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Thesis title:

Study of Flavonoids and Oils from Some Medicinal Plants

A Thesis submitted in a fulfillment of the requirement for the PhD degree in chemistry

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الاستهلال

قال تعالي:

(اللَّهُ لاَ إِلَهَ إِلاَّ هُوَ الْحَيُّ الْقَيُّومُ لاَ تَأْخُذُهُ سِنَةٌ وَلاَ نَوْمٌ لَّهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الأَرْضِ مَن ذَا الَّذِي يَشْفَعُ عِندَهُ إِلاَّ بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلاَ يُحِيطُونَ بِشَيْءٍ مِّنْ عِلْمِهِ إِلاَّ بِمَا شَاء وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالأَرْضَ وَلاَ يَؤُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ)

صدق الله العظيم

الاية (255)- سورة البقرة

Dedication

I dedicated this work, to:

My father

My mother

My wife

My brother and sisters

Acknowledgements

First,Iwould like to thank the must Merciful, Omnipotent Almighty for the great help blessing during my whole life and especially in research.

It is my pleasure to express my gratitude to my supervisor **professorMohamed Abdel karimMohamed**, who has given me much of this time for suggestions and supervision of this work.

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ABSTRACT

This study aimed to extract and investigate the physiochemical properties of oil seeds of some medicinal plants, in addition to investigate the flavonoid types present in *F.vulgare.* The physiochemical properties of the extracted oil of *D. Microcarpum* seeds showed the Viscosity (56CP), Density gm/cm^3 (0.915), Reflective index (1.470),Iodine value ml/g (150.74),colour(yellow/red) (2.30/0.11),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (63.87),Acid value mg KOH/g oil(3.88) and Saponification value mg KOH /g oil (160.58). In addition, the GC-MS analysis results revealed the presence of 17 components, the major constituents were 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.23%); 9-octadecenoic acid (Z)-, methyl ester (21.69%); Hexadecanoic acid, methyl ester (17.85%) and Methyl stearate (14.80%).The physiochemical properties of the extracted oil of *A.amara*seeds indicated the Viscosity (53CP), Density gm/cm^3 (0.917), Reflective index (1.473),Iodine value ml/g (176.4),colour(yellow/red)(33.35/00),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (123.7),Acid value mg KOH/g oil(1.63), and Saponification value mg KOH /g oil (94.421). In addition, The GC-MS analysis results showed24 components dominated by 9, 12 Octadecadienoic acid (Z, Z)-, methyl ester (29.23%); 9-octadecenoic acid (Z)-, methyl ester (21.69%); and Hexadecanoic acid, methyl ester (17.85%).The physiochemical properties of the extracted oil of *C.obtisufolia* seeds were shownthe Viscosity (58CP), Density gm /cm^3 (0.915), Reflectiveindex(1.472),Iodinevalueg/ml(167.85)colour(yellow/red)(10.105/.010) ,Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (81),Acid value mg KOH/g oil(6.41), and Saponification value mg KOH /g oil (152.05).Additionally The GC-MS analysis results revealed the presence of 16 components. Major constituents were 12-Octadecadienoic acid (Z, Z)-, methyl ester (38.75%); Hexadecanoic acid, methyl ester (18.50%); Methyl stearate (10.98%); and 9-octadecenoic acid (Z)-, methyl ester (10.67%).The physiochemical properties of the extracted oil of *F.Sylvatica*seeds were investigated and the obtain results were shown the Viscosity (52CP), Density gm/cm^3 (0.917), Reflective index

(1.471),colour(yellow/red)(10.10/1.40),Iodine valueg/ml(159.21),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (37.698),Acid value mg KOH/g oil(2.511), and Saponification value mg KOH /g oil (176.361). The GC-MS analysis results showed 16 components dominated by: 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (35.74%); Hexadecanoic acid, methyl ester (24.22%); Methyl stearate (15.27%); and 9-octadecenoic acid (Z)-, methyl ester (12.14%).The physiochemical properties of the extracted oil of *E.vesicaria* seeds were showntheViscosity (58CP), Density gm/cm^3 (0.916), Reflective index (1.473),colour(yellow/red)(43.2/00),Iodine valueg/ml(176.41),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (1.984),Acid value mg KOH/g oil(6.407), and Saponification value mg KOH/g oil (159. 51). The GC-MS analysis results revealed the presence of 30 components. The major constituents are 13-Docosenoic acid, methyl ester, (Z) – (33.80%); cis-13-Eicosenoic acid (13.83%); 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (12.09%); and 9-octadecenoic acid (Z)-, methyl ester (11.89%).The physiochemical properties of the extracted oil of *A.nubica* seeds were shown is Viscosity (54CP), Density gm/cm^3 (0.917), Reflectiveindex(1.471),Iodinevalueg/ml(159.29),colour(yellow/red)(44.3/1.40),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (23.90),Acid value mg KOH/g oil(1.11), and Saponification value mg KOH /g oil (160.805). The GC-MS analysis results showed 20 components dominated by: 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (44.26%); 9-octadecenoic acid (Z)-, methyl ester (22.20%); Hexadecanoic acid, methyl ester (17.17%); and Methyl stearate (6.74%).The obtained results of UV and FTIR; revealed that the flavonoid present in the extracts of *F.vulgare* perhaps is flavones.

مستخلص البحث

هدفت الدراسة الى استخلاص زيت بذور بعض النباتات الطبية و دراسة الخواص الفيزوكيميائية للزيت المستخلص، بالاضافة الي التحقق من نوع الفلفونيد الموجود في نبات الشمار. اوضحت الخواص الفيزوكيميائية للزيت المستخلص من نبات ابو ليلى ان اللزوجة 56 سنتي بويز، و الكثافة 0.915 جم/مل، و معامل الانكسار 1.470،,ورقم اليود150.74مل/جم، اللون اصفر / احمر 0.105/2.30و رقم البيروكسيد 63.87 مل/مج، و قيمة الحموضة 3.88 مل/جم، و رقم التصبن 160.58مل/جم. بالاضافة نتائج تحليل كروماتوغرافيا الغاز – مطياف الكتلة اوضحت ان الزيت يحتوي علي 17 مكونا كيميائيا و السائد منها (9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.23%), 9-octadecenoic acid (Z)-, methyl ester (21.69%)**,** hexadecanoic acid, methyl ester (17.85%), methyl stearate (14.80%)). اظهرت الخواص الفيزوكيميائية لنبات العرد ان اللزوجة 53 سنتيبويز، و الكثافة 0.917 جم/مل، و معامل الانكسار 1.473،رقم اليود 176.74مل/جم، اللون اصفر /احمر 0.00/33.35و رقم البيروكسيد 123.7مل/مج، و قيمة الحموضة 1.63مل/جم، و رقم التصبن 94.421 مل/جم. كما اظهرت نتائج تحليل كروماتوغرافيا الغاز- مطياف الكتلة ان الزيت يحتوي علي 24 مكونا و السائدة منها (9, 12 octadecadienoic acid (Z, Z)-, methyl ester (29.23%),9-octadecenoic acid (Z)-, methyl ester (21.69%), hexadecanoic acid, methyl ester (17.85%)). كما اظهرت الخواص الفيزوكيميائية لنبات الكول ان اللزوجة 58 سنتيبويز، و الكثافة 0.915 جم/مل، و معامل الانكسار 1.472 ورقم اليود 167.41مل/جم، اللون اصفر/ احمر 0.00/10.105 رقم البيروكسيد 81 جم/مج، و قيمة الحموضة 6.41 مل/جم، و رقم التصبن 152.05مل/جم. كما اظهرت نتائج تحليل كروماتوغرافيا الغاز- مطياف الكتلة ان الزيت يحتوي علي 16 مكونا و السائدة منها (**9**,12-octadecadienoic acid (Z, Z)-, methyl ester (38.75%)**,** hexadecanoic acid, methyl ester (18.50%);methyl stearate (10.98%), 9-octadecenoic acid (Z)-, methyl ester (10.67%)). و كذلك اظهرت الخواص الفيزوكيميائية لنبات الزان ان اللزوجة 52 سنتيبويز، و الكثافة 0.917 جم/مل، و معامل الانكسار 1.471،ورقم اليود 159.21مل/جم واللون اصفر/ احمر1.40/10.10 رقم البيروكسيد 37.698مل/مج، و قيمة الحموضة 2.511مل/جم، و رقم التصبن176.361مل/جم. كما اظهرت نتائج تحليل كروماتوغرافيا الغاز- مطياف الكتلة ان الزيت يحتوي علي 16 مكونا و السائدة منها (9, 12-octadecadienoic acid (Z, Z)-, methyl ester (35.74%), hexadecanoic acid, methyl ester (24.22%), methyl stearate (15.27%), 9-octadecenoic acid (Z)-, methyl ester (12.14%)). اضافتا اظهرت الخواص الفيزوكيميائية لزيت بذور نبات الجرجير ان اللزوجة 58سنتيبويز، و الكثافة 0.916جم/مل، و معامل الانكسار 1.473 ورقم اليود 176.41مل/جم واللون اصفر / احمر 0.00/43.2 ورقم البيروكسيد

1.984مل/مج، و قيمة الحموضة 6.407مل/جم، و رقم التصبن 159.51مل/جم. كما اظهرت نتائج تحليل كروماتوغرافيا الغاز- مطياف الكتلة ان الزيت يحتوي علي 30 مكونا و السائدة منها (13-docosenoic acid, methyl ester, (Z) – (33.80%), cis-13-eicosenoic acid, methyl ester (13.83%), 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (12.09%), 9-octadecenoic acid (Z)-, methyl ester (11.89%)).كما اظهرت الخواص الفيزوكيميائية لزيت بذور نبات اللعوت ان اللزوجة 54سنتيبويز، و الكثافة 0.917جم/مل، و معامل الانكسار 1.471 ورقم اليود 159.29مل/جم واللون اصفر / احمر 1.40/44.3رقم البيروكسيد 23.90مل/مج، و قيمة الحموضة 1.11مل/جم، و رقم التصبن 160.805مل/جم. كما اظهرت نتائج تحليل كروماتوغرافيا الغاز- مطياف الكتلة ان الزيت يحتوي علي 20 مكونا و السائدة منها (9, 12-octadecadienoic acid (Z, Z)-, methyl ester (44.26%), 9-octadecenoic acid (Z)-, methyl ester (22.20%), hexadecanoic acid, methyl ester (17.17%), methyl stearate (6.74%)). بالاضافة الي ان النتائج المتحصل عليها من مطياف الاشعة فوق البنفسجية و الاشعة تحت الحمراء ان الفلافونيد الموجود في نبات الشمار ربما يكون الفلافون.

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Natural product is a chemical compound or substance produced by a living organism—that is, found in nature(Anulika et al., 2016, Boland and Donnelly, 1998, Dean, 1963). In the broadest sense, natural products include any substance produced by life(Finar, 2006, Geissman, 1962). Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients (Harborne, 1967). Within the field of organic chemistry, the definition of natural products is usually restricted to mean purified organic compounds isolated from natural sources that are produced by the pathways of primary or secondary metabolism (Harborne et al., 1975). Within the field of medicinal chemistry, the definition is often further restricted to secondary metabolites (Harborne, 2013, Harborne and Mabry, 2013). Secondary metabolites are not essential for survival, but nevertheless provide organisms that produce them an evolutionary advantage (Harborne, 1989). Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as "chemical warfare" agents against prey, predators, and competing organisms (Harborne, 2013). Natural products sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating diseases. As such, natural products are the active components not only of most traditional medicines but also many modern medicines. Furthermore, because the structural diversity of natural products exceeds that readily achievable by chemical synthesis, and synthetic analogs can be prepared with improved potency and safety, natural products are often used as starting points for drug discovery. In fact, natural products are the inspiration for approximately

one half of U.S. Food and Drug Administration-approved drugs (Harborne, 2013).

1.2. Problem statement

The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The major source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or premature deaths, searching for available foods for the treatment of diseases (Dias et al., 2012, Bull). There is an urgent need to search for new antimicrobial active compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new infections disease as well as development of resistance to the antibiotics in use (Gulfraz et al., 2008).

1.3. Research objectives

The objectives of this study were:

1. To extract oil from the seeds of potential medicinal plants
2. To investigate the physiochemical properties of the extracted oils
3. To extract the major flavonoid from *Foeniculumvulgare*
4. To suggest a partial structure for the isolated flavonoid

1.4. Research scope

The scope of this study covers extraction of seeds oil of six potential medicinal plants (*ErucaVesicaria, DetariumMicrocarpum, FagusSylvatica, Cassia Obtusifolia, Acacia Nubica and Albiza Amara*), and then investigation of physiochemical properties of the extracted oils. In addition, extract the major flavonoid from *Foeniculumvulgare* and suggest a partial structure for the isolated flavonoid.

CHAPTER TWO

LITTERATEUR REVIEW

2.1. Oils

Oil is any no polar chemical substance that is a viscous liquid at ambient temperatures and is both hydrophobic (does not mix with water literally "water fearing") and lipophilic (mixes with other oils, literally "fat loving"). Oils have a high carbon and hydrogen content and are usually flammable and surface active. Most oils are unsaturated lipids that are liquid at room temperature.

The general definition of oil includes classes of chemical compounds that may be otherwise unrelated in structure, properties, and uses. Oils may be animal, vegetable, or petrochemical in origin, and may be volatile or non-volatile. They are used for food (e.g., olive oil), fuel (e.g., heating oil), medical purposes (e.g., mineral oil), lubrication (e.g. motor), and the manufacture of many types of paints, plastics, and other materials. Specially prepared oils are used in some religious ceremonies and rituals as purifying agents.

2.2. Fixed Oils

Fixed oils are oily substances obtained mainly from plant sources. They are produced in various organs of plants. Chemically, fixed oils arc glyceryl esters of various fatty acids. Different fixed oils differ from each other in the types of the fatty acids that form the oil. Chemically, they are similar to fats but differ in their physical state of occurrence. Fixed oils are usually oily liquids and the fats are generally solid or semi-solid substances. Fixed oils are non-volatile in nature and leave permanent translucent greasy spot on paper. Fixed oils are freely soluble in ether, chloroform and light petroleum. They are insoluble in and immiscible with water. Their specific gravity is less than 1.0, as a result of which they Goat on water. Many fixed oils are in use in pharmacy both as pharmaceutic necessities (excipients) and as solvents or carriers of a number of medicinal substances. Many of them also possess medicinal properties, e.g., Castor oil is used in the treatment of a number of disease.

2.3. Essential Oils

The term essential oil dates back to the sixteenth century and derives from the drug *(Quinta essential*), named by Paracelsus von Hohenheim of Switzerland(Hu et al., 2004). Numerous authors have attempted to provide a definition of essential oils. The French Agency for Normalizationdefines essential oils as follows:The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes.

An essential oil is a liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as oil of clove. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majorities of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits.All parts of aromatic plants may contain essential oils

2.4 oils extraction methods

There are several methods of extraction of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused around the globe (Herbert, 1985).

i. Hydrodistillation (HD)

The conventional method for the extraction of essential oils is hydrodistillation, in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser.The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively.

ii.Solvent extraction

Solvent extraction, also known as liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done by using two liquids that don't mix, for example, water and an organic solvent. In the solvent-extraction method of essential oils recovery, an extracting unit is

loaded with perforated trays of essential oil plant material and repeatedly washed with thesolvent.

iii. Soxhlet extraction

A Soxhlet extractor is a piece of laboratory apparatus(Barrett and Davies, 1985), invented in 1879 by Franz von Soxhlet(Gournelis et al., 1997). It was originally designed for the extraction of a lipid from a solid material.Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid(Coppola et al., 1987).

vi. Cold pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant .Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils(Felton and Gatehouse, 1996). In this process, the outer layer of the plants containing the oil is removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

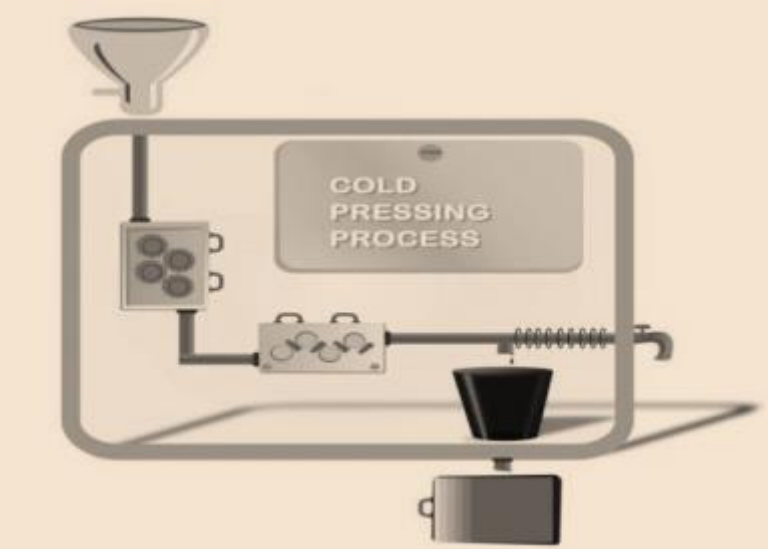
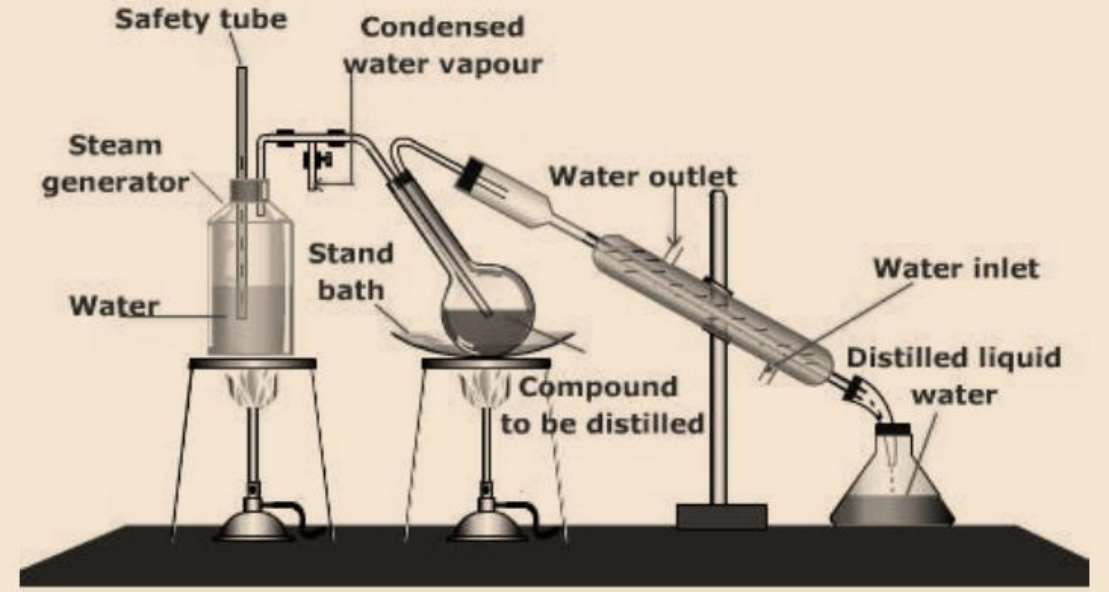


Fig (2.1): Cold Pressing Method(Felton and Gatehouse, 1996)

v. Steam Distillation

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected(Williams, 2013). The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.



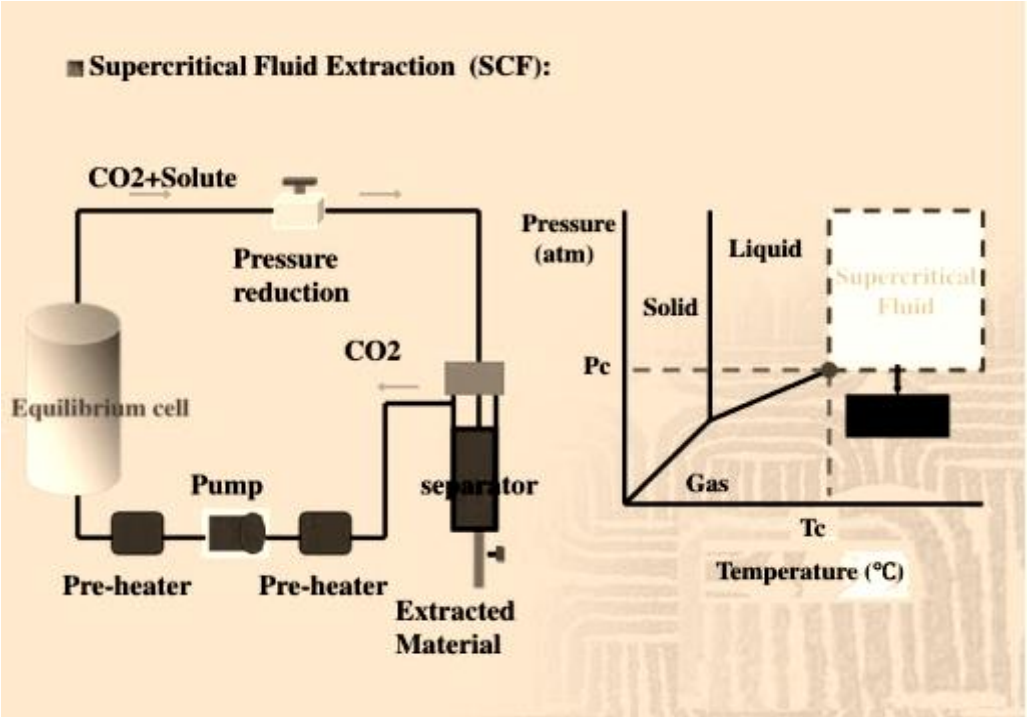
Fig(2.2): Apparatus for steam distillation (Williams, 2013)

One of the disadvantages of conventional techniques is related with the thermolability of essential oils components which undergo chemical alterations (hydrolysis, isomerization, and oxidation) due to the high applied temperatures.New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO_2 emissions.

iv. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent.Supercritical fluids (Jordan, 1991)such as essential oil extraction and metal cation extraction (Scheme 4).In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32^oC), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract(Jordan, 1991). The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes(Faulkner, 1995). These essential oils can include limonene and other straight solvents.Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE

yielded a higher antioxidant activity than extract prepared by other methods.This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of superior quality, with better functional and biological activities(Highet et al., 2012). Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

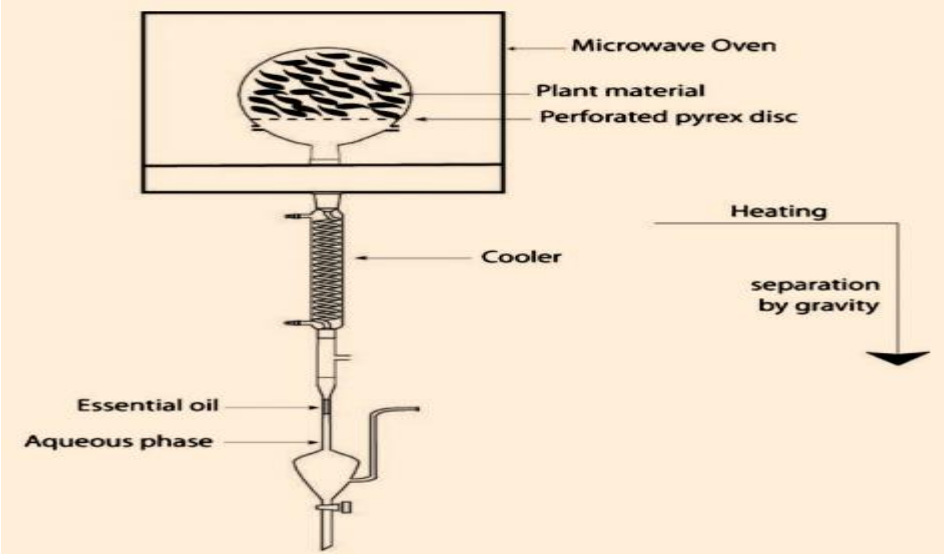


Fig(2.3):Supercritical fluid extraction set (Highet et al., 2012)

iiv. Microwave hydrodiffusion and gravity (MHG):

(MHG) Is a new green technique for the extraction of essential oils. This green extraction technique is an original microwave blend microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants (Scheme 5)(Guenther and Althausen, 1948). Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and as it

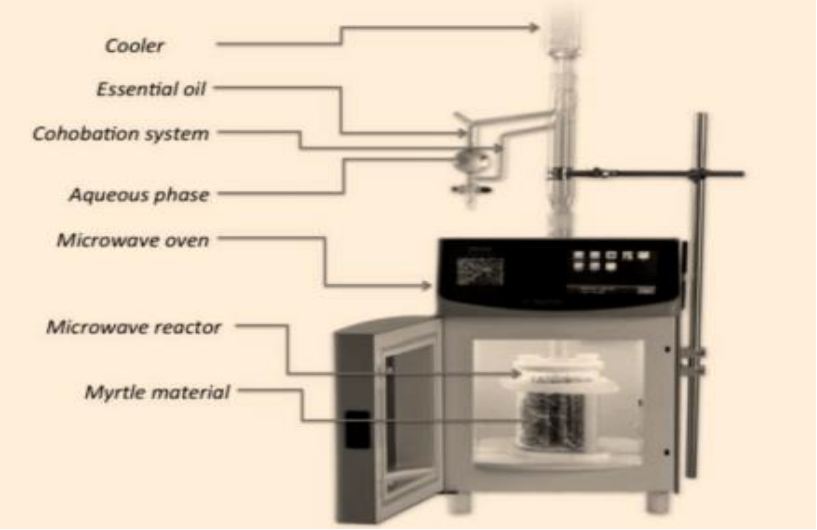
does require less energy (Likibi et al.). The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental impact and power saving (Bashir, 2019).



Fig(2.4): Microwave hydrodiffusion and gravity apparatus(Guenther and Althausen, 1948)

iiiv. Solvent-free microwave extraction

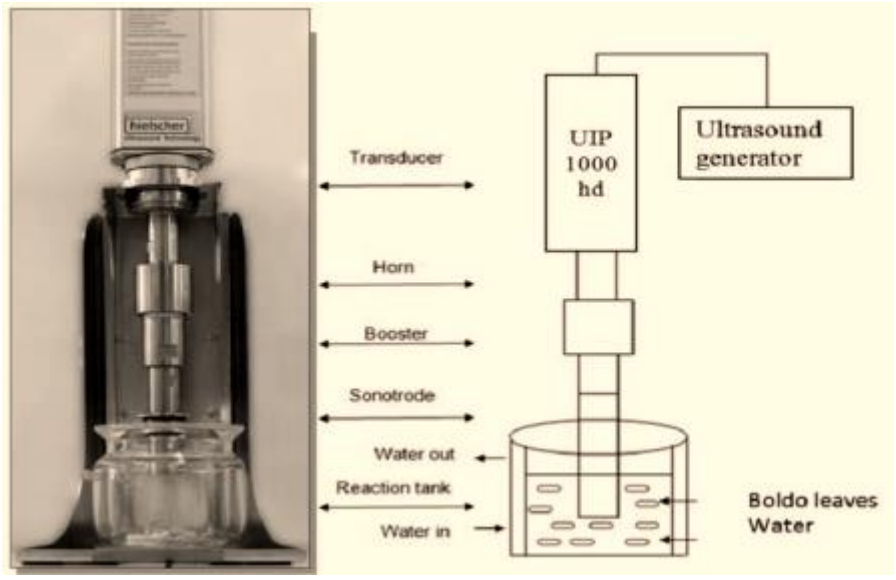
Solvent-free microwave extraction (SFME) is an extraction procedure of essential oil which is accomplished by the water of the plant material without added any solvent(Wang and Weller, 2006). Based on the integration ofdry distillation and microwave heating energy. It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent(Starmans and Nijhuis, 1996). In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature, and extraction time were controlled by the panel in the instrument.



Fig(2.5):Solvent-free microwave extraction system (Starmans et al., 1996)

x. Ultrasonic-assisted extraction

Ultrasonic-assisted extraction (UAE) is a good process to achieve high valuable compounds and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material(Khan and Dwivedi, 2018) . The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation (Scheme 7). In these applications the power ultrasonic increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer(Soxhlet, 1879). The plants raw material is immersed in water or another solvent (Methanol or ethanol or any one from the solvents) and at the same time, it is subjected to the work of ultrasound (Rassem et al., 2016). This technique has been used for the extraction of many essential oils especially from the flower, leavesor seeds (Lucchesi et al., 2004).

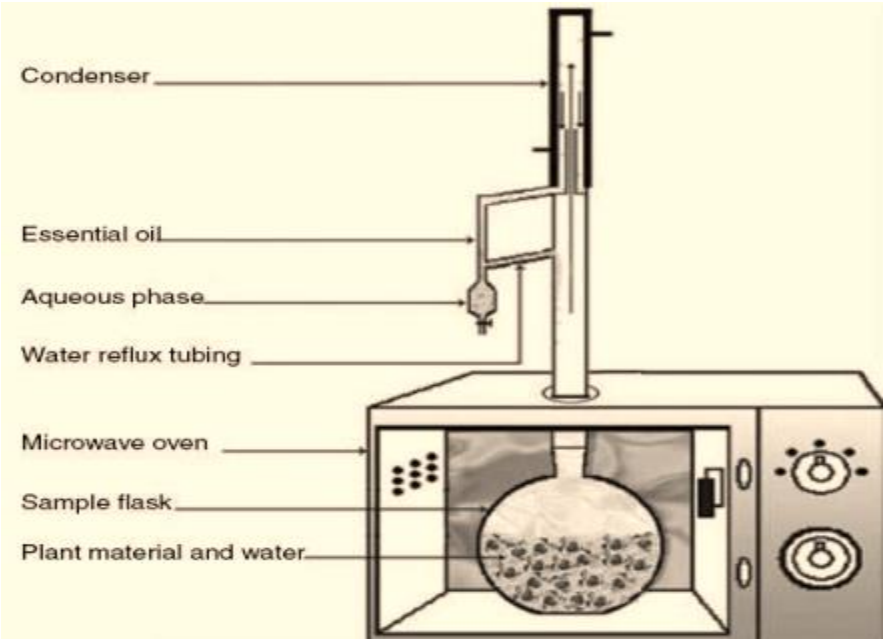


Fig(2.6):Ultrasonic-assisted extraction system (De Castro and Priego-Capote, 2010)

xi. Microwave-assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizinga microwave oven in the extraction process. The efficiency of Microwaveassisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample (Badami and Rai, 2004). High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique (Scheme8). Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere (Pourmortazavi and Hajimirsadeghi, 2007, Rozzi et al., 2002)and consuming only a fraction of the energy used in conventional extraction methods(Fadel et al., 1999).The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions. Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which

mainly are a reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process.The principle of heating using Microwave-assisted hydrodistillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously(Capuzzo et al., 2013a).



Fig(2.7): Microwave-assisted hydrodistillation apparatus (Capuzzo et al., 2013b)

2.5. Chemistry of oils

Oils are localized in the cytoplasm of certain plant cell secretions, which lies in one or more organs of the plant; namely, the secretory hairs or trichomes, epidermal cells, internal secretory cells, and the secretory pockets. These oils are complex mixtures that may contain over 300 different compounds(Vian et al., 2008).They consist of organic volatile compounds, generally of low molecular weight below 300. Their vapor pressure at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in the vapor state (Chemat et al., 2004). These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohols, aldehydes, and ketones

offer a wide variety of aromatic notes, such as fruity ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal (γ-selinene).

2.6. Gas chromatography-Mass Spectrometry

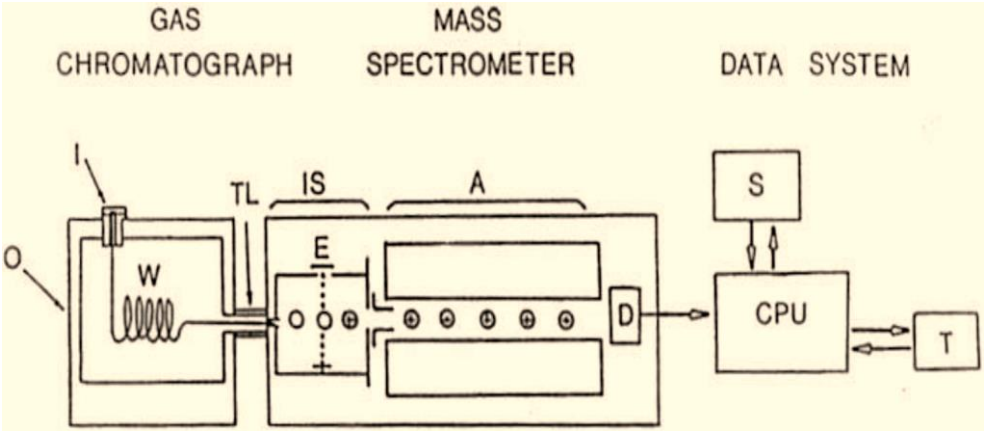
GC-MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS). It is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified and quantified, but it cannot readily separate them. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid-1950. Gas chromatography and mass spectrometry are, in many ways, highly compatible techniques. In both techniques, the sample is in the vapor phase, and both techniques deal with about the same amount of sample (typically less than 1 mg)(Hong et al., 2004).Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass spectrometry (MS) is acting as the detector for the GC. As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint. Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to

identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them(Santoro et al., 2007).

2.6.1. Gas Chromatography

All forms of chromatography involve the distribution, or partitioning, of a compound between a mobile phase and a stationary phase. In GC, the mobile phase is a gas and the stationary phase is an immobile, high molecular weight liquid which is deposited on or chemically bonded to the inner walls of long capillary tubing. The term GLC (gas-liquid chromatography) is also used to refer to this separation technique. The capillary tubing through which the sample moves is called the chromatographic or GC column. Presently, most GC columns used for this work are manufactured from fused silica. They are generally 30-60 m in length and have an internal diameter of about 0.2 mm(Rota et al., 2004). By covering the outside surface of these capillary columns with a polymeric coating, these flexible fused silica GC columns are made more durable. The analysis of effluents for organic compounds requires extraction of the organics from the water matrix, concentration of the extract, separation of individual components of the organic extract by a GC column and detection of the separated components as they are eluted from the GC column. Complex mixtures of organic compounds are extracted from effluents by using high-purity organic solvents. The low-volatility organic compounds extracted from an effluent sample can be concentrated to a small volume (typically, 1.0 mL or less) by removing the extraction solvent through evaporation. This concentration step is necessary in order to obtain detection limits in the low part-per-billion (ppb: 10-6 g/L). Some compounds of concern may be more volatile than the extraction solvent and would be lost in this process. Such compounds are removed from the sample by directly purging the aqueous sample using an inert gas and collecting the purged volatile compounds on an adsorbent trap designed for this purpose. In

either case, organic compounds from the sample are separated from the bulk aqueous matrix and concentrated for GC analysis(Si et al., 2006). The organic compounds are introduced into the GC column by injecting a few microlitres (μL) of the concentrated solvent extract into an injection port (non-volatile organics) or by heating the sorbent trap (volatile organics). An inert carrier gas (He, N_2, H_2), is used to sweep the extracted organic compounds, which are now in the vapor state, through the GC column. Compounds that have different solubilities in the liquid phase of the GC column will take different times to traverse the length of the column. For a specific set of experimental conditions, the time it takes a compound to travel through a GC column is a physical property of that compound - called its retention time. Generally, higher molecular weight compounds will have greater retention times than lower molecular weight compounds. Also, compounds that have a similar polarity to that of the liquid phase will be more soluble in the phase and will have greater retention times than compounds less soluble in the liquid phase. Therefore, organic compounds in a mixture can be separated from each other by using gas chromatography, and the retention times of these compounds can be used to assist in their identification. Some environmental samples are so complex that there are hundreds of compounds present in their concentrated organic extracts. There are currently no GC columns available that can completely separate all components of such complex mixtures from each other. However, in most cases the principal sample components can be detected individually(Sonboli et al., 2006) .



Fig(2.8): GC-MS System (Mirjalili et al., 2006)

In another design, ions travel through a magnetic field where their momentum is affected by the magnetic field strength. Conditions can be controlled to allow the analyzer to scan across a range of m/z to form a mass spectrum. This design is called a magnetic sector mass analyzer. An important concept in GC-MS is resolution. In GC, resolution refers to the ability of the GC column to separate components in a mixture from each other. In mass spectrometry, mass resolution refers to the ability of an analyzer to separate ions that have similar m/z(El Hadri et al., 2010).

i. Gas supply

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure(Moon et al., 2006).

ii. Injector

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.

iii. Column

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal(Moon et al., 2006).

vi. Oven

Gas chromatography has ovens that are temperature programmable.

2.6.2. Mass spectrometry

As the separated sample components elute from the GC column, they are monitored using any of a large number of detectors developed for this purpose. The most versatile of these detectors is the mass spectrometer (MS). When an MS detector is used to detect the compounds that elute from a GC column, the combined technique is called gas chromatography-mass spectrometry (GC-MS). Initially, molecules enter the source chamber of the mass spectrometer maintained under high vacuum, where they are bombarded by electrons. The energy transferred to molecules in this process causes them to ionize and dissociate into various fragment ions. Ions may be singly or multiply-charged. The positive ions formed are made to traverse an analyzer section. After the ions traverse the analyzer section where they are separated according to their mass-to-charge ratio (m/z), they are detected by an extremely sensitive device called an electron multiplier. By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. The mass spectrum of a compound is like a fingerprint that can be used to identify the original organic structure. It consists of a bar graph representation of the m/z of the ions and their abundances normalized to the most abundant ion (base peak). By matching the GC retention time of a sample component and its mass spectrum with those of a standard reference compound analyzed under the same conditions, a positive identification of the sample component is obtained. Several different mass analyzers have been developed. One of the most common designs consists of a square array of four parallel metal rods. By controlling radio-frequency (RP and DC voltages) to these rods, an oscillating electric field is generated and this allows ions to be filtered according to their m/z. At a specific setting of voltages, only ions of the desired m/z will have a stable trajectory and will be able to reach the electron multiplier. By changing the applied voltages in a specified manner, the mass spectrum of a compound can be generated as the ions of various m/z

are scanned. The entire process is performed in about one second. This design is called (QP) a quadruple mass analyzer.

i. Ion source

In the ion source, the products are ionized prior to analysis in the mass spectrometer

ii. Mass analyzer

There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis.

iii. Vacuum system

Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way.

vi. Detector

The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges)(Karasek and Clement, 2012).

2.7 UV-Vis Spectroscopy

Spectroscopy Spectroscopy involves investigating the interaction of electromagnetic field with matter. Historically, it originated as the study of wavelength based dispersion of visible light by a prism. The idea was expanded to include all the interactions involving radiative energy variation with wavelength or frequency. Spectroscopic data is represented as an emission spectrum of wavelength or frequency dependent response. Spectroscopy can be broadly classified into two categories – (a) techniques based on energy transfer between photon and sample, and (b) reflections, refraction, diffraction, dispersion, or scattering from the sample altering the amplitude, phase angle, polarization, or direction of propagation of the electromagnetic radiation. Over the years, spectroscopy has evolved as a potential tool for experiments and

analyses conducted in research laboratories and industries. This technique is essentially considered by analysts as an apparent solution. The objective should also be to use these spectroscopic techniques in control and industrial laboratories and to develop fully recognised spectroscopic techniques. This chapter reviews the interactions of ultraviolet, visible, and infrared radiations with matter. Irrespective of differences in the instrumentation, all spectroscopic techniques have many common attributes. The similarities as well as differences between various spectroscopic techniques have been outlined.

1. Principles and instrumentation for UV-Vis spectroscopy is an important physical tool which exploits light in ultraviolet, visible, and near infrared range of electromagnetic spectrum. Beer-Lambert law establishes a linear relationship between absorbance, concentration of absorbers (or absorbing species) in the solution and the path length. Therefore, UV-Vis spectroscopy can be employed for determining the concentration of the absorbing species, for a fixed path length. This is a very simple, versatile, fast, accurate and costeffective technique. Instrument employed for ultraviolet−visible (or UV-Vis) spectroscopy is called UV−Vis Spectrophotometer. This can be used to analyze liquids, gases and solids by using radiative energy corresponding to far and near ultraviolet (UV), visible (Vis) and near infrared (NIR) regions of electromagnetic spectrum. Consequently, predetermined wavelengths in these regions have been defined as: UV: 300 - 400 nm; Vis: 400 - 765 nm.

2.7.1 Principle:

A light beam is passed through an object and wavelength of the light reaching the detector is measured. The measured wavelength provides important information about chemical structure and number of molecules (present in intensity of the measured signal). Thus, both quantitative and qualitative information can be gathered. Information may be obtained as transmittance, absorbance or reflectance of radiation in 160 to 3500 nm wavelength range .The absorption of incident energy promotes electrons to excited states or the anti-bonding orbitals. For this transfer to occur, photon energy must match the energy

needed by electron to be promoted to next higher energy state. This process forms the basic operating principle of absorption spectroscopy.

Potentially, there may be three types of ground state orbitals involved:

i. σ (bonding) molecular orbital

ii. π (bonding) molecular orbital

iii. n (non-bonding) atomic orbital

Besides, the anti-bonding orbitals are:

i. σ* (sigma star) orbital

ii. ii. π* (pi star) orbital

The UV-Vis spectrum can by recorded via the following types of absorbance instruments:

i. Single beam spectrometer

ii. Double beam spectrometer

iii. Simultaneous spectrometer

Light source (mostly tungsten lamps), small holder and detector are common to all the three type of spectrometers. However, a filter may be used, in addition, to select one wavelength at a time. This filter is often termed as the monochromator. Single beam spectrometer includes a monochromator between the light source and specimen. The specimen is analysed individually for all wavelengths. Double beam spectrometer uses a single light source, monochromator, a splitter and a series of mirrors, to direct the beam towards the reference and the sample under investigation. Whereas, a simultaneous spectrometer uses an array of diodes for simultaneous detection of absorbance at all wavelengths. This is the fastest and most efficient of the three.

2.7.2 Instrumentation:

The basic components of a spectrometer include: light source (UV and visible), monochromator (wavelength selector),sample stage, and detector. A tungsten filament, continuous over UV region is generally used as light source. Detector is usually a photodiode or CCD. Photodiodes go with monochromators to filter light of a particular wavelength, to be fed to the detector. While monitoring the

absorbance in UV spectrum, the visible lamp must be turned off, and vice-versa. Figure 6 includes schematic UV−Vis Spectrometer.

Instrumental components

i. UV Source The power of radiating source should not vary in its operating wavelength range. Continuous UV spectrum is produced by electrically exciting deuterium or hydrogen at low pressures. The mechanism for generation of UV light includes creating an excited molecular species, that breaks into two atomic species and a UV photon. The emission wavelengths of both deuterium and hydrogen lamps are in 160 to 375 nm range. The material of the cuvettes needs to selected such that it does not absorb the light incident, because this will result in errors in obtained absorption spectrum. Thus, quartz is usually used.

ii. Visible Light Source Tungsten filament lamp is used as visible light source. This lamp can produce light in 350 to 2500 nm wavelength range. In a tungsten filament lamp, energy emitted is proportional to the fourth power of the operating voltage. Thus, in order to get stable emission, a highly stable voltage must be applied to the lamp. The stability of voltage is ensured by using electronic voltage regulators or constant-voltage transformers. Tungsten/halogen lamps include small quantities of iodine embedded within a quartz ‘envelope’, which also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, and produces a volatile compound WI2. As WI2 molecules hit the filament, they decompose, and redeposit tungsten back on the filament. The tungsten/halogen lamps usually have lifetime twice to the conventional tungsten filament lamp. Tungsten/halogen lamps are used in modern spectrophotometers owing to their high efficiency, and their output extends to UV region as well.

iii. Cuvettes Monochromator source is used; before reaching sample, light is divided in two parts of similar intensity with a half-mirror splitter. One part (or sample beam), travels via the cuvette having the solution of material to be examined in transparent solvent. Second beam, or reference beam, travels via

similar cuvette having only solvent. Reference and sample solution containers have to be transparent towards passing beam.

vi. Detectors Detector detects intensity of light transmitted by cuvettes and sends this data to a meter to record and display the values. Electronic detectors calculate and compare the intensities of light beams. Several UV−Vis spectrophotometers have two detectors – a phototube and a photomultiplier tube, and reference and sample beams are monitored simultaneously. The photomultiplier tube is the extensively used detector in UV-Vis instruments. It includes a photoemissive cathode (electrons are emitted from the cathode when photons strike it), several dynodes (a dynode emits multiple electrons when one electron strikes it) and an anode. The incident photon, after entering the tube, strikes the cathode. The cathode then emits multiple electrons, which are then accelerated towards the first dynode (whose potential is 90V more positive than cathode). The electrons strike the first dynode, leading to the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. All the electrons are eventually collected at the anode. By this time, each original photon has produced 106 – 107 electrons. The resulting current is amplified and measured. Photomultipliers are highly sensitive towards UV and visible radiations and have fast response times. However, photomultipliers are used only at low power radiation as high power light may damage them(Gandhimathi et al., 2012).

2.8. FT-IR Instrument

Infrared (IR) spectroscopy is a chemical analytical technique, which measures the infrared intensity versus wavelength (wavenumber) of light. Based upon the wavenumber, infrared light can be categorized as far infrared (4 ~ 400cm-1), mid infrared (400 ~ 4,000cm-1) and near infrared (4,000 ~ 14,000cm-1). Infrared spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter, chemical bonds will stretch, contract and bend. As a result, a chemical functional group

tends to adsorb infrared radiation in a specific wavenumber range regardless of the structure of the rest of the molecule. For example, the C=O stretch of a carbonyl group appears at around 1700cm-1 in a variety of molecules. Hence, the correlation of the band wavenumber position with the chemical structure is used to identify a functional group in a sample. The wavenember positions where functional groups adsorb are consistent, despite the effect of temperature, pressure, sampling, or change in the molecule structure in other parts of the molecules. Thus the presence of specific functional groups can be monitored by these types of infrared bands, which are called group wavenumbers. The early-stage IR instrument is of the dispersive type, which uses a prism or a grating monochromator. The dispersive instrument is characteristic of a slow scanning. A Fourier Transform Infrared (FTIR) spectrometer obtains infrared spectra by first collecting an interferogram of a sample signal with an interferometer, which measures all of infrared frequencies simultaneously. An FTIR spectrometer acquires and digitizes the interferogram, performs the FT function, and outputs the spectrum. Schematic illustration of FTIR system An interferometer utilizes a beamsplitter to split the incoming infrared beam into two optical beams. One beam reflects off of a flat mirror which is fixed in place. Another beam reflects off of a flat mirror which travels a very short distance (typically a few millimeters) away from the beamsplitter. The two beams reflect off of their respective mirrors and are recombined when they meet together at the beamsplitter. The re-combined signal results from the “interfering” with each other. Consequently, the resulting signal is called interferogram, which has every infrared frequency “encoded” into it. When the interferogram signal is transmitted through or reflected off of the sample surface, the specific frequencies of energy are adsorbed by the sample due to the excited vibration of function groups in molecules. The infrared signal after interaction with the sample is uniquely characteristic of the sample. The beam finally arrives at the detector and is measure by the detector. The detected interferogram can not be directly interpreted. It has to be “decoded” with a well-known mathematical

technique in term of Fourier Transformation. The computer can perform the Fourier transformation calculation and present an infrared spectrum, which plots adsorbance (or transmittance) versus wavenumber. When an interferogram is Fourier transformed, a single beam spectrum is generated. A single beam spectrum is a plot of raw detector response versus wavenumber. A single beam spectrum obtained without a sample is called a background spectrum, which is induced by the instrument and the environments. Characteristic bands around 3500 cm-1 and 1630 cm-1 are ascribed to atmospheric water vapor, and the bands at 2350 cm-1 and 667 cm-1 are attributed to carbon dioxide. A background spectrum must always be run when analyzing samples by FTIR. When an interferogram is measured with a sample and Fourier transformed, a sample single beam spectrum is obtained. It looks similar to the background spectrum except that the sample peaks are superimposed upon the instrumental and atmospheric contributions to the spectrum. To eliminate these contributions, the sample single beam spectrum must be normalized against the background spectrum. Consequently, a transmittance spectrum is obtained as follows. %T = I/Io Where %T is transmittance; I is the intensity measured with a sample in the beam (from the sample single beam spectrum); Io is the intensity measured from the back ground spectrum The absorbance spectrum can be calculated from the transmittance spectrum using the following equation.

A = -log10 T Where A is the absorbance.

The final transmittance/absorbance spectrum should be devoid of all instrumental and environmental contributions, and only present the features of the sample. If the concentrations of gases such as water vapor and carbon dioxide in the instrument are the same when the background and sample spectra are obtained, their contributions to the spectrum will ratio out exactly and their bands will not occur. If the concentrations of these gases are different when the background and sample spectra are obtained, their bands will appear in the sample spectrum(Smith, 2011).

2.9. Physical properties of oils

i. Density

Density is the mass of a unit volume of a substance. The density of an oil or fat is usually measured by determining its specific gravity.

ii. Refractive Index

The velocity of light changes as it travels from one medium to another and the light is then said to have undergone refraction. In effect, the rays of light are bent at the interface between the two media. The refractive index of a substance is the ratio of the velocity of light in a vacuum to the velocity of light in the substance. For practical measurement, air is used as the reference rather than vacuum. For oils and liquid fats, AOCS Method Cc 7-25 is used to measure the refractive index.

iii. Colour

Most oil products are preferred as colourless as possible. In the oils and fats trade, the colour of oils is usually measured by the Lovibond Comparator. Alternatively, a spectrophotometer may be used to record the variation of light absorbence over the visible wavelength region of the spectrum.

vi. Viscosity

The viscosity of a fluid is a measure of its resistance to deformation at a given rate. For liquids, it corresponds to the informal concept of "thickness": for example syrup has a higher viscosity than water.

2.8 Chemical properties of oils

i. Acid Value

This is defined as the number of milligrams of potassium hydroxide required to neutralise the free fatty acids in one gram of fat. Since acid value is an indication of the extent of hydrolysis and deterioration, oils with low acid value are sought after.

ii. Peroxide Value

Fats consist of saturated and unsaturated acids. The unsaturated acids are susceptible to oxidation, that is oxygen, can add to the fatty chain to form

peroxides or hydroperoxides. The peroxide value is a measure of the amount of these products. It is usually expressed as the milli-equivalents of peroxide-oxygen combined per kilogram of fat. The determination of peroxides is based primarily on their ability to liberate iodine from potassium iodide in glacial acetic acid followed by titration of the liberated iodine with sodium thiosulphate using starch as indicator.

iii. Saponification Value

The saponification value is defined as the number of milligrams of potassium hydroxide required to saponify completely one gram of the substance. It is a measure of the free and combined fatty acids present. By deducting the acid value from the saponification value, we obtain the ester value representing only the combined fatty acids in the neutral oil. The mean molecular weight of the fat can be calculated from the saponification value. The amount of glycerine in the neutral oil can be calculated from the ester value. The saponification value is measured by saponifying a known weight of an oil or fat with an excess of an alcoholic solution of potassium hydroxide followed by titration of the remaining alcoholic alkali with standard acid.

vi. Iodine Value

Iodine value is a measure of the total number of unsaturated double bonds present in an oil. Determination of iodine value involves the addition of an excess of halogen to the sample, reaction of the excess halogen with potassium iodide and titration of liberated iodine with standard sodium thiosulphate using starch solution as the indicator. Of the many procedures available, the most widely used are the Wijs (iodine monochloride) and Hanus (iodine) methods.

2.9. Flavonoids

The flavonoid compounds can be regarded as C_6-C_3-C_6 compounds, in which each C_6 moiety is a benzene ring, the variation in the state of oxidation of the connecting C_3 moiety is determining the properties and class of each such compounds; the different classes of flavonoids are shown in figure (1, 2 and 3) (Harborne, 2013).

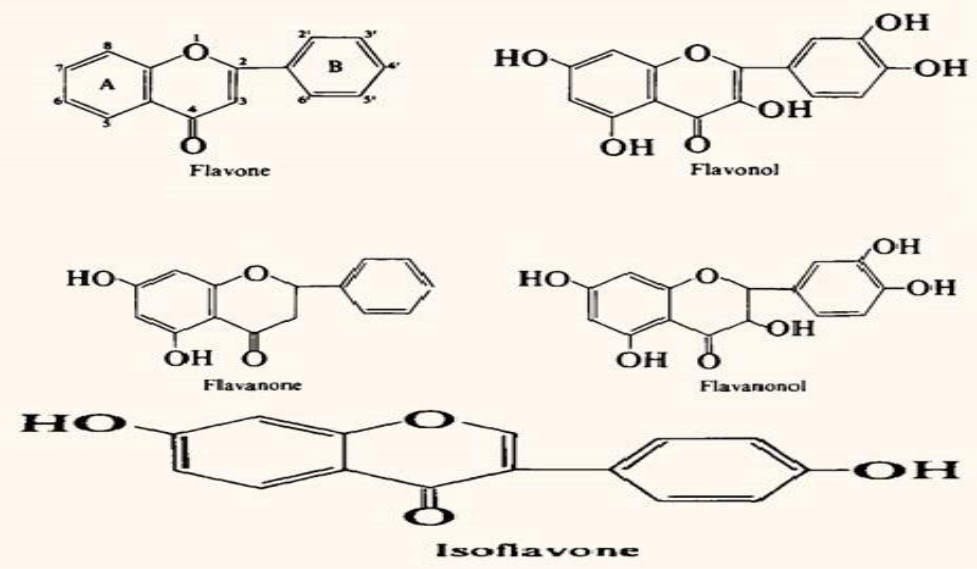


Figure (2.9): flavone, flavonol, flavanone, flavanonol and isoflavone

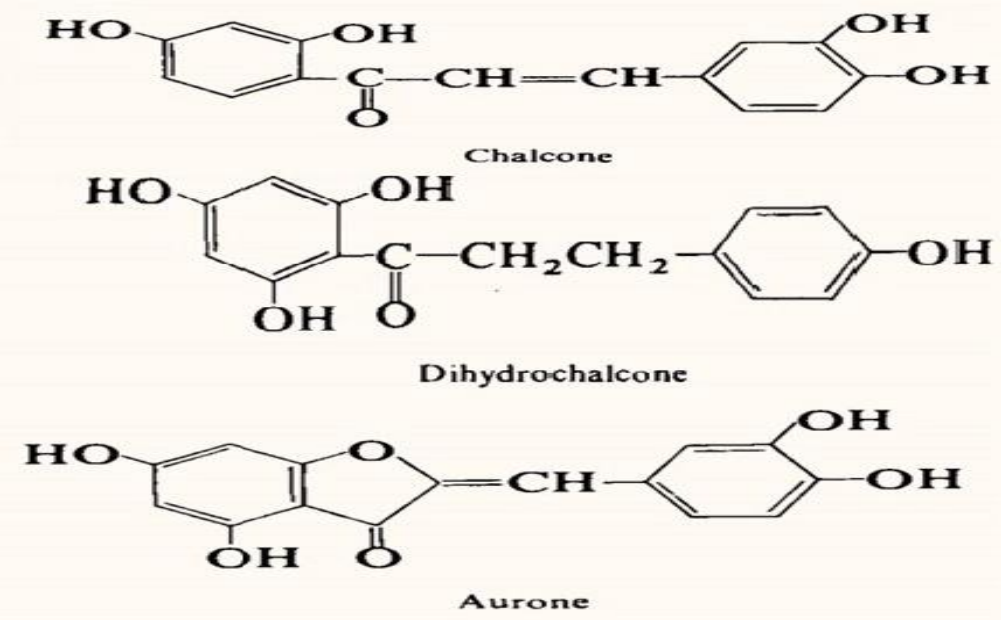


Figure (2.10): Chalcone, Dihydrochalcone and Aurone

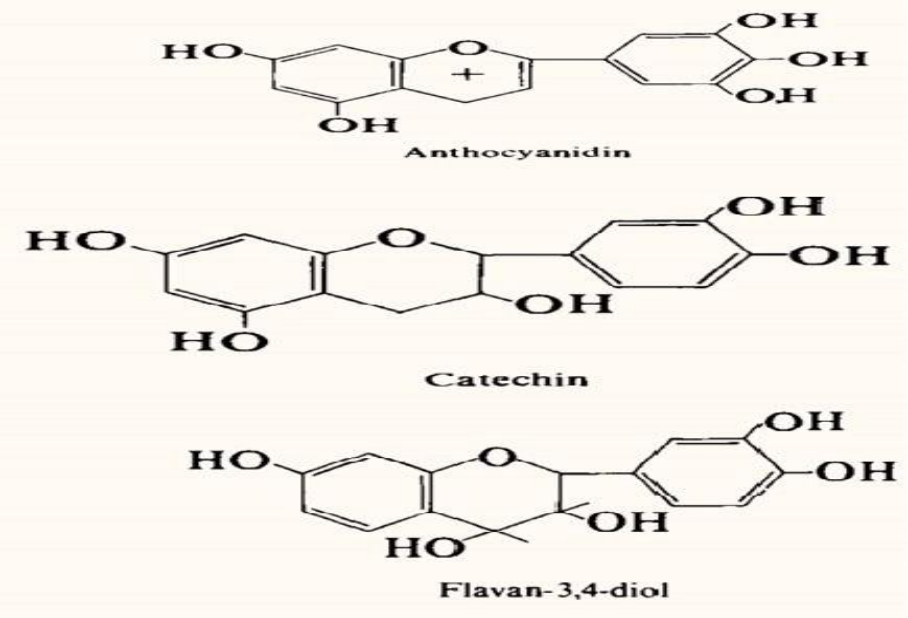


Figure (2.11): Anthocyanidin, Catechin and Flavan-3,4-diol

Flavonoid compounds and the related coumarins usually occur in plants as glycosides in which one or more of the phenolic hydroxyl groups are combined with sugar residues. The hydroxyl groups are nearly always found in positions 5 and 7 in ring A, while ring B commonly carries hydroxyl or alkoxyl groups at the 4'-position, or at both 3'- and 4'-positions. Glycosides of flavonoid compounds may bear the sugar on any of the available hydroxyl groups. Flavonoids occur in all parts of plants, including the fruit, pollen, roots, and heartwood. Numerous physiological activities have been attributed to them. Thus, small quantities of flavones may act as cardiac stimulants; some flavones, e.g., hesperidin, appear to strengthen weak capillary blood vessels; highly hydroxylated flavones act as diuretics and as antioxidants for fats. It is also claimed that flavones behave like auxins in stimulating the germination of wheat seeds. The possible function of this coloring matter in insect-pollinated flowers and edible fruits is to make these organs more conspicuous in order to aid seed dispersion by animals (Harborne, 2013).

2.10. Flavones

In flavones, ring (C) is basic and forms a pyrylium salt with hydrochloric acid as in figure (4).Consequently, the carbonyl group of flavone does not react

normally with some carbonyl reagents such as hydroxylamine. However, it does react normally with Grignard reagents.

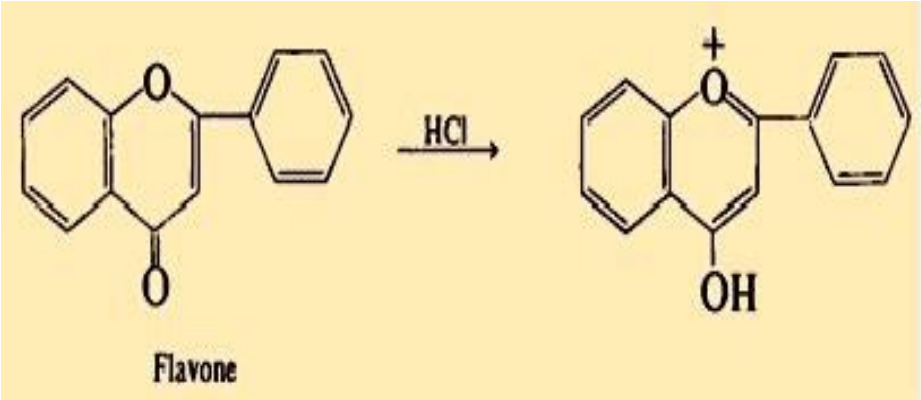


Figure (2.12): Pyrylium salt

Examples of flavones are: fisetin, primuletin and the most widespread flavone is quercetin as infigure (12), (Harborne, 2013).

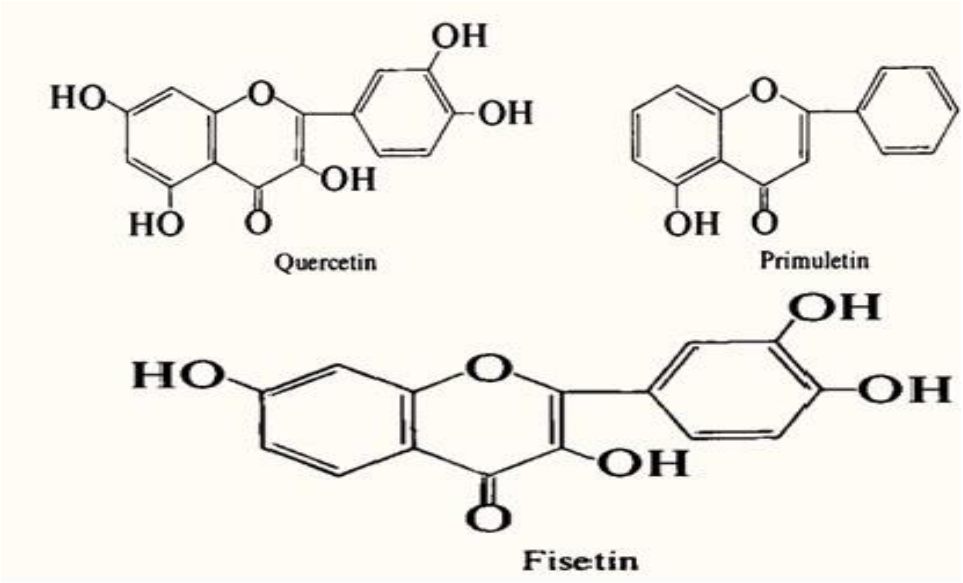


Figure (2.13): Fisetin, Primuletin and Quercetin

2.11. Flavonols

Flavones are a class of polyhydroxy flavonoid based on the structure of Flavone (2-phenyl-4H-1-benzopyran-4-one or phenylchromone) which itself occurs naturally as a farina on Primula plants. Flavonols are flavones with a 3-hydroxy substituent and they share the same nomenclature. It is convenient to separate these two classes, mainly because so many structures are known; some 1000 aglycones and over 2,000 glycosides. They differ in their spectroscopic and chromatographic properties and can readily be distinguished by these means.

They are biosynthetically distinct, flavones being formed by oxidation of flavanones, flavonols by oxidation of dihydroflavonols. There are also differences in the way they occur naturally; C-glycosides are common in the flavone series but rare among flavonols. In the DNP Type of Compound Index they are subdivided according to the number of O substituents (including methylenedioxy groups): C-methylation and C-prenylation is relatively common. Free lipophilic flavones and flavonols occur at the upper surface of leaves in the wax or in bud exudates. There are also many O-glycosides, which are found within the leaf in the cell vacuole and in other parts of the plant. There are at least 200 different glycosides of quercetin and 250 of the related flavonol, kaempferol(Harborne, 2013).

2.12. Anthocyanins

The innumerable shades of blue, purple, and violet, and nearly all the reds that appear in the cell sap of flowers, fruits, leaves, and stems of plants are due to anthocyanin pigments in the dissolved state. The sugar-free pigments are calledanthocyanidins. The structure common to all anthocyanidins is the flavylium (2-phenylbenzopyrylium) ion (figure 6).

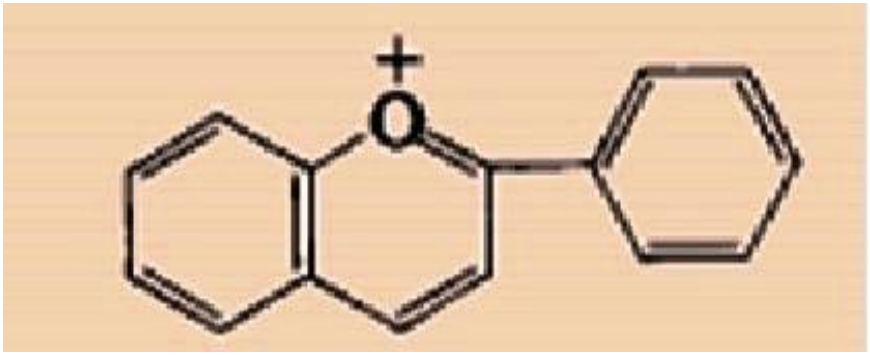


Figure (2.14): The structure of anthocyanins

The natural anthocyanidinsmay be classified into three groups: pelargonidin, cyanidin, and delphinidin.Various anthocyaninscan be distinguished by partition between two immiscible solvents, by their absorption spectra, and by their colors in buffer solutions of graded pH. For structure determination, anthocyanidinsare treated with alkali, whereupon they form phloroglucinol and a phenolic acid as

in figure (7).Several factors, such as the pH, complex-forming metals, and tannins, affect the colors of the anthocyanins(Harborne, 2013).

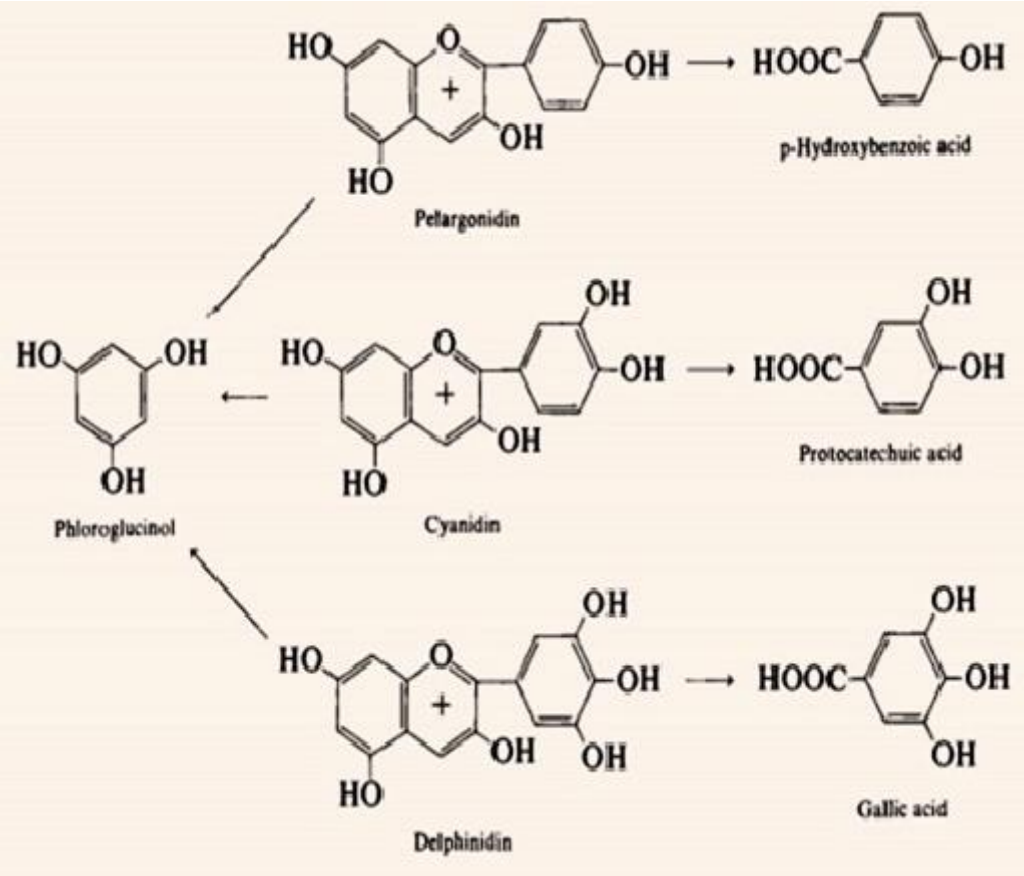


Figure (2.15): Reaction of anthocyanidins with alkali

2.13. Flavanones

Flavanones are 2,3-dihydro-2-phenyl-4H-1-benzopyran-4-ones. The simplest known natural flavanone is the 7-hydroxy derivative, while the commonest is 4′,5,7-trihydroxyflavanone (Naringenin). Flavanones are isomeric with chalcones and arise biosynthetically from them by a reaction catalysed by an isomerase. They have a centre of chirality at C-2 and usually occur in optically active forms with the 2S-configuration. They commonly occur as glycosides. A variety of more complex derivatives with methyl and/or prenyl substituents has been described. Flavanones have a wide occurrence in plants (Harborne, 2013).

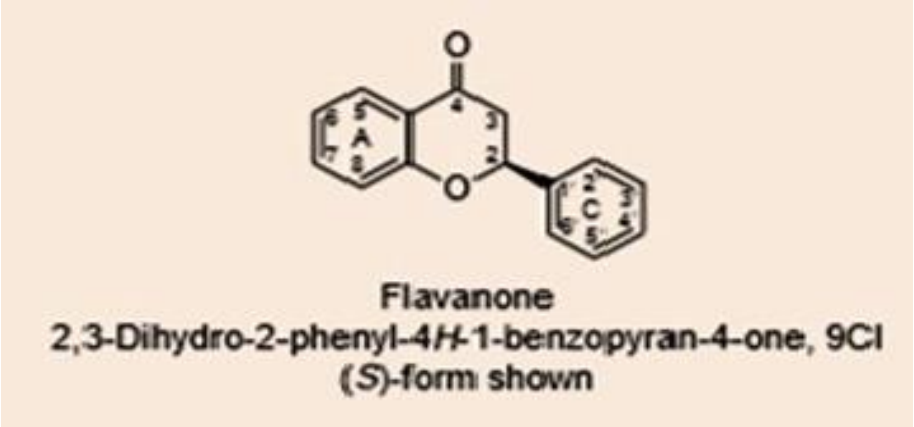


Figure (2.16): The structure of Flavanones

2.14. The target Plants

2.14.1.*Erucavesicaria*

Besides its importance as green salad available all over the year, *Erucavesicaria* (rocket) seeds contain oil that is promising to be medicinal oil.

- **Taxonomy**

Kingdom: *Plantae*

Family: *Brassicaceae*

Order: *Brassicales*

Species: *E. vesicaria*

Nail and his collogues investigated the proximate composition, oil content, oil physicochemical properties, and constituents of *Eruca sativa* seeds. Their results reveal that the composition of seeds contains; il content (20%), moisture content (3.64c), Crude protein (31.0), Crude fibers (20.376%), Total ash (4.33%) and Total carbohydrates (23.07). Physiochemicals of Eruca sativa oil are; relative density (90,77%), refractive index (1.469), relative viscosity (38cp), peroxide value (10), Acid value (2.24), Colour (red 5.4–yellow blue 0.4), saponnification value (165.495), unsaponnification value (2.648%), iodine value (63.63). on other hand GC-MS results showed that28 oil constituents in Eruca sativa oil. Palmatic acid (hexadecanoic acid), Stearic acid (octadecanoic acid), Oleic acid (Octadecenoic acid), Linoleic acid as (Octadecadienoic acid), Erucic acid (Docosenoic acid).

2.14.2. *Detariummicrocarpum*

*Detariummicrocarpum*Guill.&Perr. is a deciduous tree of the family Legumunoseae, subfamily Ceasalpinioideae(Watson and Dallwitz, 1993). It is found in semi-arid sub-Saharan Africa from Senegal to Cameroon, extending east to the Sudan. It has an irregular distribution, but it can be locally very common. Typically, it is found in high rainfall savanna areas, dry forests and fallow lands on sandy or iron rich hard soils as well as scattered trees on farms. It also occurs in dry savanna as a more stunted tree with smaller fruits (Akpata and Miachi, 2001) reaching ca. 10 m high and with a dense rounded crown; in wet areas it can grow up to 25 m tall. The fruits that are drupe-like, circular and disc shaped, containing fibers are edible and rich in vitamin C, potassium and calcium. The seeds, singly embedded within the hard fruits are used to thicken soups (Arbonnier, 2009).*D. microcarpum*is classified as a major African medicinal plant. The roots, stems, bark, leaves and fruits are all used to treat ailments such as tuberculosis, meningitis, itching, syphilis and diarrhea (Bationo et al., 2001, Kaboré, 2005, Sawadogo, 2007). In the previous study Air-dried *D.Microcarpum* seeds were pulverized to a coarse powder and the fixed oil extracted exhaustively (8 hours) in asoxhlet apparatus at 40-60 °C using petroleum ether (40-60°C) as solvent. Oil yield was estimated gravimetrically, and somephysicochemical properties of the seed oil were determined. Depot injection formulations of haloperidol in *D. microcarpum* seed oil (DMO), arachis oil and aqueous medium, respectively, stored at different temperatures (28, 60 and 1°C) for 180 days were assayedfor drug content via non-aqueous titration; to ascertain product stability. Results were compared, using univariate analysis of variance (ANOVA). The free fatty acid, saponification, peroxide, iodine and ester values of DMO were: 0.11 mgKOH/g, 198.80 mgKOH/g, 1.62 milliEqui./kg, 121.60 g iodine/100 g and 198.69 mgKOH/g, respectively. Other physicochemical parameters of DMO were melting point (2.50 °C), relative density (0.92), and refractive index (1.46). DMO has low deterioration rate and good edibility; thereby being adequate for consumption, industrial uses and

pharmaceutical applications. The statistical analysis indicated that the results obtained with DMO, in the accelerated stability study, were comparable to those of arachis oil ($p < 0.05$). Thus, DMO is recommended for use, in place of arachis oil, in the formulation of haloperidol injection and other oil based medicinal injections(Okorie et al., 2010).

2.14.3. *FagusSylvatica*

Fagus (beech, Fagaceae) is a small genus of 10 monoecious tree species in the northern hemisphere (Meunier et al., 2008, Shen, 1992). It is the most abundant broad-leaved forest tree in Europe and western Asia and forms an important component of mixed broad-leaved evergreen–deciduous forests in North America and East Asia (Denk, 2003, Zhou and Li, 1994).

- **Taxonomy**

Class: Magnoliopsida

Superorder: Rosanae

Order: Fagales

Family: Fagaceae

Genus:*Fagus* L. – beech

2.14.4.*Cassia obtusifolia*

Cassia obtusifolia, syn. *Cassia tora* (family Leguminous) is a wild African plant found in wastelands in the rainy season. Its leaves can be fermented (named Kawal) and is used by people from the eastern part of Chad and the southern part of Sudan as a meat replacer or a meat extender. The genus *Cassia*has been subjected to extensive phytochemical investigations because of their medicinal properties (Lee et al., 2001, Manos et al., 2001, Sui-Ming et al., 1989, Yun-Choi et al., 1990).Cassia obtusifolia is a medicinally important plants belonging to the family of Fabaceae. It has been used extensively by ayurvedic practitioner and also reported to contain anthraquinones and flavonoids, these constituents are well documented to possess anti-inflammatory activity. The fatty acid compositions of the petroleum ether extract of seeds of this plant weredetermined by gas chromatography-massspectrophotometer. 4 compounds

were identified from the extract of seeds (99.98%) and they are palmitic acid, stearic acid, oleic acid and linoleic acid of methyl ester. Among all fatty acids of linoleicacid showed the highest concentration (50.65%)(Nail et al., 2017).

2.14.5. *Acacia nubica*

Acacia Nubica is the one of the species in the family Gummifereae(Bentham, 1875). The structural evidence suggests that *A. nubica*gum molecules possess highly branched D-galactan frameworks, to which are attached D-glucuronic acid residues and L-arabinose-containing side-chains, some of which are at least six units long. The gum contains the largest proportion of L-arabinose in any of the Acacia gum exudates studied to date (Anderson and Cree, 1968).

- **Taxonomy**

Plantae: Kingdom

Species: Taxonomic Rank

Synonym(s): Acacia verekGuill.&Perr

Common Name(s): gum arabic [English]

Genus: *Acacia nubica* Mill

Acacia senegal) which used in this study is cultivated in Kordofan (Central Sudan) and Damazin (Blue Nile, Western Sudan). Physicochemical properties of gum samples were studied (moisture, ash,nitrogen, total soluble fiber, specific rotation, relative viscosity, refractive index and pH). Results show that many significant differences in moisture content, protein content and relative viscosity between Kordofan and Damazin gums. Damazin gum contained higher protein (3%) and characterized by higher viscosity (24.81) compared to Kordofan gum(El-Kheir et al., 2008).

2.14.6.*Albizaamara*

The genuses*Albizia*is represented by more than 100 species and are mainly confined to tropical and sub- tropical regions of Asia, Africa and Australia. About 16 species of *Albizia* are indigenous to the Indian subcontinent and have been cultivated as avenue trees, shade trees in tea and coffee

plantation.*Albizia*species are socially significant as high quality timber yielding and as a valuable resource for gum. Most importantly, *Albiziajulibrissin, Albizialebbeck, Albiziaprocera and Albiziaamara* are some of the most considered species in Ayruvedic medicine .*Albiziaamara*belongs to the family leguminaceae, is a valuable medicinal and multipurpose drought tolerant tree commonly found in dry forests of India. The wood of *Albiziaamara*is purplish brown with lighter bands, very hard and strong and used for making cabinets in building and agriculture purpose. The extracts of *A. amara* are used extensively in traditional medicine (Gamble and Fischer, 1935). In this work, the fatty acid profiles of the seed oils of Albizialebbeck and Albiziasaman (Samaneasaman) were reported. The oils were analyzed by GC, GC-MS and NMR. The most prominent fatty acid in both oils is linoleic acid (30–40%), followed by palmitic acid and oleic acid for A. lebbeck and oleic acid and behenic acid for A. saman. Both oils contain slightly more than 30% saturated fatty acids with stearic,eicosanoic and tetracosanoic acids present as well as odd-numbered saturated fatty acids in minor amounts. Furthermore, for the first time, epoxy fatty acids were reported in the fatty acid profiles of Albiziaseed oils. Coronaric acid (9,10-epoxy-12(Z)-octadecenoic acid) is the major epoxy fatty acid at approximately 3–4% of the fatty acid profiles with minor amounts of vernolic (12,13-epoxy-9(Z)-octadecenoic acid) and 9,10-epoxystearic acids also detected. The results are compared to previous literature on the fatty acid profiles of other Albizia seed oils. The coronaric acid content of Albizia resembles that of Acacia species with both genera belonging to the Fabaceae family. The mass spectrum of

thepyridylcarbinol (picolinyl) ester of coronaric acid is reported. Knothe, G., Phoo, Z.W.M.M., de Castro, M.E.G. and Razon, L.F., 2015. Fatty acid profile of Albizialebbeck and Albiziasaman seed oils: Presence of coronaric acid(Knothe et al., 2015) .

-Taxonomy

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Unranked: Rosids

Order: Fabales

Family: Fabaceae

Subfamily: Mimosaceae

Tribe: Ingae

Genus: Albizia

Species:*Albizaamara*

- **Skin Diseases**

The seed oil contain high content of linoleic acid and palamitic acid and low content of capric, lauric acid and lignoceric acid and is used for the treatment of leprosy and leucoderma. Paste of leaf and root bark of *Albiziaamara* is used to cure both skin diseases and poisonous bites (Mar et al., 1991) .The flowers of *Albiziaamara*have been applied to boils, eruptions and swellings .It is used as a remedy for dandruff (Ayyanar and Ignacimuthu, 2005).

2.14.7.*Foeniculumvulgare*

Fennel (Foeniculumvulgare) is a hardy, perennial herb with yellow flowers and feathery leaves. It is indigenous to the Mediterranean's shores but has become widely naturalized in many parts of the world, especially on dry soils near the seacoast and on riverbanks. It is a highly aromatic and flavourful herb used in cooking and, along with the similar-tasting anise, is one of the primary ingredients of absinthe. Florence fennel or finocchio (UK: /fɪˈnɒkiəʊ/, US: /-ˈnoʊk-/, Italian: [fiˈnɔkkjo]) is a selection with a swollen, bulblike stem base that is used as a vegetable.

- **Taxonomy**

Kingdom: *Plantae*

Clade: *Tracheophytes*

Clade: *Angiosperms*

Clade: *Eudicots*

Clade: *Asterids*

Order: *Apiales*

Family: *Apiaceae*

Genus: *Foeniculum*

Species: *F. Vulgare*

Binomial name: *Foeniculumvulgare Mill*.

- **Traditional uses**

Fennel is used as a traditional alternative and balancing medicine in Arabic, Roman, Indian, European, Iranian, and traditional Chinese medicines. The whole part plant of the fennel can be used medicinally in different forms to treat a variety of diseased conditions. Fennel has been many traditional uses in treating various diseases, such as cancer, fever, abdominal pains, flatulence, gastralgia, gastritis, insomnia, liver pain, mouth ulcer, stomachache, etc. Fennel seeds have been shown a potential drug for the treatment of hypertension (FENNEL).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

Seeds of *Detariummicrocarpum, Albizaamara,Cassiaobtisufolia, FagusSylvatica, Erucavesicaria*, *Acacia nubica* and *Foeniculumvulagre* were purchased from the local market-Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, Khartoum-Sudan.

3.1.2 Chemicals and Reagents

Chemicals and reagents used in this research were:

Alcoholic sulphuric acid, supersaturated sodium chloride ,normal hexane 95% , alcoholic potassium hydroxide solution ,Hydrochloric acid ,Potasium iodide , sodium acetate , sodium thioslphate , starch solution phenolphthalein indicator , Aluminium chloride solution, Potassium hydroxide solution, Ferric chloride solution, Than layer chromatography, sodium methoxide, Boric acid.

3.1.3. Apparatus and laboratory glassware

The apparatus and laboratory glassware used in this research were flasks, beakers, test tubes, glass bottles, putri dish, measuring cylinders, filters papers, hotplate burette, volumetric flask, and pipette.

3.1.4. Instruments

Instruments used in this research were Shimadzu GC-MS-QP2010 Ultra, Shimadzu FTIR and ShimadzuUV/Vis instruments.

3.2 Methods

3.2.1 Extraction of oils

Powdered seeds of studied plant (350g) were macerated with n-hexane. The solvent was removed under reduced pressure and the oil was kept in the fridge at $4^{o}C$ for further work.

The oil (2mL) was placed in a test tube and 7mL of alcoholic sodium hydroxide (0.2M) were added followed by 7ml Lof alcoholic sulphuric acid (0.2M). The

tubes stoppered and shaken vigorously for five minutes and then left overnight, 2mL of supersaturated sodium chloride were added, then 2mL of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated.5µL of the hexane extract were mixed with 5mL diethyl ether. The solution was filtered and the filtrate (1µL) was injected in the GC-MS instrument.

3.2.3 Physiochemical properties of Target plant

i. Density (Dn)

The density of the oil was determined using the standard method. The mass of the empty cylinder, empty cylinder plus the oil and the volume of the oil used were recorded.

The calculation procedure was as follows:

Density = M_1-M_2/V

Where**:**

M_1= mass of empty cylinder

M_2= mass of oil + cylinder

V= volume of oil used

ii. Viscosity (VS)

Viscosity was determined using Oswald viscometer. A definitive volume of the oil was poured into the bulb C with a pipette. The oil was sucked to the top of the left limb with the help of rubber tubing attached to it. The oil was then released to flow back into the bulb C. The time (t_1) from A to B was noted with a stop watch. The apparatus was then cleaned and the experiment was repeated with equal volume of water. The time of flow of water (t_2) from A to B was also recorded. The density of the oil and water were determined using a pyknometer. The relative viscosity coefficient was calculated from the expression (Bahl A et al. –Essential of physical chemistry, 2010).

$$\frac{\eta}{\eta_w} = \frac{d\ t_1}{d_w\ t_2}$$

Where:

η= viscosity coefficient of liquid

η_w= known viscosity coefficient of water

d = density of liquid

dw = density of water

t_1= time flow of liquid

t_2= time flow of water

iii. Refractive index

Refractive Index was measured by Abbey Refractometer. The instrument was charged by opening the double prism by means of screw head and a few drops of the sample were placed on the prism or the prisms were opened slightly by turning screw head and few drops of the sample were poured into funnel shaped aperture between prisms. The prism was closed firmly by tightening screw head and the instrument was left to stand for few minutes before reading so that the temperature of the sample and instrument were the same. The prism was cleaned between readings by wiping off oil with soft cloth then with cotton pad moistened with solvent (toluene, petroleum ether) and left to dry. Method of measurement was based upon observation of position of borderline of total reflection in relation to faces of flint glass prism. The borderline was brought into the field of vision of telescope by rotating double prisms by means of alidade backward or forward until the field of vision is divided into light and dark portion. Line dividing these portions is “border line” and as rule it isn’t a sharp line but band of colour. Colours were eliminated by rotating screw head of compensator until a sharp colourless line was obtained. The border line was adjusted so that it fell on point of intersection of cross hairs. The value n of the substance was read directly on scale est g 4th decimal place.3 or more readings were taken approaching intersection alternatively from one field to another the average was taken.

vi. colour

It was essential that the determination be carried out in subdued ambient light, i.e., not facing a window or in direct sunlight. The prepared sample was poured into a cell which was clean and dry and was pre warmed so that no solid matter separates from the sample during the determination. The cell containing the sample was placed within the black metal heath gamest the side of the liephlening cabinet holding the colour racks. The colour was then immediately determined, initially by using the colour racks in the ratio of 10 yellow to 1 red. Then was corrected until an accurate colour match was obtained.

v. Saponification Value (SV)

Five grams of oil was weighed and placed in a 200-300ml erlnmeyer flask.50 ml of alcoholic KOH solution was pipette into the flask. The flask was connected with an air condenser and the oil boiled until it was completely saponified (30 mins) It was then cooled and titrated with 0.5N HCl using phenolphthalein. Blank determination was conducted along that of the sample by using the pipette which was used for measuring KOH solution and draining at the same time (Chemists and Chemists, 1980).

Saponification value (mg KOH to saponify 1g of fat) = 28.05(B-S)/g sample

Where:

B= ml 0.5N HCl required by blank and S=ml 0.5N HCl required for sample

iv. Peroxide Value (PV)

The peroxide value is the number of milli-equivalents of active oxygen that expresses the amount of peroxide contained in 1000g of the substance(M Amin Mir. 2017).

Five grams sample was put in a 250ml Erlenmeyer flask,30 ml of HOAcCHCl$_3$ was added to it and swirled to dissolve.0.5 ml of standard KI solution was added from Mohr pipette and the mixture was shaken for 1min,30 ml of H_20 was added slowly and the mixture titrated with 0.1N $Na_2S_2O_3$ with vigorous shaking until the yellow colour almost was almost gone.0.5 ml of 1% starch solution was added and the titration was continued while being shaken vigourously to release

from $CHCl_3$ layer until the blue colour disappears. Blank determination was done and its value was subtracted from the sample titration.

Peroxide value (miliequivalent,peroxide/kg sample) =S ×N×1000/g sample where:

S = $Na_2S_2O_3$ (Blank corrected)(ml)

N= Normality of $Na_2S_2O_3$solution

iiv. Acid Value

Five grams of oil was weighed in a 250ml Erlenmeyer flask, 100ml of neutralized ethanol and 2ml of phenolphthalein indicator were then added.The mixture was shaken to dissolve it completely then titrated with a standard base (e.g 0.1N NaOH) until the endpoint was reached which was indicated by a slight pink color which persisted for 30seconds.The volume of the titrate used was then recorded (S.SuzanneNielsen, Food Analysis Laboratory manual, Third Edition, 2017).

Acid value; $AC=\frac{V\times N\times 56}{W}$

Where:

V= Volume of KOH (ml)

N= Strength of OH (mol/100ml)

56= Molecular weight of KOH

W= Weight of oil used

iiiv. Iodine Value

The iodine value is the number which expresses in grams the quantity of halogen, calculated as iodine, which is observed by 100g of the substance under the desired condition (M. Amin Mir. 2017).

This is also measure of the proportion of unsaturated acid or fat and oil present, but the test measures the amount of iodine absorbed per gram of sample. The determination of iodine value measures the reaction of the double bonds with halogen (M. Amin Mir. 2017).

Iodine value = $(V_b - V_s)$ x 1.269/w

Where:

V_b = Concordant Reading

Vs = Concordant reading

3.3.4. GC-MS analysis

The extracted oils were analyzed using Shimadzu GC-MS-QP2010 Ultra instrument equipped with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness). Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program was 150 ^{o}C for 1 min hold time, then 300 ^{o}C.The injection mode was split with injection temperature 300 ^{o}C. The column flow was 1.54 mL/min with total flow as 50 mL/min and pressure 139.3 KPa; In addition to purge flow, 3.0 mL/min.

3.4. Flavonoid test reagents

i. Aluminium chloride solution

One gram of aluminum chloride was dissolved in 100 ml methanol.

ii. Potassium hydroxide solution

One gram of potassium hydroxide was dissolved in 100 ml water.

iii. Ferric chloride solution

One gram of ferric chloride was dissolved in 100 ml methanol.

3.4.1 Test for flavonoids

Twenty ml of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with acetic acid her and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

i. To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.

ii. To 3 ml. of the filtrate few drops of aluminium chloride solution were added.

iii. To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

3.4.2. Extraction and isolation of flavonoids

One kg of powdered air-dried plant material was macerated with 95% ethanol (5L) for 48hr at room temperature. The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure at 40^{o} C yielding a crude product. This crude product was applied on silica gel plates as narrow zones. The plates were developed with macitic acid: water (3:2,v:v). After the usual workup chromatographically pure compounds I were isolated from *Foeniculumvulagre*.

3.4.3. UV shift reagents

The Stepwise procedures for UV shift reagents were as follows:

i. The UV spectrum of the compound in methanol was first recorded.

ii. Three drops of NaOMe reagent were added to the sample and the NaOMe spectrum was recorded, and after 8 minutes the NaOMe spectrum was re-recorded.

iii. Six drops of $AlCl_3$ reagent were added to the fresh sample and the $AlCl_3$ spectrum was recorded, 3 drops of HCl were added and after mixing , the $AlCl_3$/ HCl spectrum was recorded.

vi. Powdered NaOAC was then added to the fresh sample, the mixture was shaked and the NaOAC spectrum was recorded. NaOAC/ H_3BO_3 spectrum was then recorded after adding H_3BO_3.

3.5. FTIR analysis of the *Foeniculumvulagre* extracts

FTIR spectra were obtained (Shimadzu, IR-Prestige spectro-meter) as the result of the accumulation of 32 scans with the interferometer mirror speed of 2.8 mm/s, a resolution of 8 cm1anda detector with temperature control (DLATGS) that can cover mid-infrared regions in the range of 4000–650 cm1. In addition, we use an ATR cell (PIKE, Miracle) equipped with a diamond crystal with a simple reflection refractive index of 2.4. The cell was pre-aligned to the optical system of the IR team, before taking spectra. The angle of incidence of the IR beam was 451. Each spectrum was converted to ASCII format to be further processed.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physiochemical properties of *D. Microcarpum*seed oil

The physiochemical properties of the extracted oil of *D. Microcarpum* seeds were investigated and the obtain results were shown in Table 4.1.

Table (4.1): Physiochemical results of *D. Microcarpum* seeds oil

Test	**Results**
Viscosity CP	56
Density gm /cm^3	0.915
colour (yellow/red)	2.30/0.105
Reflective index	1.470
Iodine value ml/g	150.74
Peroxide value ml$Na_2S_2O_3$. 5H_2O/mg oil	63.87
Acid value mg KOH/g oil	3.88
Saponification value mg KOH /g oil	160.58

4.1.2 GC-MS analysisof *D. Microcarpum* seed oil

The extracted seeds oil of*D. Microcarpum*oil has been analyzed by GC-MS instrument and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis results revealed the presence of 17 components (Table 4.2).

Table (4.2): GC-MS analysis results of *D. Microcarpum* seed oil

No.	**Name**	**Common name**	**Chemical formula**	**Area%**
1	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.08
2	Pentadecanoic acid, methyl ester	Methyl pentadecanoate	$C_{16}H_{32}O_2$	0.02
3	7-Hexadecenoic acid,	(Z)-Methyl hexadec-	$C_{17}H_{32}O_2$	0.04

	methyl ester, (Z)-	7-enoate		
4	9-Hexadecenoic acid, methyl ester, (Z)-	methyl palmitioleate	$C_{17}H_{32}O_2$	0.58
5	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	17.85
6	cis-10-Heptadecenoic acid, methyl ester	(Z)-Methyl Heptadec-10-enoate	$C_{18}H_{34}O_2$	0.06
7	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.11
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	29.23
9	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	21.69
10	Methyl stearate	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	14.80
11	9,12-Octadecadienoyl chloride, (Z,Z)-	Linioleic acid chloride	$C_{18}H_{31}ClO$	0.76
12	cis-11-Eicosenoic acid, methyl ester	11-Eicosenoic acid, methyl ester, (Z)-	$C_{21}H_{40}O_2$	3.71
13	Eicosanoic acid, methyl ester	Methyl eicosanoate	$C_{21}H_{42}O_2$	6.51
14	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	$C_{22}H_{44}O_2$	0.12
15	Docosanoic acid, methyl ester	Behenic acid, methyl ester	$C_{23}H_{46}O_2$	3.44
16	Tricosanoic acid, methyl ester	Methyl tricosanoate	$C_{24}H_{48}O_2$	0.18
17	Tetracosanoic acid, methyl ester	Methyl tetracosanoate	$C_{25}H_{50}O_2$	0.82

4.1.3 Physiochemical properties of *A.amara* seeds oil

The physiochemical properties of the extracted oil of *A. amara*seeds were investigated and the obtain results were shown in Table 4.3.

Table (4.3): Physiochemical properties of *A. amara*seed oil

Test	**Result**
Viscosity CP	53
Density gm /cm^3	0.917
Reflective index	1.173
colour (yellow/Red)	33.35/0.00
Iodine value ml/g	176.41
Peroxide value mlNa2S2O3. 5H2O/mg oil	123.7
Acid value mg KOH/g oil	1.63
Saponification value mg KOH /g oil	94.421

4.1.4 GC-MS analysis of *A.amara* seeds oil

The oil was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis results revealed the presence of 24 components as shown in Table 4.4.

Table (4.4): GC-MS analysis results of *A. amara* seeds oil

No.	**Name**	**Common name**	**Chemical formula**	**Area%**
1	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.04
2	Pentadecanoic acid, methyl ester	Methyl pentadecanoate	$C_{16}H_{32}O_2$	0.03
3	7,10-Hexadecadienoic acid, methyl ester	7,10-Hexadecadienoate	$C_{17}H_{30}O_2$	0.03
4	7-Hexadecenoic acid, methyl ester, (Z)-	(Z)-Methyl hexadec-7-enoate	$C_{17}H_{32}O_2$	0.19
5	9-Hexadecenoic acid,	Methyl palmitoleate	$C_{17}H_{32}O_2$	0.14

	methyl ester, (Z)-			
6	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	9.45
7	cis-10-Heptadecenoic acid, methyl ester	(Z)-Methyl Heptadec-10-enoate	$C_{18}H_{34}O_2$	0.16
8	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.19
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	22.65
10	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	18.97
11	Methyl stearate	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	3.72
12	9,12-Octadecadienoyl chloride, (Z,Z)-	Linoleic acid chloride	$C_{18}H_{31}ClO$	0.67
13	Oxiraneoctanoic acid, 3-octyl-, methyl ester	Cis-9-10-Ehoxystearic acid,methyl ester	$C_{19}H_{36}O_3$	1.39
14	cis-13-Eicosenoic acid, methyl ester	cis-13-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	6.92
15	Eicosanoic acid, methyl ester	Methyl eicosanoate	$C_{21}H_{42}O_2$	2.02
16	PGH1, methyl ester	PGH1, methyl ester	$C_{22}H_{38}O_4$	0.12
17	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	$C_{22}H_{44}O_2$	0.17
18	cis-10-Nonadecenoic acid, methyl ester	cis-10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	3.55
19	Docosanoic acid, methyl ester	Behenic acid, methyl ester	$C_{23}H_{46}O_2$	14.37
20	Tricosanoic acid, methyl ester	Methyl tricosanoate	$C_{24}H_{48}O_2$	0.82

21	15-Tetracosenoic acid, methyl ester, (Z)-	cis-15-Tetracosenoic acid, methyl ester	$C_{25}H_{48}O_2$	0.33
22	Tetracosanoic acid, methyl ester	Methyl tetracosanoate	$C_{25}H_{50}O_2$	10.91
23	Pentacosanoic acid, methyl ester	Methyl pentacosanoate	$C_{26}H_{52}O_2$	0.57
24	Hexacosanoic acid, methyl ester	Methyl hexacosanoate	$C_{27}H_{54}O_2$	2.59

4.1.5 Physiochemical properties of *C. Obtisufolia* seeds oil

The physiochemical properties of the extracted oil of *C. Obtisufolia*seeds were investigated and the obtain results were shown in Table 4.5.

Table (4.5): Physiochemical properties of *C. Obtisufolia* seed oil

Test	**Result**
Viscosity CP	58
Density gm /cm^3	0.915
Reflective index	1.472
colour (yellow/red)	10.11/0.10
Iodine value mg/g	167.85
Peroxide value mlNa2S2O3.5H2O/mg oil	81
Acid value mg KOH/g oil	6.41
Saponification value mg KOH /g oil	152.05

4.1.6 GC-MS analysis of *C. Obtisufolia* seeds oil

The oil from *C. Obtusifolia*was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis revealed the presence of 16 components as in Table 4.6.

Table (4.6): GC-MS analysis results of *C. Obtisufolia* seeds oil

No.	Name	Common name	Chemical formula	Area%
1	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.23
2	7-Hexadecenoic acid, methyl ester, (Z)-	(Z)-Methyl hexadec-7-enoate	$C_{17}H_{32}O_2$	0.07
3	9-Hexadecenoic acid, methyl ester, (Z)-	Methyl palmitoleate	$C_{17}H_{32}O_2$	0.80
4	Hexadecanoic acid, methyl ester	Palmitic acid	$C_{17}H_{34}O_2$	18.50
5	cis-10-Heptadecenoic acid, methyl ester	(Z)-Methyl Heptadec-10-enoate	$C_{18}H_{34}O_2$	0.14
6	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.24
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid	$C_{19}H_{34}O_2$	38.75
8	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid	$C_{19}H_{36}O_2$	10.67
9	9-Octadecenoic acid, methyl ester, (E)-	Elaidic acid	$C_{19}H_{36}O_2$	3.21
10	Methyl stearate	Octadecanoic acid	$C_{19}H_{38}O_2$	10.98
11	cis-11-Eicosenoic acid, methyl ester	11-Eicosenoic acid, methyl ester, (Z)-	$C_{21}H_{40}O_2$	1.99
12	Eicosanoic acid, methyl ester	Methyl arachisate	$C_{21}H_{42}O_2$	4.83
13	Docosanoic acid, methyl ester	Methyl docosanoate	$C_{23}H_{46}O_2$	5.09
14	Tetratriacontane	n-Tetratriacontane	$C_{34}H_{70}$	1.16
15	Tetracosanoic acid,	Methyl lignocerate	$C_{25}H_{50}O_2$	2.21

	methyl ester			
16	Hexatriacontane	n-Hexatriacontane	$C_{36}H_{74}$	1.13

4.1.7 Physiochemical properties of *F. Sylvatica* seeds oil

The physiochemical properties of the extracted oil of *F. Sylvatica*seeds were investigated and the obtain results were shown in Table 4.6.

Table (4.7): Physiochemical properties of *F. Sylvatica*seed oil

Test	**Result**
Viscosity CP	52
Density gm /cm^3	0.917
Colour (yellow/red)	10.10/1.4
Reflective index	1.471
Peroxide value mlNa2S2O3. 5H2O/mg oil	37.698
Acid value mg KOH/g oil	2.511
Saponification value mg KOH /g oil	176.361
Iodine value ml/g	159.21

4.1.8 GC-MS analysis of *F. Sylvatica* seeds oil

The oil was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis revealed the presence of 16 components as shown in Table 4.7.

Table (4.8): GC-MS analysis results of *F. Sylvatica* seeds oil

No.	**Name**	**Common name**	**Chemical formula**	**Area%**
1	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.18
2	Pentadecanoic acid, methyl ester	Methyl n-pentadecanoate	$C_{16}H_{32}O_2$	0.06
3	7-Hexadecenoic acid, methyl ester, (Z)-	(Z)-Methyl hexadec-7-enoate	$C_{17}H_{32}O_2$	0.09

4	9-Hexadecenoic acid, methyl ester, (Z)-	Methyl palmitoleate	$C_{17}H_{32}O_2$	1.04
5	Hexadecanoic acid, methyl ester	Palmitic acid	$C_{17}H_{34}O_2$	24.22
6	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.24
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid	$C_{19}H_{34}O_2$	35.74
8	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid	$C_{19}H_{36}O_2$	12.14
9	Methyl stearate	Octadecanoic acid	$C_{19}H_{38}O_2$	15.27
10	9,12-Octadecadienoyl chloride, (Z,Z)-	Octadecanoic acid	$C_{19}H_{38}O_2$	0.45
11	cis-11-Eicosenoic acid, methyl ester	11-Eicosenoic acid, methyl ester, (Z)-	$C_{21}H_{40}O_2$	1.60
12	Eicosanoic acid, methyl ester	Methyl arachisate	$C_{21}H_{42}O_2$	4.52
13	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	$C_{22}H_{44}O_2$	0.47
14	cis-10-Nonadecenoic acid, methyl ester	cis-10-Nonadecenoic acid, methyl ester cis-10-Nonadecenoic acid	$C_{20}H_{38}O_2$	0.19
15	Docosanoic acid, methyl ester	Methyl docosanoate	$C_{23}H_{46}O_2$	2.53
16	Tetracosanoic acid, methyl ester	Methyl lignocerate	$C_{25}H_{50}O_2$	1.26

4.1.9 Physiochemical properties of *E.vesicaria* seeds oil

The physiochemical properties of the extracted oil of *E. vesicaria* seeds were investigated and the obtain results were shown in Table 4.8.

Table (4.9): Physiochemical properties of *E. vesicaria* seed oil

Test	Result
Viscosity CP	58
Density gm /cm^3	0.916
Reflective index	1.473
Colour (yellow/ Red)	43.2/0.00
Iodine value ml/g	176.41
Peroxide value mlNa2S2O3. 5H2O/mg oil	1.984
Acid value mg KOH/g oil	6.407
Saponification value mg KOH /g oil	159. 51

4.1.10 GC-MS analysis of *E. vesicaria* seeds oil

The oil was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis revealed the presence of 30 components Table 4.9.

Table (4.10): GC-MS analysis results of *E. vesicaria*seeds oil

NO	**Name**	**Common Name**	**Chemical formula**	**Area%**
1	L.alpha-Terpineol	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, (S)-	$C_{10}H_{18}O$	0.09
2	Dodecanoic acid ,methyl ester	Lauric acid	$C_{13}H_{26}O_2$	0.02
3	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.23
4	5-Octadecanoic acid , methyl ester	5-Octadecanoic acid	$C_{13}H_{24}o_2$	0.01
5	cis-5-Dodecenoic acid,	cis-5-Dodecenoic acid	$C_{13}H_{24}O_2$	0.01

	methyl ester			
6	Pentadecanoic acid, methyl ester	Methyl n-pentadecanoate	$C_{16}H_{32}O_2$	0.03
7	7, 10-Hexadecadienoic acid, methyl ester	7, 10-Hexadecadienoic acid	$C_{17}H_{30}O_2$	0.07
8	cis,cis,cis-7,10,13-Hexadecatrienal	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	0.14
9	9-Hexadecenoic acid, methyl ester, (Z)-	Methyl palmitoleate	$C_{17}H_{32}O_2$	0.31
10	Hexadecenoic acid, methyl ester, (Z)-	Palmitic acid	$C_{17}H_{34}O_2$	6.62
11	cis-10-Heptadecenoic acid, methyl ester	(Z)-Methyl Heptadec-10-enoate	$C_{18}H_{34}O_2$	0.06
12	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.05
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid	$C_{19}H_{34}O_2$	12.09
14	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid	$C_{19}H_{36}O_2$	11.89
15	9,12,15-9-Octadecenoic acid, methyl ester	Linolenic acid	$C_{19}H_{32}O_2$	6.80
16	Methyl stearate	Octadecanoic acid	$C_{19}H_{38}O_2$	2.03
17	gamma.-Linolenic acid, methyl ester	Linolenic acid	$C_{19}H_{32}O_2$	0.22
18	cis-13-Eicosenoic acid, methyl ester	11-Eicosenoic acid	$C_{21}H_{40}O_2$	13.83
19	cis-11-Eicosenoic acid, methyl ester	11-Eicosenoic acid, methyl ester, (Z)-	$C_{21}H_{40}O_2$	2.25

20	Eicosanoic acid, methyl ester	Methyl arachisate	$C_{21}H_{42}O_2$	1.55
21	11-Octadecenoic acid, methyl ester	Methyl 11-octadecenoate	$C_{19}H_{36}O_2$	0.06
22	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	$C_{22}H_{44}O_2$	0.05
23	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	$C_{23}H_{32}O_2$	0.06
24	13-Docosenoic acid, methyl ester, (Z)-	Methyl (Z)-13-docosenoate	$C_{23}H_{44}O_2$	33.80
25	Docosanoic acid, methyl ester	Behenic acid	$C_{23}H_{46}O_2$	2.08
26	9-Octadecenoic acid, methyl ester	9-Octadecenoic acid	$C_{19}H_{34}O_2$	0.18
27	Tricosanoic acid, methyl ester	Methyl tricosanoate	$C_{24}H_{48}O_2$	0.10
28	15-Tetracosenoic acid, methyl ester, (Z)-	Methyl nervonate; cis-15-Tetracosenoic acid	$C_{25}H_{48}O_2$	3.88
29	Tetracosanoic acid, methyl ester	Methyl lignocerate	$C_{25}H_{50}O_2$	0.91
30	Stigmasterol	Stigmasta-5,22-dien-3-ol, (3β,22E)-	$C_{29}H_{48}O$	0.60

4.1.11 Physiochemical properties of *A. Nubica* seeds oil

The physiochemical properties of the extracted oil of *A. Nubica*seeds were investigated and the obtain results were shown in Table 4.10.

Table (4.11): Physiochemical properties of *A. Nubica* seed oil

Test	**Results**
Viscosity CP	54
Density gm /cm^3	0.917
Reflective index	1.471
Colour (Yellow/Red)	44.3/1.40)
Iodine value ml/g	159.29
Peroxide value mlNa2S2O3. 5H2O/mg oil	23.90
Acid value mg KOH/g oil	1.11
Saponification value mg KOH /g oil	160.805

4.1.12 GC-MS analysis of *A. Nubica* seeds oil

The oil was analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra. The GC-MS analysis revealed the presence of 20 components Table 4.11.

Table (4.12): GC-MS analysis results of *A. Nubica* seeds oil

No.	**Name**	**Common name**	**Chemical formula**	**Area%**
1	Methyl tetradecanoate	Tetradecanoic acid	$C_{15}H_{30}O_2$	0.15
2	Pentadecanoic acid, methyl ester	Methyl n-pentadecanoate	$C_{16}H_{32}O_2$	0.04
3	7-Hexadecenoic acid, methyl ester, (Z)-	Methyl (7E)-7-hexadecenoate	$C_{17}H_{32}O_2$	0.05
4	9-Hexadecenoic acid, methyl ester, (Z)-	Methyl palmitoleate	$C_{17}H_{32}O_2$	0.24
5	Hexadecanoic acid, methyl ester	Palmitic acid	$C_{17}H_{34}O_2$	17.17

6	cis-10-Heptadecenoic acid, methyl ester	(Z)-Methyl Heptadec-10-enoate	$C_{18}H_{34}O_2$	0.10
7	Heptadecanoic acid, methyl ester	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.15
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid	$C_{19}H_{34}O_2$	44.26
9	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid	$C_{19}H_{36}O_2$	22.20
10	Methyl stearate	Octadecanoic acid	$C_{19}H_{38}O_2$	8.74
11	9,12-Octadecadienoyl chloride, (Z,Z)-	Linoleic acid	$C_{19}H_{34}O_2$	1.01
12	cis-11-Eicosenoic acid, methyl ester	11-Eicosenoic acid, methyl ester, (Z)-	$C_{21}H_{40}O_2$	0.62
13	Eicosanoic acid, methyl ester	Methyl arachisate	$C_{21}H_{42}O_2$	1.88
14	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	$C_{22}H_{44}O_2$	0.07
15	Docosanoic acid, methyl ester	Methyl behenate	$C_{23}H_{46}O_2$	1.52
16	Tricosanoic acid, methyl ester	Methyl tricosanoate	$C_{24}H_{48}O_2$	0.23
17	Tetracosanoic acid, methyl ester	Methyl tetracosanoate	$C_{25}H_{50}O_2$	0.82
18	Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-	24-Ethyl-5-α-cholest-7,25-dien-3-β-ol	$C_{29}H_{48}O$	0.16
19	Hexatriacontane	n-Hexatriacontane	$C_{36}H_{74}$	0.33
20	Tetracontane	n-Tetracontane	$C_{40}H_{82}$	0.26

4.1.13 UV-Vis and FT-IR F. vulgare analysis results

The fractions obtained from the extracts of *F. Vulgare* over silica gel subjected to analysis using UV and FTIR instruments; the obtained results were shown in Figures (9-14).

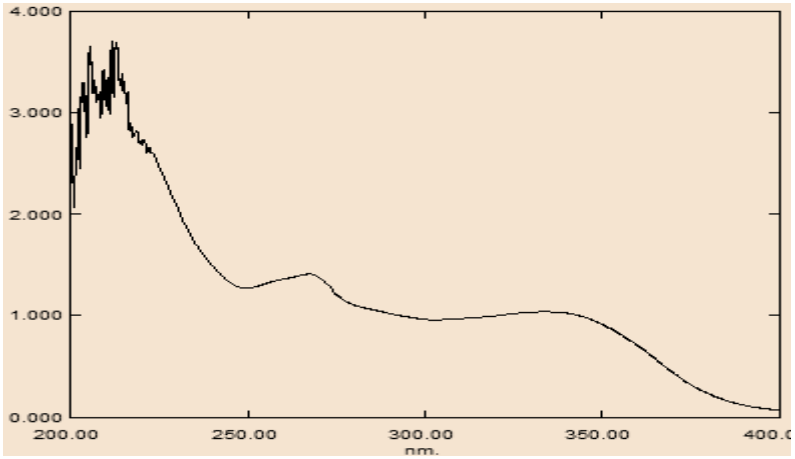


Figure (4.1): UV spectrum of *F.Vulgare*

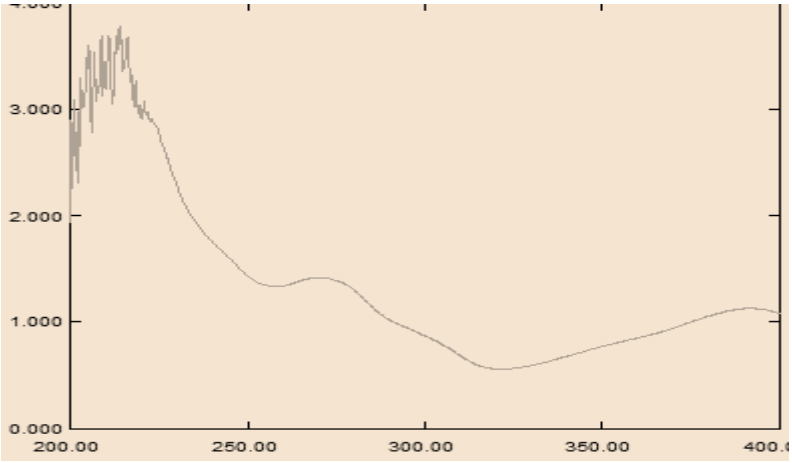


Figure (4.2): Sodium methoxide spectrum of *F.Vulgare*

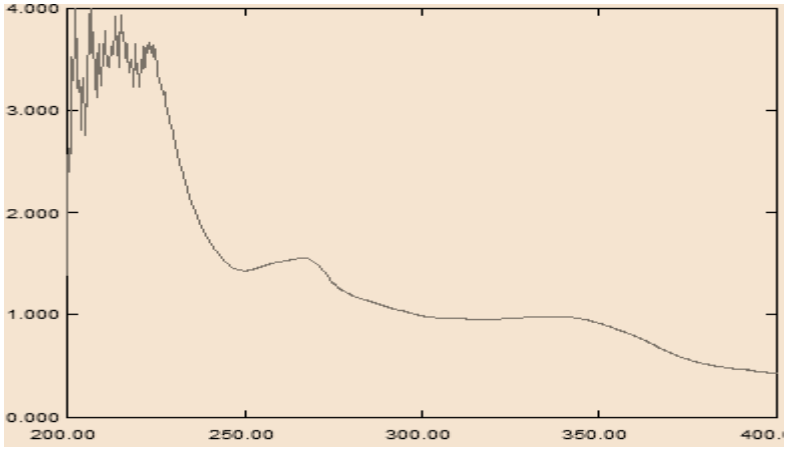


Figure (4.3): Sodium acetate spectrum of *F. Vulgare*

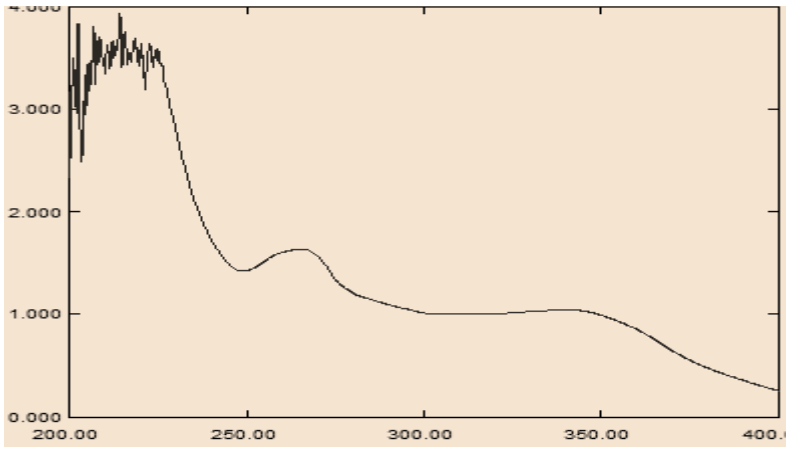


Figure (4.4): Boric acid spectrum of *F.Vulgare*

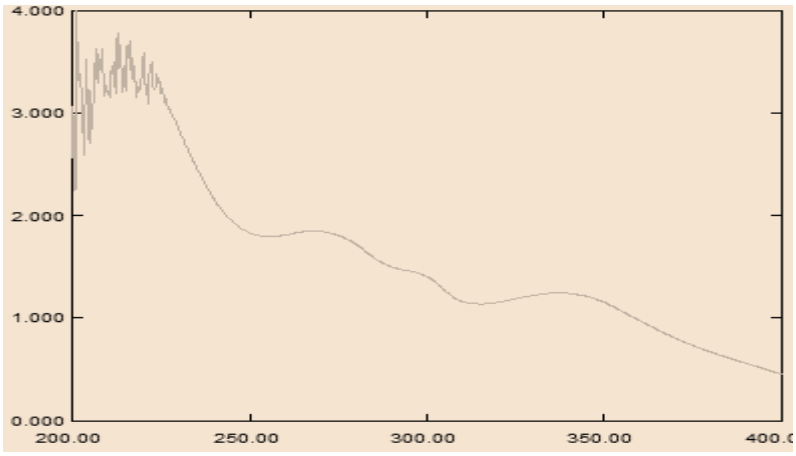


Figure (4.5): Aluminium chloride spectrum of *F.Vulgare*

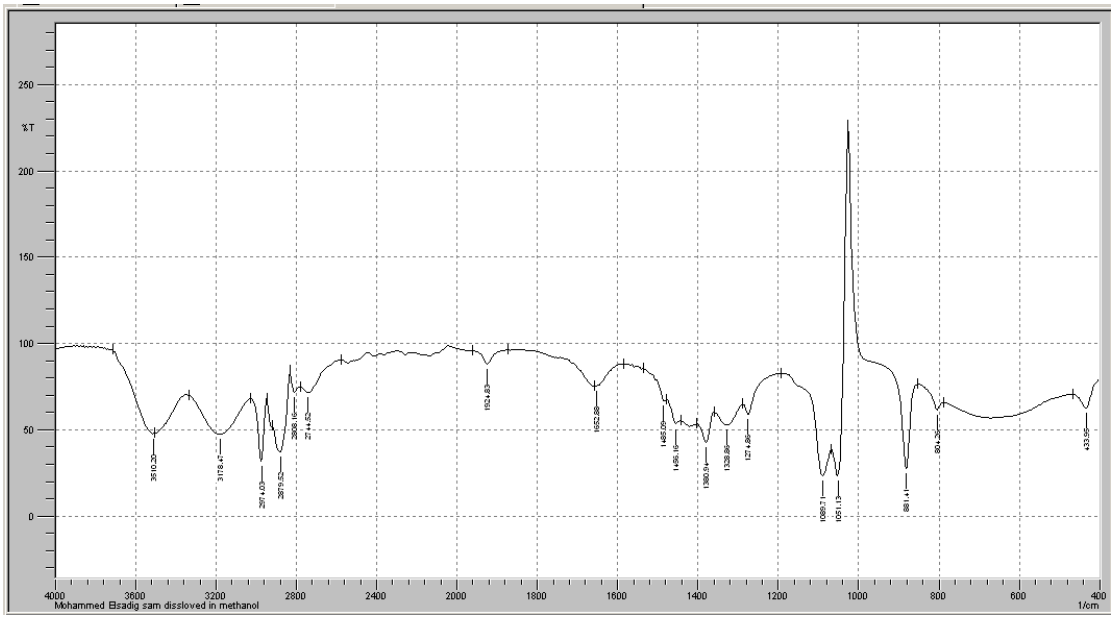


Figure (4.6): FTIR spectrum of *F. Vulgare*

4.2 DISCUSSION

This study aimed at some of the natural renewable resources plants in Sudan, that it could provide natural drugs that can cure many diseases. This stimulates the search for curable drugs in the chemical characterization. The physiochemical properties of the extracted oil of *D. Microcarpum* seeds investigated. The obtain results were showed the Viscosity (56CP), Density gm/cm^3 (0.915),Iodine value ml/g (150.74),colour(yellow/red) (2.30/0.11) Reflective index (1.470), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (63.87),Acid value mg KOH/g oil (3.88) and Saponification value mg KOH /g oil (160.58).Also, The GC-MS analysis results of the extracted oil of *D. Microcarpum* revealed the presence of 17 components, the major constituents were 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.23%); 9-octadecenoic acid (Z)-, methyl ester (21.69%); Hexadecanoic acid, methyl ester (17.85%) and Methyl stearate (14.80%).The obtained results were found in the range of the obtained results of the previous studies mentioned in chapter two.

The physiochemical properties of the extracted oil of *Albizaamara*seeds were investigated and the obtain results indicted the Viscosity (53CP), Density gm/cm^3 (0.917), Reflective index (1.473), Iodine value ml/g (176.4),colour(yellow/red)(33.35/00),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (123.7),Acid value mg KOH/g oil(1.63), and Saponification value mg KOH /g oil (94.421). In addition, The GC-MS analysis results of *Albizaamara* seed oil showed 24 components dominated by 9, 12 Octadecadienoic acid (Z, Z)-, methyl ester (29.23%); 9-octadecenoic acid (Z)-, methyl ester (21.69%); and Hexadecanoic acid, methyl ester (17.85%).

The physiochemical properties of the extracted oil of *Cassia obtisufolia* seeds were investigated and the obtain results were shown is Viscosity (58CP), Density gm/cm^3(0.915),Reflectiveindex(1.472),Iodinevalueg/ml(167.85)colour(yellow/red)(10.105/.010),Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (81),Acid value mg KOH/g oil(6.41), and Saponification value mg KOH /g oil (152.05).Additionally The GC-MS analysis results of *Cassia obtisufolia* seeds oil revealed the presence

of 16 components. Major constituents were 12-Octadecadienoic acid (Z, Z)-, methyl ester (38.75%); Hexadecanoic acid, methyl ester (18.50%); Methyl stearate (10.98%); and 9-octadecenoic acid (Z)-, methyl ester (10.67%).

The physiochemical properties of the extracted oil of *F.Sylvatica*seeds were investigated and the obtain results were shown the Viscosity (52CP), Density gm/cm^3(0.917), colour (yellow/red) (10.10/1.40), Iodine value g/ml (159.21) Reflective index (1.471), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (37.698), Acid value mg KOH/g oil (2.511), and Saponification value mg KOH /g oil (176.361).

The GC-MS analysis results of *F. Sylvatica* seed oilshowed 16 components dominated by: 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (35.74%); Hexadecanoic acid, methyl ester (24.22%); Methyl stearate (15.27%); and 9-octadecenoic acid (Z)-, methyl ester (12.14%).

The physiochemical properties of the extracted oil of *E. vesicaria* seeds were investigated and the obtain results were shown is Viscosity (58CP), Density gm/cm^3 (0.916), Reflective index (1.473), colour(yellow/red)(43.2/00),Iodine valueg/ml(176.41)Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (1.984),Acid value mg KOH/g oil(6.407), and Saponification value mg KOH/g oil (159. 51).The GC-MS analysis results of *E. vesicaria* seeds oil revealed the presence of 30 components. The major constituents are 13-Docosenoic acid, methyl ester, (Z) – (33.80%); cis-13-Eicosenoic acid, methyl ester (13.83%); 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (12.09%); and 9-octadecenoic acid (Z)-, methyl ester (11.89%).

The physiochemical properties of the extracted oil of *A.nubica* seeds were investigated and the obtain results were shown is Viscosity (54CP), Density gm/cm^3 (0.917), Reflective index (1.471), Iodine value g/ml (159.29), colour (yellow/red) (44.3/1.40), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (23.90), Acid value mg KOH/g oil(1.11), and Saponification value mg KOH /g oil (160.805).The GC-MS analysis results of *Acacia nubica*seeds oil showed 20 components dominated by: 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

(44.26%); 9-octadecenoic acid (Z)-, methyl ester (22.20%); Hexadecanoic acid, methyl ester (17.17%); and Methyl stearate (6.74%).

In the UV results,the fraction of *F. vulgare*was absorbed at λ_{max}267 and 334nm (Fig.9), such absorption is characteristic of flavones. Thensome UV shift reagents have investigated the hydroxylation pattern on the bucleus of the flavones. The shift reagent sodium methoxide induces a bathochromic shift in the presence of a 3- or 4`-OH group. In both cases it gives bathochromic shifts but with decrease in intensity in case of a 3-OH function. When a methanolic solution of compound I was treated with this shift reagent a bathochromic shift without decrease in intensity was observed (Fig.10) indication a 4-OH group. Another useful shift reagent is sodium acetate that gives bathochromic shifts diagnostic of a 7-OH group. The sodium acetate spectrum of compound I (Fig.11) did not reveal any bathochromic shift indicating absence of a 7-OH function. Boric acid is a UV shift reagent, which is diagnostic of catechol systems whether being in ring A or ring B. However, the boric acid spectrum (Fig.12) failed to show any bathochromic shift suggesting absence of catechol moieties. Aluminum chloride is a shift reagent, which is diagnostic of 3- and 5-OH groups. However, the aluminum chloride spectrum (Fig. 13) did not reveal any bathochromic shift indicting absence of 3- and 5-OH groups.

The FTIR spectrum of *F.vulgare* indicated the type of function group present in the obtained fraction of *F.vulgare* (fig.14). Based on the obtained results of UV and FTIR; the flavonoid that present the extracts of *F.vulgare* perhaps is flavones.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study aimed at some of the natural renewable resources plants in Sudan, that it could provide natural drugs that can cure many diseases. This stimulates the search for curable drugs in the chemical characterization and biological activity. The physiochemical properties of the extracted oil of *D. Microcarpum* seeds investigated. The obtain results were showed the Viscosity (56CP), Density gm/cm^3 (0.915), Reflective index (1.470), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (63.87),Acid value mg KOH/g oil(3.88) and Saponification value mg KOH /g oil (160.58).In addition, The GC-MS analysis results of the extracted oil of *D.Microcarpum*revealed the presence of 17 components. The physiochemical properties of the extracted oil of *Albizaamara*seeds were investigated and the obtain results indicted the Viscosity (53CP), Density gm/cm^3 (0.917), Reflective index (1.473), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (123.7), Acid value mg KOH/g oil(1.63), and Saponification value mg KOH /g oil (94.421). In addition, The GC-MS analysis results of *Albizaamara* seed oil showed 24 components. The physiochemical properties of the extracted oil of *Cassia obtisufolia* seeds were investigated and the obtain results were shown is Viscosity (58CP), Density gm /cm^3 (0.915), Reflective index (1.472), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (81),Acid value mg KOH/g oil(6.41), and Saponification value mg KOH /g oil (152.05).Additionally The GC-MS analysis results of *Cassia obtisufolia* seeds oil revealed the presence of 16 components. The physiochemical properties of the extracted oil of *F.Sylvatica* seeds were investigated and the obtain results were shown the Viscosity (52CP), Density gm/cm^3 (0.917), Reflective index (1.471), Peroxide value ml$Na_2S_2O_3.5H_2O$/mg oil (37.698),Acid value mg KOH/g oil(2.511), and Saponification value mg KOH /g oil (176.361). The GC-MS analysis results of *F. Sylvatica* seed oilshowed 16 components. The physiochemical properties of the extracted oil of *E.vesicaria* seeds were

investigated and the obtain results were shown is Viscosity (58CP), Density gm/cm^3 (0.916), Reflective index (1.473), Peroxide value ml$Na_2S_2O_{3.}5H_2O$/mg oil (1.984),Acid value mg KOH/g oil(6.407), and Saponification value mg KOH/g oil (159. 51). The GC-MS analysis results of *E. vesicaria* seeds oil revealed the presence of 30 components. The physiochemical properties of the extracted oil of *A.nubica* seeds were investigated and the obtain results were shown is Viscosity (54CP), Density gm/cm^3 (0.917), Reflective index (1.471), Peroxide value ml$Na_2S_2O_{3.}5H_2O$/mg oil (23.90),Acid value mg KOH/g oil(1.11), and Saponification value mg KOH /g oil (160.805).The GC-MS analysis results of *Acacia nubica*seeds oil showed 20 components. The obtained results of UV and FTIR; revealed that the flavonoid present in the extracts of *F.vulgare* perhaps is flavones.

5.2 Recommendation

We recommended the following:

1. In vivo studies on the targeted oils are highly recommended.
2. Extract the major flavonoid from *ErucaVesicaria, DetariumMicrocarpum, FagusSylvatica, Cassia Obtusifolia, Acacia Nubica and Albiza Amara*.
3. Once the in vivo studies gave positive indications a formulation process may be attempted.

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