-ol compound present in the two essential oils of the studied plants in ratio of 0.11% (*O. basilicum*) and 0.32% (*C. rotundus*) is very important compound used and could be used in wide area of industrial applications as reported previously, antibacterial, antifungal, antiinflammatory and cytotoxic activities (Arunkumar et al., 2014), insecticidal Repellent (Guo et al., 2016; Nour et al., 2017).

The compound Caryophyllene which in exist in basil (*O. basilicum*) essential oil at 0.99% is very important compound used and could be used in wide area of industrial applications as reported previously, anti-inflammatory, anti- mutagenic and anti-carcinogenic activities (Filho et al., 2010). The compound β-Elemene which is present in the two essential oils of the studied plants in ratio of 2.44% (*O. basilicum*) and 0.53% (*C. rotundus*) is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial activities (Sieniawska et al., 2016). Insecticidal effects on S. cerealella (Adjalian et al., 2015). Antineoplastic medicine (Wang et al., 2012).

β-Cubebene which is present in the two essential oils of the studied plants in ratio of 0.55% (*O. basilicum*) and 0.65% (*C. rotundus*) is very important compound used and could be used in wide area of industrial applications as reported previously, anti-oxidative activities (Filho et al., 2010). The compound Cubedol which in exist in basil (*O. basilicum*) essential oil at 0.77% is very important compound used and could be used in wide area of industrial applications as reported previously, active against the growth of Gram positive than the Gram negative bacterial tested (Omoruyi and Muchenje, 2017).

Moreover, Cinnamic acid which in exist in basil (*O. basilicum*) fixed oil at 0.05% is very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant, hepatoprotective, anxiolytic, antidiabetic, anti-inflammatory, Antifungal and anticholesterolemic (Korosec et al. 2013, Pontiki et al. 2014, Sharma, 2011). Cosmetics (Krzyżak et al., 2018). Anticancer, antitumor agents (Asif and Mohd, 2019; Prithwiraj et al., 2011).

Furthermore, compound Myristic acid which is present in the three fixed oils of the studied plants in ratio of 0.11% (*O. basilicum*), 0.28% (*A. digitata*) and 0.23% (*M. olrifera*) are very important compound used and could be used in wide area of industrial applications as reported previously, Used in the food industry as a flavor ingredient (Burdock and Carabin, 2007). Antibacterial and antifungal properties (Agoramoorthy et al., 2007), antitrypanosomal (Doering et al., 1994).

The compound Isomyristic acid which in exist in basil (*O. basilicum*) fixed oil at 0.02% is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic

properties (Belakhdar et al., 2015). This compound Pentadecylic acid which is present in the three fixed oils of the studied plants in ratio of 0.04% (*O. basilicum*), 0.08% (*A. digitata*) and 0.02% (*M. olrifera*) are very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic properties (Belakhdar et al., 2015). This compound Palmitoleic acid which is present in the three fixed oils of the studied plants in ratio of 0.78% (*O. basilicum*), 0.36% (*A. digitata*) and 2.85% (*M. olrifera*) are very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant, anti-inflammatory, anticancer, antitumour, antiarthritic, cancer preventive, antibacterial (Parthiban et al., 2014).

This compound Palmitic acid which is present in the three fixed oils of the studied plants in ratio of 13.38% (*O. basilicum*), 22.87% (*A. digitata*) and 9.20% (*M. olrifera*) are very important compound used and could be used in wide area of industrial applications as reported previously, activity against the test microorganisms (Abubakar and Majinda, 2016). Antioxidant, anti-inflammatory, antifungal, anticancer, antitumour, antiarthritic, cancer preventive, antibacterial, decrease blood cholesterol (Agoramoorthy et al., 2007; Belakhdar et al., 2015; Parthiban et al., 2014). This compound Margaric acid which is present in the three fixed oils of the studied plants in ratio of 0.20% (*O. basilicum*), 0.28% (*A. digitata*) and 0.86% (*M. olrifera*) are very important compound used and could be used in wide area of industrial applications as reported previously, activity against the test microorganisms (Abubakar and Majinda, 2016).

The compound Linoleic acid which is present in the three fixed oils of the studied plants in ratio of 32.18% (*O. basilicum*), 30.63% (*A. digitata*) and 0.86% (*M. olrifera*) is very important compound used and could be used in

wide area of industrial applications as reported previously, activity against the test microorganisms (Abubakar and Majinda, 2016). Antioxidant, anti-inflammatory, anticancer, antitumour, antiarthritic, cancer preventive, antifungal antibacterial (Agoramoorthy et al., 2007; Parthiban et al., 2014).

The compound Linolenic acid which in exist in basil (*O. basilicum*) fixed oil at 43.92% is very important compound used and could be used in wide area of industrial applications as reported previously, antibacterial and antifungal properties (Agoramoorthy et al., 2007). The compound Stearic acid which is present in the three fixed oils of the studied plants in ratio of 8.46% (*O. basilicum*), 5.89% (*A. digitata*) and 0.86% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, activity against the test microorganisms (Abubakar and Majinda, 2016). Antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic properties (Agoramoorthy et al., 2007; Belakhdar et al., 2015).

The compound arachidic acid which is present in the three fixed oils of the studied plants in ratio of 0.72% (*O. basilicum*), 1.43% (*A. digitata*) and 6.41% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic properties (Agoramoorthy et al., 2007; Belakhdar et al., 2015). The compound behenic acid which is present in the three fixed oils of the studied plants in ratio of 0.17% (*O. basilicum*), 0.64% (*A. digitata*) and 10.54% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic properties (Agoramoorthy et al., 2007; Belakhdar et al., 2015; Gideon, 2015).

The compound vebenone which in exist in *C. rotundus* essential oil at 0.33% is very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant activity (Hu et al., 2017). The compound Caryophyllene oxide which in exist in *C. rotundus* essential oil at 0.61% is very important compound used and could be used in wide area of industrial applications as reported previously, anti-inflammatory, anti-microbial, anti-fungal, antioxidants, growth regulating properties, analgesis, antidiabetic, hypotensive and splasmolytic (Kumar et al., 2017).

The compound o-Cymene which in exist in *C. rotundus* essential oil at 0.19% is very important compound used and could be used in wide area of industrial applications as reported previously, repellency against German cockroaches (Nour et al., 2017). The compound α-Copaene which in exist in *C. rotundus* essential oil at 3.72% is very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant activity (Hu et al., 2017).

The compound γ-Muurolene which in exist in *C. rotundus* essential oil at 1.92% is very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant activity (Hu et al., 2017).The compound p-Cymene which in exist in *C. rotundus* essential oil at 0.06% is very important compound used and could be used in wide area of industrial applications as reported previously, antioxidant activity (Hu et al., 2017).

The compound Carvone which in exist in *C. rotundus* essential oil at 0.12% is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial (Labiod et al., 2015), antioxidant activity (Hu et al., 2017).

The compound Myrtenal which in exist in *C. rotundus* essential oil at 2.01% is very important compound used and could be used in wide area of industrial applications as reported previously, a prominent role in preventing the liver cancer (Srivastava et al., 2014). Anti-inflammatory, anti-microbial, anti-fungal, antioxidants, growth regulating properties, analgesis, antidiabetic, hypotensive and splasmolytic (Kumar et al., 2017).

The compound Pinocavreol which in exist in *C. rotundus* essential oil at 1.73% is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial (Labiod et al., 2015), antioxidant activity (Hu et al., 2017). The compound α-Muurolene which in exist in *C. rotundus* essential oil at 0.55% is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial (Labiod et al., 2015) antioxidant activity (Hu et al., 2017).

The compound T-Calamenene which in exist in *C. rotundus* essential oil at 1.59% is very important compound used and could be used in wide area of industrial applications as reported previously, antimicrobial (Labiod et al., 2015), antioxidant activity (Hu et al., 2017). The compound Rotundene which in exist in *C. rotundus* essential oil at 1.91% is very important compound used and could be used in wide area of industrial applications as reported previously, anti-inflammatory, anti-microbial, anti-fungal, antioxidants, growth regulating properties, analgesis, antidiabetic, hypotensive and splasmolytic (Kumar et al., 2017), antibacterical, antifungal (Bhawna et al., 2013).

The compound Longifolenaldehyde which in exist in *C. rotundus* essential oil at 5.16% is very important compound used and could be used in wide area of industrial applications as reported previously, anti-inflammatory, anti-microbial, anti-fungal, antioxidants, growth regulating properties, analgesis, antidiabetic, hypotensive and splasmolytic (Kumar et al., 2017). The

compound Longiverbenone which in exist in *C. rotundus* essential oil at 5.61% is very important compound used and could be used in wide area of industrial applications as reported previously, anti-inflammatory, anti-microbial, anti-fungal, antioxidants, growth regulating properties, analgesis, antidiabetic, hypotensive and splasmolytic (Kumar et al., 2017).

This compound oleic acid which is present in the tow fixed oils of the studied plants in ratio of 23.34% (*A. digitata*) and 51.74% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, activity against the test microorganisms (Abubakar and Majinda, 2016). Antioxidant, anti-inflammatory, anticancer, antitumour, antiarthritic, cancer preventive, antibacterial (Agoramoorthy et al., 2007; Belakhdar et al., 2015; Marimuthu et al., 2014; Parthipan et al., 2015).

The compound Lauric acid which in exist in *M. oleifera* fixed oil at 0.02% is very important compound used and could be used in wide area of industrial applications as reported previously, antibacterial and antifungal properties (Agoramoorthy et al. 2007; Belakhdar et al., 2015). This compound Cis-10-Heptadecenoic acid which is present in the tow fixed oils of the studied plants in ratio of 0.48% (*A. digitata*) and 0.21% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, antibacterial and antifungal properties (Agoramoorthy et al., 2007). This compound Cis-10-Nonadecenoic acid which is present in the tow fixed oils of the studied plants in ratio of 2.67% (*A. digitata*) and 0.09% (*M. olrifera*) is very important compound used and could be used in wide area of industrial applications as reported previously, antibacterial and antifungal properties (Agoramoorthy et al., 2007).

CHAPTER FIVE

CONCLUSION AND RECOMMONDATIONS

5.1 CONCLUSION

This research was carried out to achieve certain objectives, which were extraction of essential oils from two medicinal plants namely, *O. basilicum* (fresh leaves, sample D) and *C. rotundus* (dry rhizomes, sample E) by steam distillation method; and then obtained fixed oils from *O. basilicum* (sample A), *A. digitata* (sample B), and *M. oleifera* (sample C), seeds. The yield and the chemical compositions in terms of the quality and quantity of the essential oils and physicochemical properties of the seed-oils were studied. Then after, the potential utility of the oils (essential oils, fixed oils) in the cockroaches' repellent activity was investigated and insect repellent product was formulated. Finally, the potential industrial applications for the studied oils were proposed. Therefore, the objectives of the study were fully achieved. The oils content of oils obtained by steam distillation were, 0.78% (D) and 0.73% (E).

Almost forty one and forty-four compounds were detected, the predominant constituents were methyl cinnamate (25.32%) and (-)-Isolongifolol (7.63%) for D and E, respectively. The Pale yellow with camphor odor, the reddish yellow with characteristic odor, and golden yellow with characteristic odor oils were extracted from the seeds of A, B, and C. The obtained fixed oils have the following properties: freezing point, -2, -14 and 0 °C; melting point, 5, 8 and 21 °C; boiling point, 215, 227 and 225 °C; refractive index (25 °C), 1.48532, 1.436 and 1.447; iodine value, 108.6, 98.3 and 96.6 g/100 g of oil; peroxide value, 4.6 , 4.3 and 7.6 meq. O_2/kg of oil; free fatty acids, 0.20, 0.34 and 0.07%; acid value, 4.0 , 6.8 and 1.4 mg of KOH/g of oil; saponification value, 164.2, 180.7 and 185.2 mg KOH/g of oil; unsaponifiable matter, 1.6, 1.7 and 3.2; moisture and volatile value, 4.97, 14.79

and 4.91(wt%); density, 0.914, 0.867 and 0.900g/cm^3; viscosity, 10.29, 35.03 and 60.99 mm^2/s; specific gravity, 0.921, 0.874 and 0.907; the major fatty acid were linolenic- (43.92%), oleic- (51.74%) and linoleic acid(30.63%)), respectively.

In bioassay test, the repellency of the oils against cockroaches increase with concentration of oil increased. According to the research above, it can be concluded that the studied oils (essential and fixed) has repellent effect against American cockroaches when tested in individual or synergistic combinations. The IC_{50} and IC_{90} values for the formulation were 6.0 v/v% and 14.2%. Hence these oils are promising repellent and may play a vital role in cockroaches' repellent agent. This oils formulation would practical for use as cockroaches repellent as it requires very low dose (20%) to be effective 100% repellency for more than 72 hrs.

5.2 RECOMMENDATIONS

There are some recommendations can be considered for future researches. According to the findings achieved from the present study. Depending on the current study, the following are some recommendations:

1. It is highly recommended to use other extraction methods such as microwave assisted hydrodistillation (MAHD) and SFE extraction. Because the quality and quantity of the oils were significant for different extraction methods used. The biological activities or other uses of the essential oils are depends on the quantitative and qualitative of the constituents of essential oils.
2. Beside this, the oils (essential and fixed) may be used to test for the cockroaches with other species such as German cockroaches, Oriental cockroaches and Tropical cockroaches.
3. The oils can also be used to test for the repellency of other insect such as mosquito, beetles and many other household pests in order to increase the utility of these oils.

4. Therefore, may warrant further study to identify the bioactive compound(s) and to confirm whether the chemical components of studied oils could act individually as repellent agents. Furthermore, new studies on the studied oils (especially essential oils) are required for their proper formulations, potential industrial applications and commercialization as a cheap, natural and environmental safe repellent product(s).

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Appendix (A) MS Library of *O. basilicum* essential oil

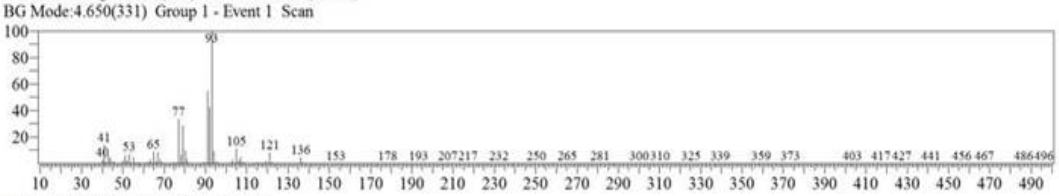
Library

<< Target >>

Line#:1 R.Time:4.705(Scan#:342) MassPeaks:277

RawMode:Single 4.705(342) BasePeak:93.10(76105)

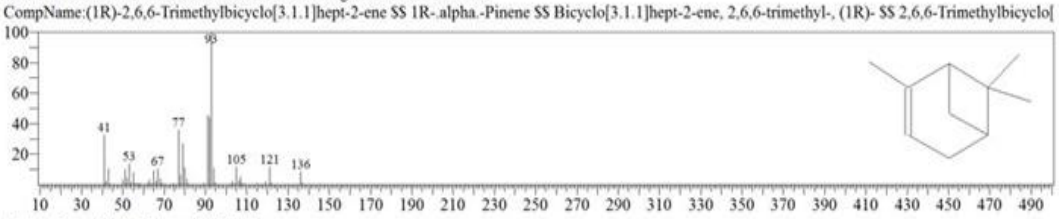
BG Mode:4.650(331) Group 1 - Event 1 Scan



Hit#:1 Entry:6665 Library:NIST11s.lib

SI:94 Formula:C10H16 CAS:7785-70-8 MolWeight:136 RetIndex:948

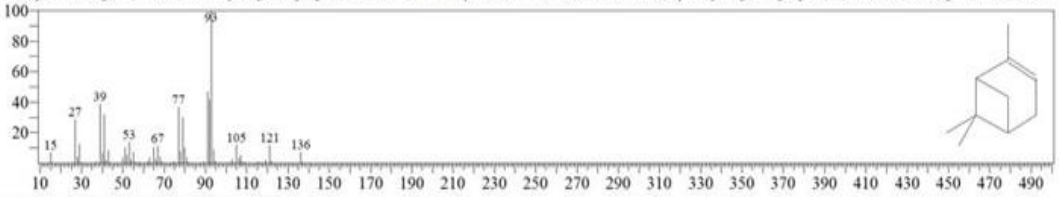
CompName:(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ 1R-.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1R)- $$ 2,6,6-Trimethylbicyclo[



Hit#:2 Entry:9809 Library:NIST11.lib

SI:94 Formula:C10H16 CAS:80-56-8 MolWeight:136 RetIndex:948

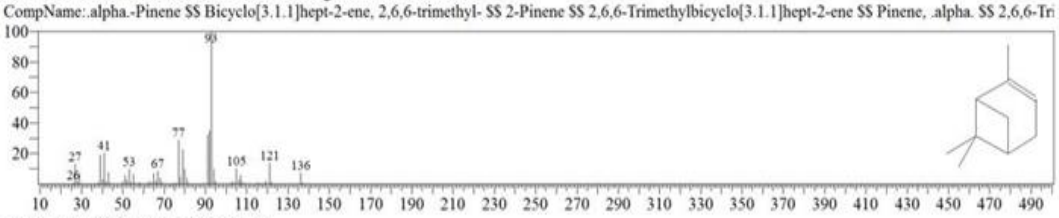
CompName:.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- $$ 2-Pinene $$ 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ Pinene, .alpha. $$ 2,6,6-Tri



Hit#:3 Entry:6669 Library:NIST11s.lib

SI:94 Formula:C10H16 CAS:80-56-8 MolWeight:136 RetIndex:948

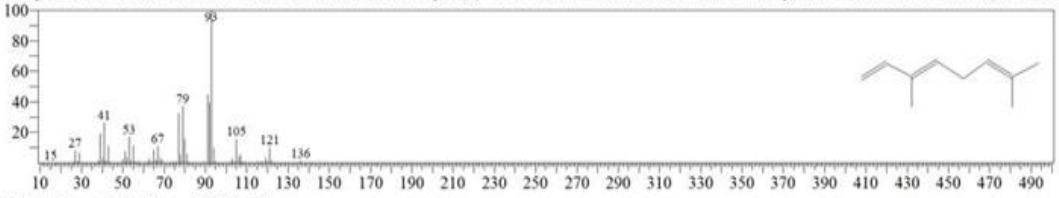
CompName:.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- $$ 2-Pinene $$ 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ Pinene, .alpha. $$ 2,6,6-Tri



Hit#:4 Entry:6664 Library:NIST11s.lib

SI:93 Formula:C10H16 CAS:3779-61-1 MolWeight:136 RetIndex:976

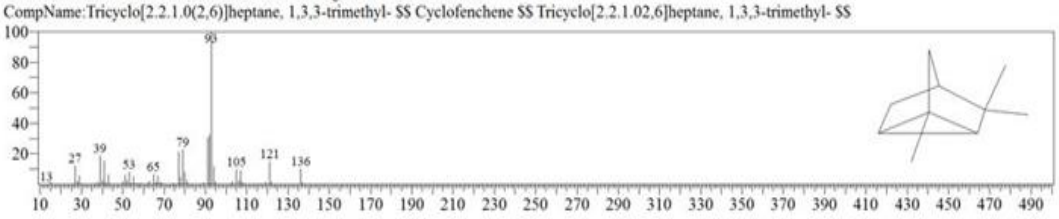
CompName:trans-.beta.-Ocimene $$ 1,3,6-Octatriene, 3,7-dimethyl-, (E)- $$ .beta.-trans-Ocimene $$ trans-3,7-Dimethyl-1,3,6-Octatriene $$ Ocimene, trans-.be



Hit#:5 Entry:6667 Library:NIST11s.lib

SI:93 Formula:C10H16 CAS:488-97-1 MolWeight:136 RetIndex:729

CompName:Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl- $$ Cyclofenchene $$ Tricyclo[2.2.1.02,6]heptane, 1,3,3-trimethyl- $$



Appendix (B) MS Library of *C. rotundus* rhizome essential oil

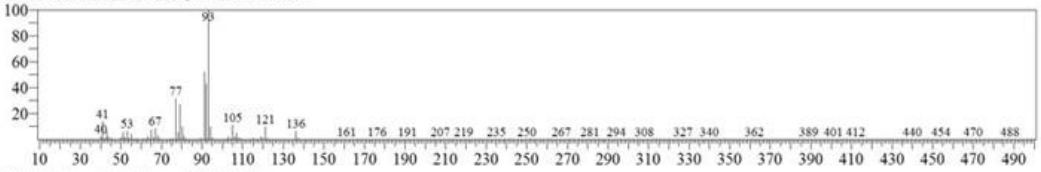
Library

<< Target >>

Line#:1 R.Time:4.700(Scan#:341) MassPeaks:278

RawMode:Single 4.700(341) BasePeak:93.10(176067)

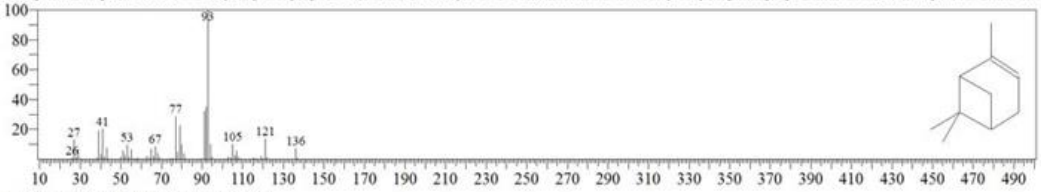
BG Mode:4.650(331) Group 1 - Event 1 Scan



Hit#:1 Entry:6669 Library:NIST11s.lib

SI:95 Formula:C10H16 CAS:80-56-8 MolWeight:136 RetIndex:948

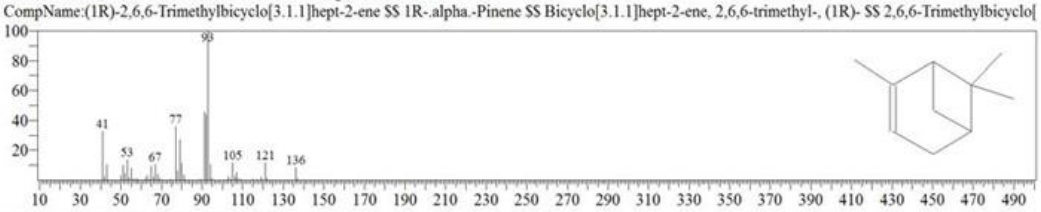
CompName:.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- $$ 2-Pinene $$ 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ Pinene, .alpha. $$ 2,6,6-Tri



Hit#:2 Entry:6665 Library:NIST11s.lib

SI:95 Formula:C10H16 CAS:7785-70-8 MolWeight:136 RetIndex:948

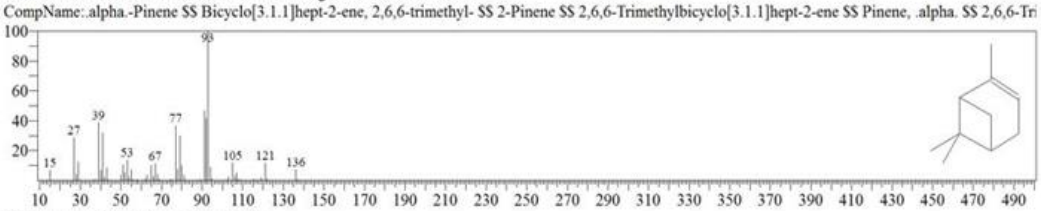
CompName:(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ 1R-.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (1R)- $$ 2,6,6-Trimethylbicyclo[



Hit#:3 Entry:9809 Library:NIST11.lib

SI:95 Formula:C10H16 CAS:80-56-8 MolWeight:136 RetIndex:948

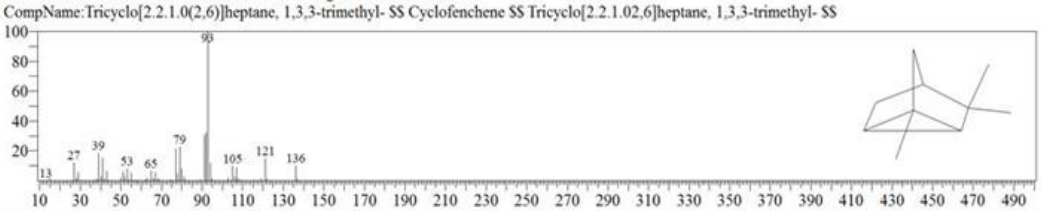
CompName:.alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- $$ 2-Pinene $$ 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ Pinene, .alpha. $$ 2,6,6-Tri



Hit#:4 Entry:6667 Library:NIST11s.lib

SI:94 Formula:C10H16 CAS:488-97-1 MolWeight:136 RetIndex:729

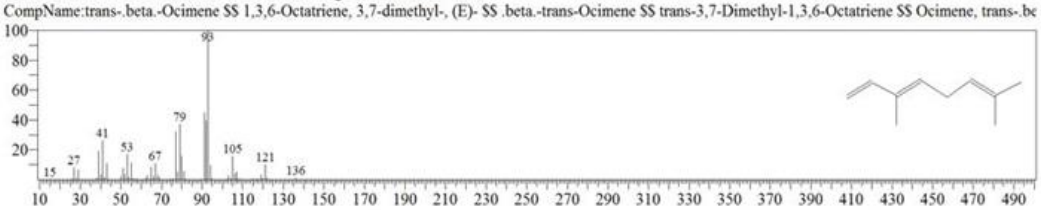
CompName:Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl- $$ Cyclofenchene $$ Tricyclo[2.2.1.02,6]heptane, 1,3,3-trimethyl- $$



Hit#:5 Entry:6664 Library:NIST11s.lib

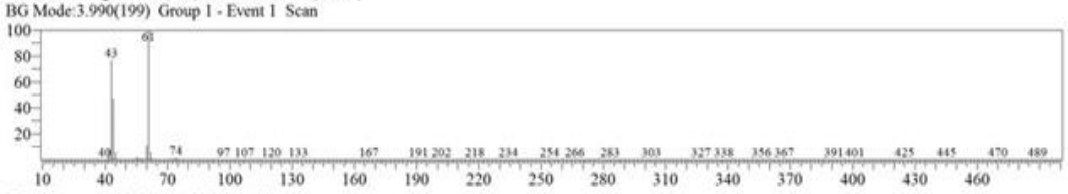
SI:94 Formula:C10H16 CAS:3779-61-1 MolWeight:136 RetIndex:976

CompName:trans-.beta.-Ocimene $$ 1,3,6-Octatriene, 3,7-dimethyl-, (E)- $$ .beta.-trans-Ocimene $$ trans-3,7-Dimethyl-1,3,6-Octatriene $$ Ocimene, trans-.be

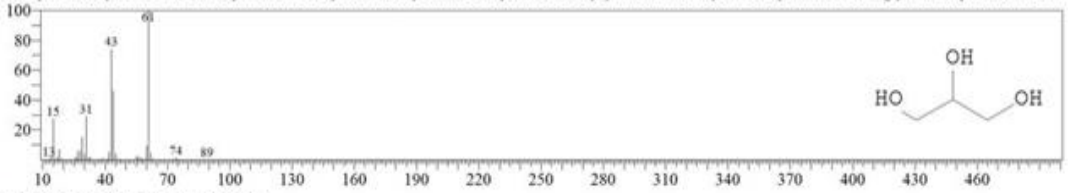


Appendix (C) MS Library of the *A. digitata* seed oil

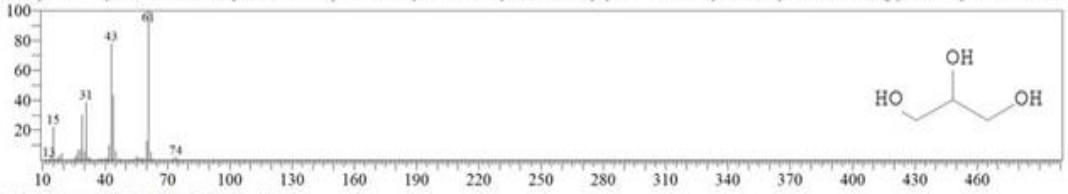
Library
<< Target >>
Line#:1 R.Time:4.030(Scan#:207) MassPeaks:265
RawMode:Single 4.030(207) BasePeak:61.00(61408)
BG Mode:3.990(199) Group 1 - Event 1 Scan



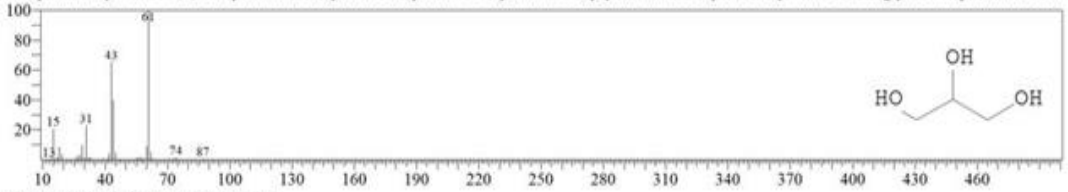
Hit#:1 Entry:1238 Library:NIST11s.lib
SI:98 Formula:C3H8O3 CAS:56-81-5 MolWeight:92 RetIndex:967
CompName:Glycerin $$ 1,2,3-Propanetriol $$ Glycerol $$ Glycerine $$ Glyceritol $$ Glycyl alcohol $$ Glyrol $$ Glysanin $$ Osmoglyn $$ Propanetriol $$ Tri



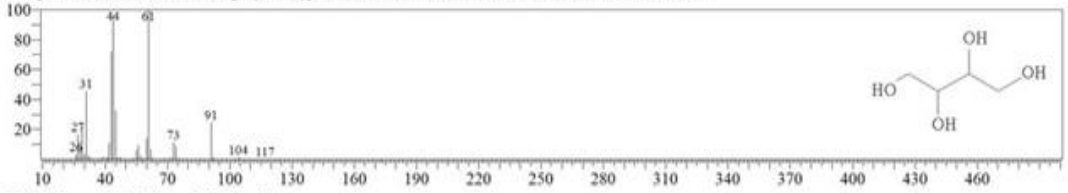
Hit#:2 Entry:1187 Library:NIST11.lib
SI:98 Formula:C3H8O3 CAS:56-81-5 MolWeight:92 RetIndex:967
CompName:Glycerin $$ 1,2,3-Propanetriol $$ Glycerol $$ Glycerine $$ Glyceritol $$ Glycyl alcohol $$ Glyrol $$ Glysanin $$ Osmoglyn $$ Propanetriol $$ Tri



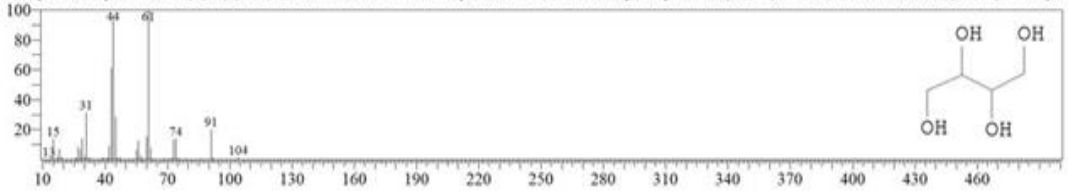
Hit#:3 Entry:1237 Library:NIST11s.lib
SI:96 Formula:C3H8O3 CAS:56-81-5 MolWeight:92 RetIndex:967
CompName:Glycerin $$ 1,2,3-Propanetriol $$ Glycerol $$ Glycerine $$ Glyceritol $$ Glycyl alcohol $$ Glyrol $$ Glysanin $$ Osmoglyn $$ Propanetriol $$ Tri



Hit#:4 Entry:4250 Library:NIST11s.lib
SI:88 Formula:C4H10O4 CAS:2319-57-5 MolWeight:122 RetIndex:1229
CompName:1,2,3,4-Butanetetrol, [S-(R*,R*)]- $$ Threitol, L- $$ l-Threitol $$ L-1,2,3,4-Butanetetraol $$



Hit#:5 Entry:4249 Library:NIST11s.lib
SI:87 Formula:C4H10O4 CAS:149-32-6 MolWeight:122 RetIndex:1229
CompName:Erythritol $$ 2(R),3(S)-1,2,3,4-Butanetetrol $$ meso-Erythritol $$ 1,2,3,4-Tetrahydroxybutane, (R*,S*)- $$ 1,2,3,4-Butanetetrol, (R*,S*)- $$ Erytl



Appendix (D) MS Library of the *O. basilicum* seed oil

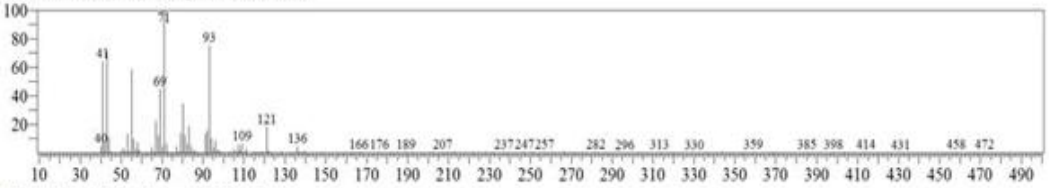
Library

<< Target >>

Line#:1 R.Time:5.600(Scan#:521) MassPeaks:266

RawMode:Single 5.600(521) BasePeak:71.05(13432)

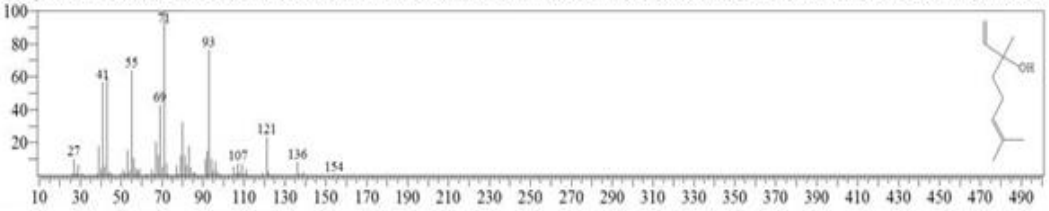
BG Mode:5.640(529) Group 1 - Event 1 Scan



Hit#:1 Entry:17562 Library:NIST11.lib

SI:97 Formula:C10H18O CAS:78-70-6 MolWeight:154 RetIndex:1082

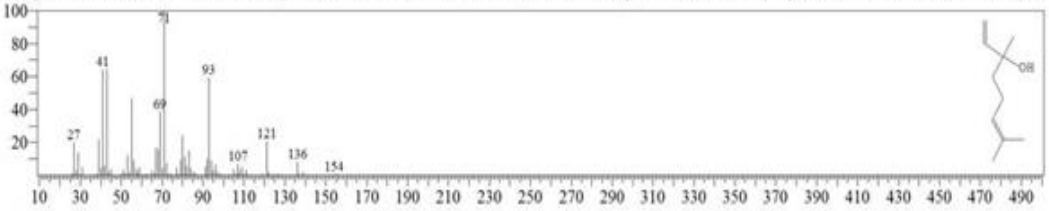
CompName:1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl-2,7-octadien-6-ol $$ allo-Ocimenol $



Hit#:2 Entry:9979 Library:NIST11s.lib

SI:95 Formula:C10H18O CAS:78-70-6 MolWeight:154 RetIndex:1082

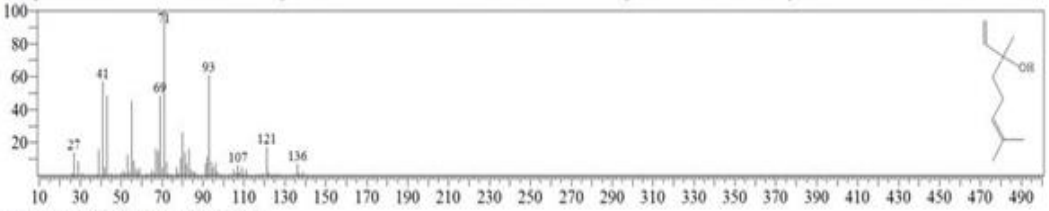
CompName:1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl-2,7-octadien-6-ol $$ allo-Ocimenol $



Hit#:3 Entry:9983 Library:NIST11s.lib

SI:94 Formula:C10H18O CAS:78-70-6 MolWeight:154 RetIndex:1082

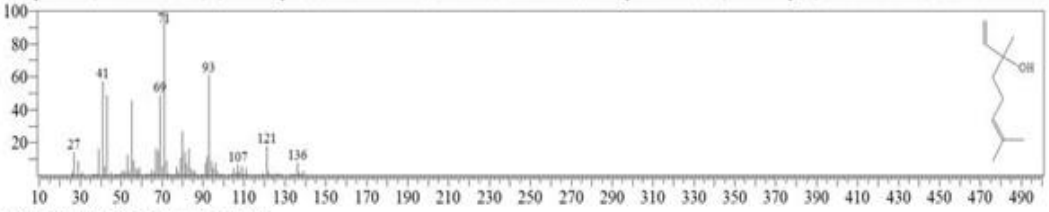
CompName:1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl-2,7-octadien-6-ol $$ allo-Ocimenol $



Hit#:4 Entry:9984 Library:NIST11s.lib

SI:94 Formula:C10H18O CAS:78-70-6 MolWeight:154 RetIndex:1082

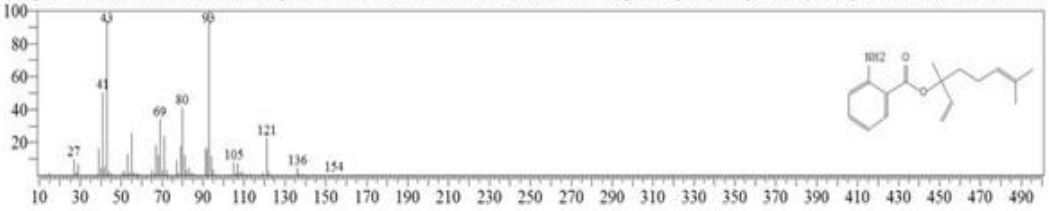
CompName:1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl-2,7-octadien-6-ol $$ allo-Ocimenol $



Hit#:5 Entry:97575 Library:NIST11.lib

SI:87 Formula:C17H23NO2 CAS:7149-26-0 MolWeight:273 RetIndex:2157

CompName:1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate $$ Anthranilic acid, 1,5-dimethyl-1-vinyl-4-hexenyl ester $$ Linalyl anthranilate $$ Anthranilic



Appendix (E) MS Library of *M. oleifera* seed oil

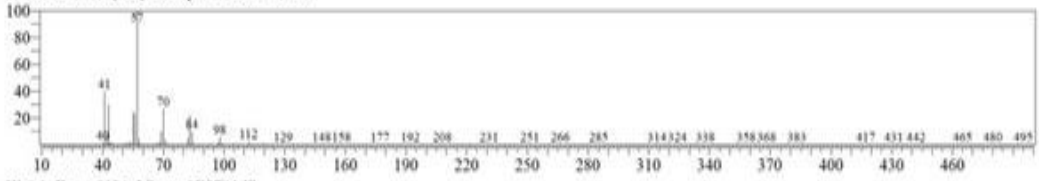
Library

<< Target >>

Line#:1 R.Time:4.620(Scan#:325) MassPeaks:258

RawMode:Single 4.620(325) BasePeak:57.05(75744)

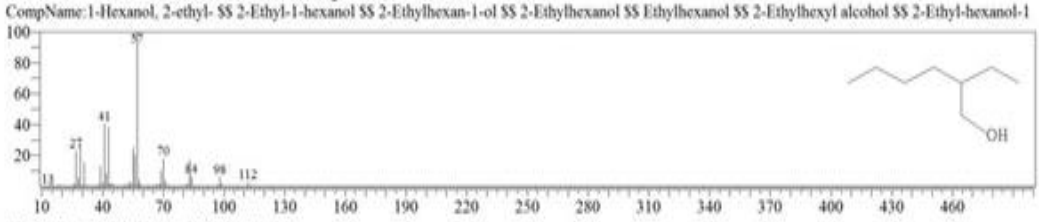
BG Mode:4.585(318) Group 1 - Event 1 Scan



Hit#:1 Entry:8034 Library:NIST11.lib

SI:96 Formula:C8H18O CAS:104-76-7 MolWeight:130 RetIndex:995

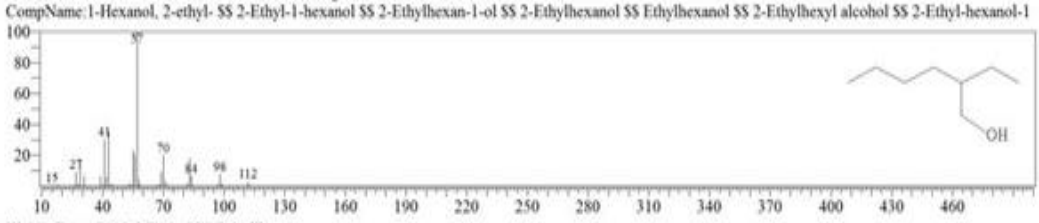
CompName:1-Hexanol, 2-ethyl- $$ 2-Ethyl-1-hexanol $$ 2-Ethylhexan-1-ol $$ 2-Ethylhexanol $$ Ethylhexanol $$ 2-Ethylhexyl alcohol $$ 2-Ethyl-hexanol-1



Hit#:2 Entry:5630 Library:NIST11s.lib

SI:96 Formula:C8H18O CAS:104-76-7 MolWeight:130 RetIndex:995

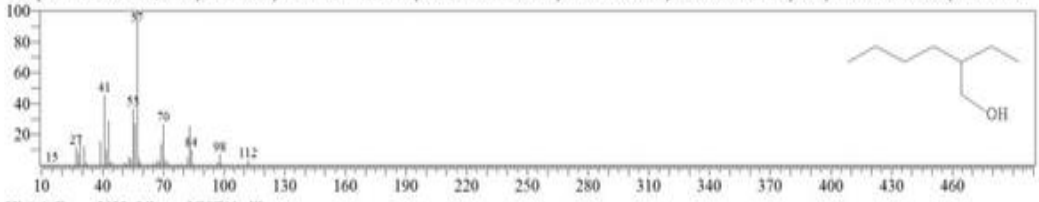
CompName:1-Hexanol, 2-ethyl- $$ 2-Ethyl-1-hexanol $$ 2-Ethylhexan-1-ol $$ 2-Ethylhexanol $$ Ethylhexanol $$ 2-Ethylhexyl alcohol $$ 2-Ethyl-hexanol-1



Hit#:3 Entry:5626 Library:NIST11s.lib

SI:96 Formula:C8H18O CAS:104-76-7 MolWeight:130 RetIndex:995

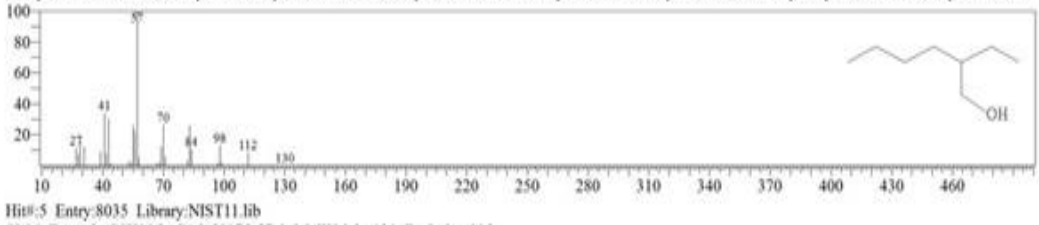
CompName:1-Hexanol, 2-ethyl- $$ 2-Ethyl-1-hexanol $$ 2-Ethylhexan-1-ol $$ 2-Ethylhexanol $$ Ethylhexanol $$ 2-Ethylhexyl alcohol $$ 2-Ethyl-hexanol-1



Hit#:4 Entry:5622 Library:NIST11s.lib

SI:96 Formula:C8H18O CAS:104-76-7 MolWeight:130 RetIndex:995

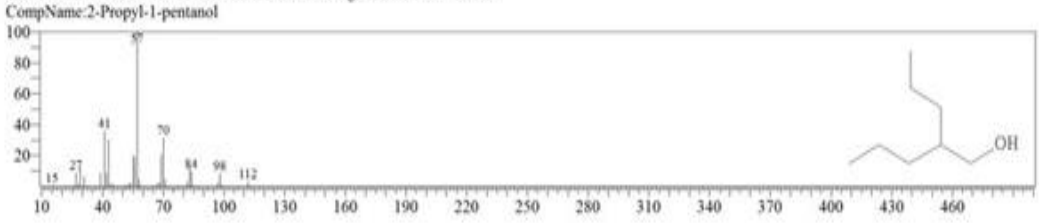
CompName:1-Hexanol, 2-ethyl- $$ 2-Ethyl-1-hexanol $$ 2-Ethylhexan-1-ol $$ 2-Ethylhexanol $$ Ethylhexanol $$ 2-Ethylhexyl alcohol $$ 2-Ethyl-hexanol-1



Hit#:5 Entry:8035 Library:NIST11.lib

SI:96 Formula:C8H18O CAS:58175-57-8 MolWeight:130 RetIndex:995

CompName:2-Propyl-1-pentanol



LIST OF PUBLICATIONS

1. Azhari H. Nour, Aini N. Mohamed, **Abeer A. Idris**, Awadalla B. Omer and Mohammed B. Sulieman. Distribution of Fatty Acids In Fresh and Thermally Treated Oxidized Vegetable-Cooking Oils, Journal of Sciences, 5:15-36, **2018**.

2. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa and Omer A. Omer Ishag. Physicochemical Properties and Fatty Acids Composition of Sudanese Baobab (Adansonia Digitata L.) Seed Oil, International Journal of Pharma and Bio Sciences, 11(1): 34-42, **2020.**

3. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa, Omer A. Omer Ishag and Abdurahman H. Nour. Physicochemical Properties and Chemical Composition of Basil (*Ocimum Basilicum* L.) Seed Oil, Asian Journal of Applied Chemistry Research, 8(1): 1-12, **2020**.

4. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa and Omer A. Omer Ishag. Physicochemical Properties and Fatty Acids Composition of *Moringa oleifera* Seed Oil, Journal of the Turkish Chemical Soction A: Chemisrty, 7(3): 911-920, **2020**.

5. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa and Omer A. Omer Ishag. Chemical Composition and Repellent Activity of Methyl Cinnamate Rich Basil (Ocimum basilicum L.) Essential Oil (Submitted).

6. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa and Omer A. Omer Ishag. Chemical Composition and Repellent Activity of *Cyperus rotundus* Essential Oil (Submitted).

7. **Abeer A. Idris**, Azhari H. Nour, Mahmoud M. Ali, Ibrahim Y. Erwa and Omer A. Omer Ishag. Formulation and cockroach repellent product form combination of essential and fixed oils (in progress).