Sudan University of Science and Technology

College of Graduate Studies

GC-MS Analysis and Antioxidant Activity of Mentha *Piperita* Oil

تحليل الكروموتوغرافيا الغازية – طيف الكتلة واختبار مضاد الأكسدة لزيت النعناع

A Thesis Submitted in Fulfillment for the Requirements of the

M.Sc. Degree in Chemistry

By

Sadia Abdel Rhaman Abdel Gabar Mohamed

(B.Sc. Chemistry)

Supervisor

Prof. Mohammed Abdelkarim

March, 2022

الاستهلال

بسم الله الرحمن الرحيم

قَالَ تَعَالَى: ﴿ وَقُلِ ٱعْمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمْ وَرَسُولُهُۥ وَٱلْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ
إِلَىٰ عَٰلِمِ ٱلْغَيْبِ وَٱلشَّهَٰدَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴿١٠٥﴾ ﴾

صدق الله العظيم

سورة التوبة الآية (١٠٥)

Dedication

To …

My parents

Husband

Son

Brothers and sisters

Acknowledgement

First of all would like to thank Allha Almighty for giving me the ability and strength to accomplish this work

I would like to express my gratitude and respect to my supervisor prof

Mohamed Abdel Kareem for his interest ,close supervision and continuous advice

Thanks for the staff , Dept .of chemistry ,sudan University of London science and Technology for all facilities .

Also thanks are extended to the technical staff of the Dept . of Ta\xanomy ,

Medicinal and Aromatic plants Research Institute , Khartoum ,Sudan for their help
Deep thanks to my family for their infinite support .

Abstract

This study was designed to investigate the oil from the potential medicinal plant *Mentha piperita*. The oil has been studied by GC. MS and the antimioxidant potential has been screened. Gas chromatography–mass spectrometry anaiysis showed 11 constituents. The oil was dominated by: i) Ievo-menthol (74.34%) ii) p-menthone (9.02%).

Mentha piperita oil has been evaluated for antioxidant activity using the DPPH assay. The oil showed significant radical scavenging activity (RSA76%) compared to the positive control-propy I gaiiate.

المستخلص

صمم هذا البحث لدراسة مكونات زيت النعناع وتقدير فعالية مضاد الأكسدة. اثبت تحليل الكرموتوغرافيا الغاوية – طيف الكتلة وجود ١١ مركبا بالزيت اهمها :

I)levo-menthol (74.34%) –ii) p-menthone (9.02%)

أجرى اختبار مضاد الأكسدة بطريقة (DPPH) وقد أبدى الزيت فعالية عالية كمضاد للأكسدة بلغت (RSA 76%) مقارنة بالمادة القياسية بروبيل قاليت.

Table of Contents

Subject		No.
	الاستهلال	I
	Dedication	II
	Acknowledgment	III
	Abstract	IV
	المستخلص	V
	Table of Contents	VI
Chapter One		
1	Introduction	1
1.1	Natural product	1
1.2	Essential oils	3
1.2.1	Analysis of essential oils	9
1.3	Gas Chromatography	9
1.4	The hyphenated technique: GC.MS	10
1.5	Pharmacological effects and traditional uses of volatile oils	10
1.6	Chemistry of essential oils	16
1.7	The targeted plant species	19
Chapter Two		
2.	Materials and Methods	22
2.1	Materials	22
2.1.1	Plant Material	22
2.1.2	Instruments	22
2.2	Methods	22
2.2.1	Extraction of oil	22
2.2.2	GC-MS Analysis	23
2.2.3	Antioxidant Assay	24
Chapter Three		
3	Results and Discussion	26
3.1	Menthe Piperta	26
3.1.1	GC/MS Analysis of Mentha Piperta Oil	26

3.1.2	Antioxidant Activity of Mentha Piperta Oil	28
	Conclusion	
	Recommendation	
	References	

Chapter One

Introduction

1-Introductio

1.1-Natural products

The definition of the natural products is still a matter of debate.However, they are chemical compounds or substances isolated from living organism.([1]

Chemistry of the natural products comprise the following processes: extraction, identification, quantification ,biosynthesis, , structural elucidation, physical and chemical properties and reactions

Natural products are formed by the pathway of primary or secondary metabolism [(2)]. Metabolism is defined as series of enzyme-catalyzed biochemical reaction or transformation occurring within the cells of an organism which are mainly required for its growth, development and for proper response to its environment. [(3)] Metabolism can be in form of anabolism or catabolism. Metabolites are the intermediate or products of metabolism, the term metabolite is usually restricted to small molecules. [(4)]

Plant secondary metabolites are called phytochemicals. Phytochemicals are the naturally occurring, biologically active compounds found in plants which have capabilities of disease inhibiting.[(5.6)] Phytochemicals are much effective in preventing disease because of antioxidant effect. Antioxidants defend molecules from oxidation if they are attacked by free radicals. They also prevent them from reactive oxygen. In this way prevention from many diseases and food spoilage is possible.[(7.8)] Before the introduction of orthodox medicines medicinal plants were used. Flowers, leaves, stems, seeds, roots, bark and fruit are constituents of the herbal medicines. Component of phytochemicals represent medicinal value of the natural plants.[(9.10)]. Phytochemicals perform physiological actions on human body.

Important phytochemicals are tannins, alkaloids, phenolic compounds and flavonoids.[11]

There are several types of naturally occurring compounds:

a)-Natural products formed by microorganisms

Microorganisms are considered as an important source source of drug candidates. They were not explored until the discovery of penicillin in 1929[13] .Since then , a large number of terrestrial and marine microorganisms have been screened for discovery .[14]

b)- **Natural products formed by marine organisms**

Some biologically interesting molecules like spongothymidine and spongouridine were isolated from a sponge known as : *Cryptotheca crypta* in 1950s. [15] The above compounds were nucleotides and represented great potential in the form of antiviral and anticancer agents. Discovery of these compounds led to the extensive research for the identification of novel drugs from the marine source. [16] Almost 70% of earth's surface is mainly covered by oceans. A number of marine organisms show sedentary lifestyle. So they synthesize complex and potent chemicals for the purpose of their defense from the predators. These chemicals may serve as remedies for ailments like cancer. Example is discodermolide isolated from marine sponge. [17] Discodermolide is a natural product having similar action to paclitaxel and possesses antitumor activity. It also has better solubility in water compared to paclitaxol. Combination of these two drugs led to reduce the tumor growth in various types of cancers. [15.18]

c) –**Natural products formed by Animals**

Many natural products are of animals origin. These natural products have been a major source of interesting compounds which were used as drugs. Epibatidine is obtained from skin of the Ecuadorian frog which is poisonous. It is 10 times more effective than the morphine. [19] Toxins and venoms obtained from animals played an important role in the curing of several diseases. Example is Teprotide, extracted from the Brazilian viper. It led to development of the captopril and cilazapril which were effective against the hypertension. [20]

d) **Natural products isolated from plants**

To date around 35.000- 70.000 plants have been screened for their bioactivity. The use of plants in herbal medicine has a long history in the treatment of various diseases .The earliest known records for the use of plants as drugs are from Mesopotamia in 2600B.C. and these still are significant part of traditional medicine and herbal remedies[21] ..[22]

1.2-Essential Oils

Essential oils are plant constituents mainle produced by plants for needs other than nutrition (i.e. protection or attraction). These oils are complex mixtures of organic compounds that give characteristic odour and flavour to the plants.

Essential oils are mainly composed of monoterpenes and sesquiterpenes whose main metabolic pathway is through mevalonate leading to sesquiterpenes and from methylerythritol leading to monoterpenes. They are located in different parts of the plant. They can be found in the root such as that of the vetiver grass (*Vetiveria zizanioides*), in stems like that of peteribi wood (*Cordia trichotoma*) and in cense, in leaves like in eucalyptus trees (*Eucalyptus citriodora*), citronella (*Cymbopogon* *nardus*), chinchilla (*Tagetes minuta*) and lemon grass (*Cymbopogon citratus*), in flowers like lavenders (*Lavandula officinalis*), in fruit like lemon, orange (*Citrus spp*.) and even in seeds as in the case of anise (*Pimpinella Anisum*), coriander (*Coriandrum sativum*) and pepper (*Piper nigrum*), among others.[(23)] They can work as internal messengers, like defense substances or plant volatiles aimed at natural enemies but also to attract pollinating insects to their host.[(24)] Essential oil also can be defined as a "product obtained from natural raw material, either by distillation with water and steam, or from the epicarp of citrus fruits by mechanical processing. [(25.26)] Similarly, other names like essence, fragrant oil, volatile oil, etheric oil, aetheroleum or aromatic oil [(27)] have been used to describe essential oils. Essential oils can be obtained from various aromatic plants, most commonly grown in tropical and subtropical countries. They are obtained from various parts of the plants, such as seeds, buds, leaves, roots, fruits, rhizomes, barks and flowers. Oil cells, secretary ducts, cavities or in glandular hairs are some of the prominently explored cellular sources of essential oils in plants. Among many others, Apiaceae, Lauraceae, Rutaceae, Asteraceae, Pinaceae and Cup ressaceae are the well known and famous families rich in essential oil. Some of the essential oils can be found in animals sources such as musk, sperm whale, civet and can be produced by microorganism. Hydrodistillation, steam distillation, microwave-assisted distillation, solvent extraction, cold pressing and supercritical fluid extraction. [(28.29.30)] are some the applied techniques used for extraction of oils.

Historically, the ancient Romans and Greeks in 1st century described the instrumental procedures for extraction. [(31)] Clear evidence which depicts the primitive form of distillation technology, which was in use in 400 BC is found in Taxila Museum, Pakistan [(32)]. While in late 12th or early 13th century (1235–1311

AD), Arnald de Villanova compiled detailed information about the conventional hydrodistillation method. [32]

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenous layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant. Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (*Angelica archangelica*), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated factors, climatic, seasonal and geographical conditions, harvest period and extraction techniques. [33] The yield of oils from the plants can also be affected by the stages of the plant growth.

Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators.

Oxygenated components off essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers. [34.35, 36] These properties were established after the oils have been extracted from the plant materials.

Essential oils are generally of complex matrix of plant volatile principles contained in the plant and they are more or less modified during the preparation process.[37]

Plant oils are usually stored in the oil glands or sacs can be removed by either accelerated diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions.

Common techniques used for the extraction of essential oils are;

a. Hydrodistillation

b. Hydrodiffusion

c. Effleurage.

d. Cold pressing

e. Steam distillation

f. Solvent extraction

g. Microwave Assisted Process (MAP)

h. Carbondioxide extraction.

Hydrodistillation involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapour and oil are removed through a water cool condenser.

During the process of hydrodiffusion steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma.[38]

On the other hand the process of **e**nfleurage is usually employed for flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odourless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind only the essential oil; hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

Cold pressing is another technique used for the extraction of essential oil but this method has not yet found high application in scientific research . It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

However, steam distillation is the technique of choice for extracting oils. This method is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel. Next steam is added and passed through the plant that contains the plants aromatic molecules or oils. The plant releases the aromatic molecules and the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapours. As they cool, they condense and transform into a liquid state.

Another useful technique used for the extraction of essential oil is : solvent extraction .This technique of extraction includes the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used for extraction. For instance, leaves, roots, fruits are extracted with benzene or with mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and colour. After the extraction, the solvent is removed by distillation under reduced pressure leaving behind the semisolid concentrate, this concentrate are extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil [(39)].

Microwave- assisted extraction of essential oils is of common use. The role of microwave is to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants.[(39)] This technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction. [(40-44)] This technique has also been applied for the extraction of saponins from some medicinal plants [(45)].

Carbon dioxide may also be used for extraction of essential oils**.** In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition of the oil.

1.2.1 Analysis of essential oils

The analyzing essential oils is carried out in order :

(i) To characterize and quantify oil constituents .

(ii) To assess the quality of the oils .

Analysis of essential oils is generally performed using Gas chromatography-mass spectroscopy which is a qualitative and quantitative technique[46].

1.3 Gas chromatography

Gas chromatography is a technique mainly used for separating chemicals in a complex matrix. This anlaysis provides some useful spectral data. The gas chromatography instrument vapourizes the sample and then separates the constituents of the analyte . The time elapsed between injection and evaluation is called “Retention time”. Each constituent of the anlyte ideally produces a characteristic spectral signal.

In this analytical method, the analyte is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separates before eluting from the column end. The detector measure different compounds as they emerge from the column.

1.4 The hyphenated technique: GC - MS

GC - MS analysis is an analytical tool which combines two features :the features of gas chromatography and mass spectrometry . This technique can identify different components within a test sample.

GC - MS instrument is composed mainly of two parts: The gas chromatography (GC) portion which separates the chemical mixture into pulses of pure components and mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed through the GC, the chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions. This is important because the particles must be charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent, so that it can be put into various uses.

1.5 Pharmacological effects and traditional uses of volatile oils

Volatile oils are used as raw materials in diverse fields comprising : aromatherapy, phytotherapy , perfumery, cosmetics,, pharmaceuticals and insecticides. [38]

Aromatherapy is the therapeutic use of volatiles to prevent or mitigate or diseases, infection and indisposition by means of inhalation. [47].

The inhalation of volatile oils or their individual constituents has a significant role in controlling disorders of the central nervous system. For instance, aroma inhibit of storax pill essential oil and preinhalation of *Aconus gramineus* rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy.[48.49]

The fragrance compounds, cisjasmonate, which characterized the aroma of *Jasminum grandiflorum*, have a tranquilizing effect on the brain upon inhalation. [50]They significantly increased the sleeping time of mice induced by pentobarbital. Cendrol, which is a major component of card wood essential oil, shows a sedative effect and prolonged pentobarbital induced sleeping time on rats upon inhalation [51] The vapour of lavender essential oil or one of its main component linalool may also be applicable to the treatment of menopausal disorder through inhalation. [52] Lavender essential oil demonstrated an analgesic activity.[53]

Many essential oils exhibit diverse pharmacological effects including: antibacterial, fungicidal, relaxant, stimulating, antidepressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both by pathogenic and non-pathogenic diseases. Pathogenic diseases caused by bacterial, virus, and the fungi can be treated with essential oils.

Some *in vitro* studies indicated[54-58] that volatile oils can exhibit many beneficial effects such as antibacterial activity against a wide spectrum of pathogenic bacteria strains including; *Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella dysentria*, *Bacillus cerus*, and *Staphylococcus aureus*. Thyme and oregano essential oils can inhibit some pathogenic bacteria strains such as *E.coli*, *Salmonella typhimurium*, *Salmonella entritidis* and *Salmonella choleraesuis* (59), with the inhibition directly correlated to the phenolic components carvacrol and thymol. Eugenol and carvocrol showed an inhibitory effect against the growth of four strains of *Escherichia coli* and *Listeria monocytogens*(60). Also, the presence of phenolic hydroxyl group in carvacrol particularly is credited with its activity against pathogens such as *Bacillus cereus*. (61). Essential oil with high concentration of thymol and carvacrol e.g. oregano, savory and thyme, usually inhibit Gram positive more than Gram-negative pathogenic bacteria.(62) However, they show antibacterial activity against Gram- negative *Haemophilus influenza* and *Pseudomonas aeruginosa* respiratory pathogens, while Gram-positive *streptococcus pyrogens* was the most resistant to the oil (63). Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural mouth control,(64) general improvement of oral health (65), interdental hygiene (66) and to control oral mal odour (67). Mouth rinses containing essential oils with chlorohexine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decreases chances of postoperative infections, decreases oral bacterial load and decrease aerolization of bacteria (68). Mouth washes containing essential oils could also be used as part of plaque-control routine since they can penetrate the plaque biofilm, kill pathogenic-wall and inhibiting their enzymatic activity. (69) In addition, essential oil in mouth washes prevent bacterial aggregation slows the multiplication and extract bacterial endotoxins.(70) *Croton cajucarabenth* essential oil was found to be toxic to some pathogenic bacteria and fungi associated with oral cavity diseases.(71)

Essential oils have also been reported to show very interesting antiviral activities and could be used as alternatives to synthetic antiviral drugs.

Some volatile oils have demonstrated virucidal properties with the advantages of low toxicity [(72)]; *Herpes simplex* virus (type III) causes some of the most common viral infections in human and can be fatal. Synthetic antiviral drugs have been used to treat Herpes infection [(73)], but not all are efficacious in treatment of genital herpes infections. Incorporation of *Artemisia arboreseens* essential oils in Multilamella liposomes greatly improved its activity against intra cellular *Herpes simplex* virus type 1 (HSV-1) [(74)]. Due to the presence of citral and citronellal in *Melissa officinalis* L. essential oil, it also inhibits the replication of HSV-2 [(75)] and the ability to replicate of HSV-1 can be suppressed with different essential oils in vitro. [(76)] Also the effect of five essential oils on Epstein-Barr virus (EBV) (viridae) has been reported.which caused the infectious mononucleosis associated with Burkitt lymphoma and naso-pharynx carcinoma.

It has been suggested[77] that volatile oils can also be used as a natural remedy for non-pathogenic diseases. For instance, Garlic essential oil significantly lowered serum cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases [(77)]. The hypolipidemic action of garlic oil is primarily due to a decrease in hepatic cholestrogenesis [(78)].

Some volatile oils showed hypotensive effect when applied in vivo and they are now used for treating hypertension. Oral administration of combination of oregano, cinnamon, cumin, and other essential oils decreases systolic blood pressure in rats and intravenous administration of the essential oil from the aerial parts of *Mentha villosa* induced a significant dose-dependent hypotension associated with decrease in heart rate. This activity was attributed to volatile component, piperitenone oxide which represents 55.4% of the oil. The hypotensive effect induced by the oil is probably due to its direct cardiodeplesant action and peripheral vasolidation, which can be attributed to both endothelium-dependent and endothelium-independent mechanism.

Ocimum gratissium oil when administered intravenously induced a significant hypotensive effect [79]. The hypotensive activity of this oil is associated with its vasodilator effect. The oil acts directly upon vascular smooth muscles. This effect was attributed to eugenol ; but from a safety point of view, care must be taken in dealing with eugenol due to its suspected carcinogenicity and hepatotoxicity.

The alcohol : terpinen-4-ol decreased main aortic blood pressure in a dose related manner, in a DOCA-salt hypertensive rat model. The mechanism of action was related to the induction of vascular smooth muscle relaxation rather than enhanced sympathetic nervous system activity. Terpinene-4ol is a major constituent of several essential oil, particularly tea tree [80] and sweet marjoram essential oils. Some essential oils may aggravate diabetes, for instance rosemary essential oil showed hyperglycaemic and insulin release inhibitory effect in diabetic rabbits[81] ·It has been emphasised that the lipophilic fraction of aromatic plants are not generally responsible for any anti-diabetic activity showed by these plants, but it was also indicated that an oral administration of a combination of essential oils including cinnamon, cumin, oregano, fennel, myrtle besides others was able to enhance insulin sensitivity in type II diabetes, in addition to lowering circulating glucose in diabetic rats. The essential oil of *Satureja khuzestanica* results in significant decreases in fasting blood glucose level in diabetic rats [82].

Some volatile oils exhibited cancer suppressive activity when tested on a number of human cancer cells lines including glioma, tumours, breast cancer, leukaemia and others [83].

A significant effect on the treatment of glioma is by using the sesquiterpene hydrocarbon element which is found in small amounts in many essential oils, it prolonged quality survival time of patients with glioma [(84)].

Antiangiogenic therapy is one of the most promising approaches to control cancer. Perillyl alcohol (POH) which is the hydroxlated analogue of d-limonene has the ability to interfere with angiogenesis [(85)]. POH either alone or with PA (perillic acid, the major metabolite of POH in the body), has the potential use as an anticancer drug that stimulates different types of tumour to apoptosis inhibit their proliferation and overcomes their resistance to chemo/radiotherapy [(86)]. Treatment of human leukaemia cells with eucalyptus oil showed morphological changes (fragmentation of DNA) indicating an induction of apoptosis [(87)]. The essential oil of lemon balm (*Mellisa oficinalis* L) was found to be effective against a series of human cell lines(A549, MCF-7, Caco-2, HL-60, K562) and a mouse cell line (B16F10)[(88)] and that of *Artemisia annua* L. induced apoptosis of cultured SMMC-7721 hepatocarcinoma cells.[(89)] The essential oils of Australian tea tree (*Melaleuca alternifolia*) and its major monoterpene alcohol, terpinen-4-ol, were able to induce apoptosis in human melanoma M14 WT cells.[(90)]

There is evidence to suggest that the effect of the total oil of terpinen-4-ol was meditated by their interaction within the plasma membrane and subsequent reorganisation of membrane lipids. Hepatic arterial infusion with Curcuma oil had a similar positive effect in treating primary liver cancer as that of the chemical drugs [(91)].

The essential oil of *Tetraclinis articulate*, (a conifer tree) showed the hallmarks of apoptosis when tested on a number of human cancer cell lines including melanoma, breast and ovarian cancer .[(92)]

1.6 Chemistry of essential oils

Essential 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 [93]. 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. [94.95]

Essential oils contain diverse volatile compounds belonging to various chemical classes including : 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 (-seining).

Many thousands of compounds belonging to the family of terpenes have so far been identified in essential oils [96], such as functionalized derivatives of alcohols (geraniol,bisabolol), ketones (menthone, p-vetivone) , aldehydes (citronellal, sinensal), esters (tepinyl acetate, cedryl acetate), and phenols (thymol). Essential oils also contain non-terpenic compounds biogenerated by the phenylpropanoids pathway, such as eugenol, cinnamaldehyde, and safrole.

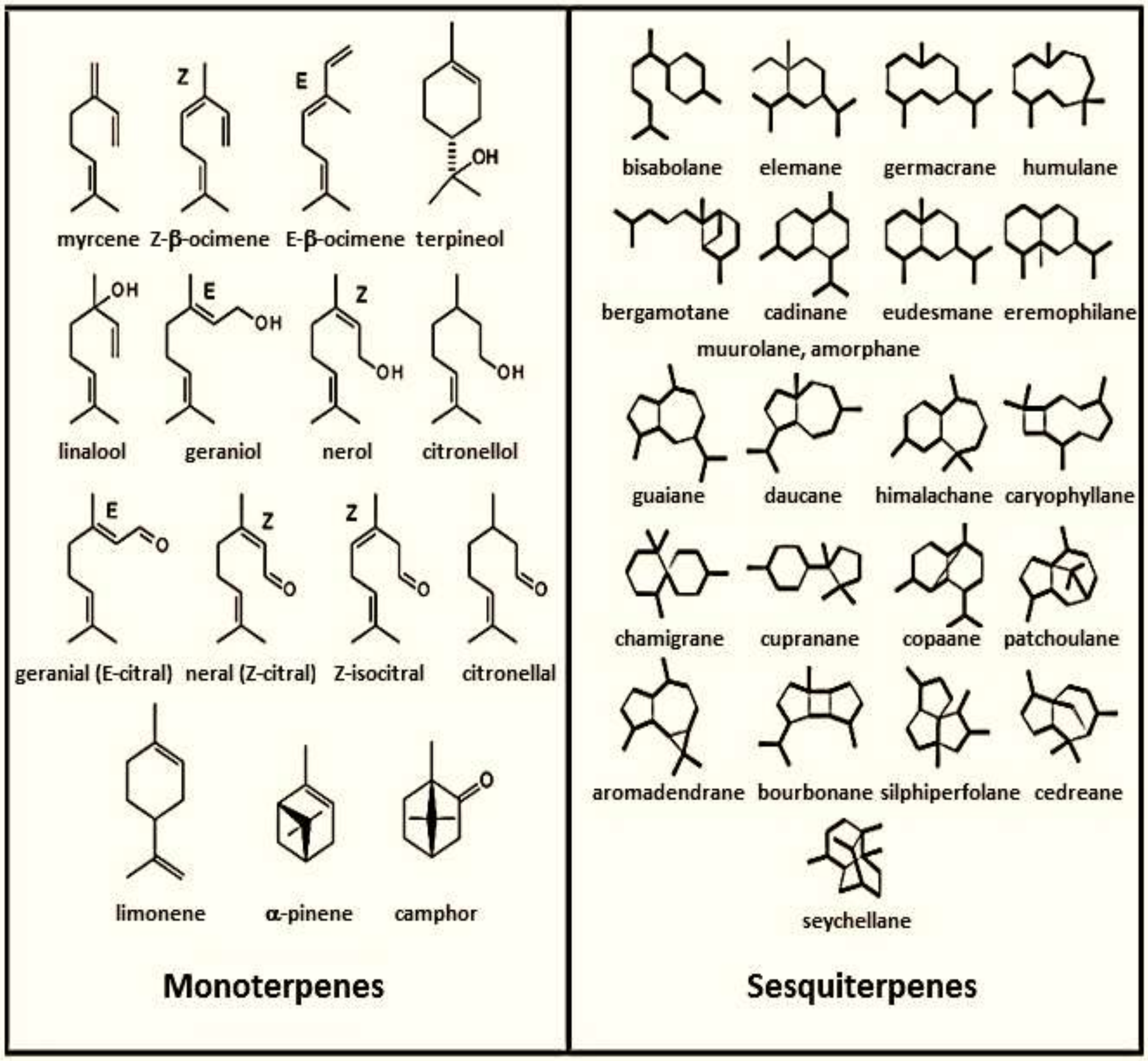

Figure 1.1 Structures of some terpenes

The chemical composition of essential oils is very diverse.Various factors are responsible for this variability and can be grouped into two categories:

(a)Intrinsic factors

Intrinsic factors are related to the plant, and its interaction with the environment (soil type and climate, etc.) and the maturity of the plant concerned, even at harvest time during the day **(b)extrinsic factors**

Extrinsic factors are related to the extraction method and the environment. The factors that determine essential oil yield and composition are numerous. In some cases, it is difficult to isolate these factors from each other as they are interrelated and influence each other. These parameters include the seasonal variations, plant organ, and degree of maturity of the plant, geographic origin, and genetics.[97.98]

There are several methods are employed for the extraction of volatiles from aromatic plants. The most often used device is the circulatory distillation apparatus described by Cocking and Middleton [99] introduced in the European Pharmacopoeia and several other pharmacopoeias. This device consists of a heated round-bottom flask into which the chopped plant material and water are placed and which is connected to a vertical condenser and a graduated tube, for the volumetric determination of the oil. At the end of the distillation process, the essential oil is separated from the water phase for further investigations. The length of distillation depends on the plant material to be investigated. It is usually fixed to 3–4 h.

A further improvement was the development of a simultaneous distillation–solvent extraction device by Likens and Nickerson in 1964[100]. The device permits continuous concentration of volatiles during hydrodistillation in one step using a closed-circuit distillation system.

1.7. The targeted plant species

Mentha piperita is an important medicinal plant in the family Laminiaceae. This plant was first described by Linnaeus in 1753.Thes plant is indogenous to Europe and is widely cultivated in tropics and subtropics. This plant has potential traditional uses besides being used as flavoring agent and as ingredient in cosmetic 101.

Mentha piperita

Herbalists regard this plant as analgesic vermifuge antiseptic carminative antiemetic antipruritic ant androgenic antiseptic

And antispasmodic. Mentha piperita contains mainly menthol

menthon and methy1 acetate 101.

Aim of this research

This study was designed to:

- Extract the oil from the seeds of the medicinal plant : Mentha piperita.
- Identify and quantify the constituents of the oil via the hyphenated technique: GC-MS.
- Evaluate the oil for its antioxidant potential using the DPPH assay.

Chapter Two

Materials and Methods

2-Materials and Methods

2.1 Materials

2.1.1 Plant materials

Seed of *Mentha piperita* were purchased from the local market-Khartoum –Sudan .the plants were identified and authenticated by the medicinal and Aromatic plants Research Institute –Khartoum –Sudan.

2.1.2 Instruments

AshimadzO GC-MS-QP2010 Uitra instrument with a RT|X-5MS column (30m,length ; 0.25mm diameter;0.25 um, thickness) was used for GC-MS analysis.

2.2 Methods

2.2.1 Extraction of oil

Powdered plant material (400g) were macerated with n-hexane for 48h. the solvent was removed under reduced pressure giving the oil .the oil was kept in fridge for work.

2.2.2 GC-MS Analysis

(2ml) of the oil was mixed thoroughly with 7ml of alcoholic sodium hydrxide that was prepared by dissolving 2g in 100ml methanol. (7ml) alcoholic sulfuric acid (1ml H_2SO_4 in 100ml methanol) was added.

The mixture was then shacked for 5 minutes. The (1ml) of supersaturated sodium hexane were added and the contents were added and the contents were shaken thoroughly for 5 min. (2ml) of normal hexane were added and the contents were shaken thoroughly for 5 minutes. (5µ) of the n-hexane were diluted with (5ml) of diethyl ether and dried over anhydrous sodium sulphite. (1µ1) of the diluted sample was injected in the GC.MS vial.

The qualitative and quantitative analysis of the sample was carried out by using a shimadzu machine-model (GC/MS-QP2010-Ultra). The sample was injected under the following chromatographic conditions: column oven temperature: 150.0^{o}C; injection temperature: 300.0^{o}C; injection mode: split; flow mode: linear velocity; linear velocity: 47.2cm/sec.; purge flow 3.0ml/min.; split ratio: -1.0, oven temperature program is presented table 1.

Table 1: oven temperature program

Rate	Temperature (^oC)	Hold time $(min)^{-1}$
-	150.0	1.00
4.00	300.0	0.00

2.2.3 Antioxodant Assay

The targeted oil has been screened for antioxidant activity against stable DPPH radicals and the decrease in absorbance at λ_{max}217nm has been measured via UV spectroscopy.

Chapter Three

Results and Discussion

3-Results and Discussion

In this research the oils from the potential medicinal plant : *Mentha piperta* has been studied by GC.MS and the antimicrobial activity has been screened.

3.1-*Mentha piperta*

3.1.1-GC/MS analysis of *Mentha piperta* oil

Gas chromatography - mass spectrometry has been used for the characterization of the *Mentha piperta* essential oil. The analysis revealed the presence of 11 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

Fig. 3.1: Total ion chromatograms

Table 3.1: Constituent of the oil

ID#	Name	Ret.Time	Area%
1.	.alpha.-Pinene	4.131	0.94
2.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	4.795	0.46

3.	.beta.-Pinene	4.853	2.07
4.	Cyclohexane, 1-methyl-4-(1-methylethyl)-, trans-	5.063	0.12
5.	D-Limonene	5.792	1.88
6.	Eucalyptol	5.835	0.52
7.	Isopulegol	8.125	0.99
8.	p-Menthone	8.258	9.02
9.	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	8.479	6.04
10	Levomenthol	8.668	74.34
11	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	10.964	3.62

The following compounds were detected in the chromatograms as major constituents:

i) Levo-menthol (74.34 %)
ii) p-Menthone (9.02 %)

The GC-MS analysis gave a spectrum(Fig.3.2) identical of levo-menthol. The peak at m/z156(RT.8.668)corresponds : M^+ $[C_{10}H_{20}O]^+$.It also showed a mass spectrum characteristic of p-menthone (Fig.3.3).The peak at m/z 154(RT.8.258) corresponds M^+ $[C_{10}H_{18}O]^+$.

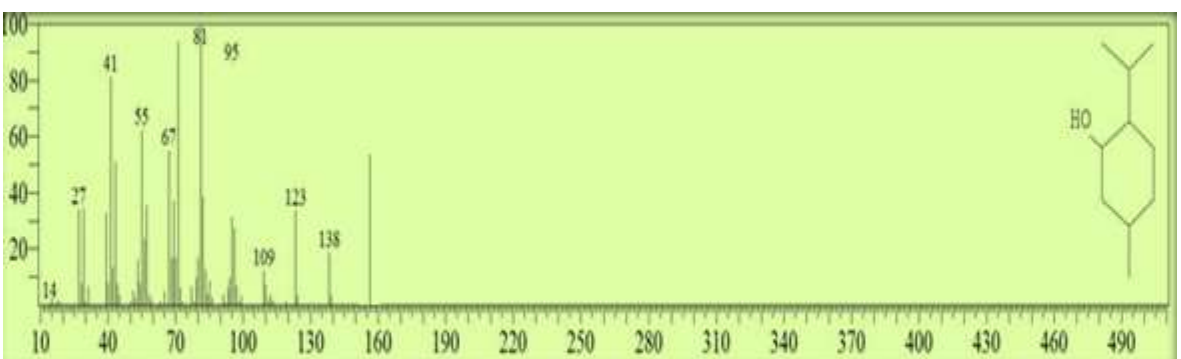

Fig. 3.2 :Mass spectrum of levo-menthol

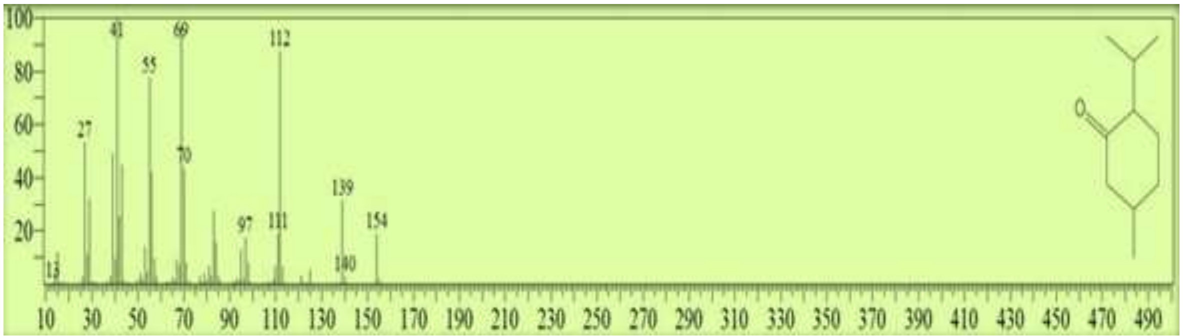

Fig. 3.2: Mass spectrum of p-menthone

3.1.2-Antioxidant activity of *Mentha piperta* oil

Mentha piperta oil was assessed for antioxidant activity using the DPPH bioassay. The oil showed significant radical scavenging capacity (RSA 76%) compared to the positive control – propyl gallate (93%).

References

References

1. Bhat, S. V., Nagasampagi, B. A. and Sivakumar, M. Chemistry of natural product. Berlin, New York. Springer, ISBN 81-7319-481-5. 650p, (2005).

2. Finar, I. L. Stereochemistry and the chemistry of natural Product, Pearson Education Ltd, Patparganj Delhi, India, **Vol.2** (5 th edn.), 942p,(2006) .

3. Cos, P. Vlietinck, A.J., Berghe D.V. and Maes. L. Anti-infective potential of natural products: how to develop a stronger in vitro ‘proof-of-concept’. *Journal of ethnopharmacology*,**106**(3): 290-302,(2006) .

4. Nussbaum, F., Brands, M., Hinzen, B., Weigand, S. and Häbich D. Antibacterial natural products in medicinal chemistry, Angewandte Chemie International Edition, **45**(31): 5072-5129,(2006).

5. Abdurraham, F. Studies on natural products chemistry of the Moraceae in Africa traditional medicine and management of psychiatry in Borno state. University of Maiduguri, Nigeria,(1992).

6. Farombi, E .Nwankwo, J. and Emerole G, Effect of methanolic extract of browned yam flour diet on 7, 12 Dimethylbenzanthracene (DMBA) and 3methylcholanthrene(3-MC)-induced toxicity in the rat Proceedings of the Federation of African Society on Biochemistry and Molecular Biology , **1**: 5-10. (1998).

7. Chetia, J., Upadhyaya, S. and Saikia, L. Phytochemical Analysis, Antioxidant and Antimicrobial Activity and Nutrient Content analysis of Ocimum Gratissimum linn. From dibrugarh, NE India. International *Journal of Pharmaceutical Sciences Review & Research*, **25**(1)(2014).

8. Koleva, I.I. , Niederländer, H.A. and T.A. van Beek, T.A. An on-line HPLC method for detection of radical scavenging compounds in complex mixtures. Analytical Chemistry, 72(10): 2323-2328, (2000) .

9. Akinmoladun, A.C. , Ibukun, E., Afor, E., Obuotor, E. And Farombi, E. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Sci. Res. Essay*, **2**(5): 163-166, (2007).

10. Pascaline, J., Charles M., Lukhoba C. and George, O. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. *J Anim Plant Sci.*, **9**: 1201-1210,(2011).

11. Kolak, U., TOPÇU, G., Birteksöz, S., Ötük, G and Ulubelen. , ATerpenoids and steroids from the roots of Salvia blepharochlaena. *Turkish Journal of Chemistry*, **29**(2): 177-186 , (2009).

12. Newman, D.J.: Cragg, G. Natural products as sources of new drugs over the last 25 years.*J.Nat. Prod*, m**70,** 461- 477,(2007).

13. Dar-Al Hamed "Chemistry of Natural products.vol.2" India Acadimic, pp1-91,(1998).

14. Chin, Y.W.: Balunas, M.J.; Chai, H.B.; Kinghom, A. D. Drug discovery from natural sources .*Aaps Journal*,***8***, 239-253,(2007).

15. Bernan, V., Greenstein, M. and Maiese. W. Marine microorganisms as a source of new natural products. Advances in applied microbiology, 43: 57-90, (1997).

16. König, G.M. , Kehraus, S., Seibert, S.F,Abdel-Lateff, A. and Müller. D Natural products from marine organisms and their associated microbes. *Chem BioChem.* **, 7**(2): 229-238,(2006).

17. Haefner, B. Drugs from the deep: marine rynatural products as drug candidates. *Drug discove today*, **8**(12): 536-544,(2003).

18.Tziveleka, L.A.,Vagias,C. and Roussis. V. Natural products with anti-HIV activity from marine organisms. Current topics in medicinal chemistry**3**(13): 1512-1535 ,(2003).

19. Cragg, G.M. , Newman, D.J. and Snader, K.M. Natural products in drug discovery and development. *Journal of natural products*, **60**(1): 52-60,(1997.)

20. Clark, A.M. Natural products as a resource for new drugs. *Pharmaceutical research*, **13**(8): 1133-1141 ,(1996).

21. Koehn, F.E; Carter, G. T. The evolving role of natural products in drug discovery. *Nat. Rev.Drug Discov* , **4, 206-** 220(2005).

22. Farmsworth, N.R., Akerele, O. Bingel, A.S. Soejarto , D. D.and Guo , Z.,Global importance of medicinal plant . *In The Conservation of Medicinal Plant*, (1991).

23. Baser, K. H.; *Handbook of Essential Oils: Science, Technology, and Applications / K. Hüsnü Can Baser, Gerhard Buchbauer* ISBN. Universitat Wien, Austria, 978-1-4200-6315-8.(2010).

24. Harrewijn, P., Van Oosten, A. M. and Piron, P. G. M. Natural Terpenoids as Messengers. Dordrecht: Kluwer Academic Publishers,(2001).

25. Schnaubelt, K. Medical aromatherapy: Healing with essential oils. Frog Books. Berkeley, California, USA.(1999).

26. ISO. International Standards Organization-http://www.iso. org/ iso/ home.htm (accessed on 12 December 2014).

27. Baser KHC, Demirci F. Chemistry of essential oils. In: Fragrance and flavours chemistry, bioprocessing and sustainability, 1st ed.; Berger, R.G., Ed.; Springer: Leipzig, Germany,(2007).

28. Fadel, O. Ghazi ,Z. Mouni, L. Benchat ,N. Ramdani, M. Amhamdi,H. Wathelet JP. Asehraou, A. Charof R Comparison of microwave-assisted hydrodistillation and traditional hydrodistillation methods for the Rosmarinus eriocalyx essential oils from Eastern Morocco. *J. Mater. Environ. Sci*, **2**(2): 112- 117,(2010).

29. Asghari J. Touli, CK. Mazaheritehrani, M. Microwave assisted hydrodistillation of essential oils from echinophora platyloba DC. *J. Med. Plants Res***, 6**: 4475-4480,(2012).

30. Mohamad, M. Shamspur, T.Mostafavi, A. Comparison of Microwave-assisted distillation and conventional hydrodistillation in the essential oil extraction of flowers Rosa damascena mill. *J. Essential Oil Res*., **25**(1): 55-61, (2013).

31. Guenther, E. The essential oils. Vol 1: History-origin in plants production analysis. Read Books Ltd., New York, USA.(2013).

32. Sell, C . Baser K.H.C., Buchbauer, G., Chemistry of essential oils. In: Handbook of essential oils: Science, technology, and applications; Eds.; CRC Press, Taylor and Francis Group: London, UK. (2010).

33. Pannizi, L., Flamini, G., Cioni P, L., Morelli I. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. *J Ethnopharmaco,* **39**: 167-170,(1993).

34. Buchauer, G., Jirovetz, L., Jager ,W *.et al.* Fragrance compounds and essential oils with sedative effects upon inhalation. *J. pharm Sc.,* **82:** 660-664,(1993)

35. Federspil, P., Wulkow, Zimmermann, T. Effect of standardized myrtol in the therapy of acute sinusitis-result of a double-bind, randomized and multi-center study compared with placebos. *Laryago Rlune-otologie,* **76**: 23-27, (1997)

36. Rajesh, D., Howard, P. Perillyl alcohol mediated radio-sensitization via augumentation of the Fast pathway in prostate cancer cells. *Prostate*, **57**: 14-23(2003).

37.Brunteon, J. Pharmacognosy, phytochemistry, medicinal plants, intercepts, LTD: Hamsphire. (1995).

38. Buchbauer ,G. The detailed analysis of essential oils leads to the understanding of their properties.*Perfumer and flavourist*, **25**:64-67,(2000)

39. Belanger, A., landry, B., dextraze, L . *et al.* Extraction et determination decomposes volatile de l' au (allicum satium) *Rivistraitalian ep,* **2**:455,(1991).

40. Pare, JR., Sigoiun, M. Lapointe J. Microwave assisted natural product extraction. Brevet App can no , 600322, 16 Mai, (1989).

41. Collin, GJ. , Lord, D, Allaire J, Gagnon D. Huiles essentielles extraits ' micro-ondes '.*Applied Environment Microbial*, **47**:229-233,(1991).

42. Bouzid, N., Vilarem, G., Graset, A. Extraction desnuiles essentielles pardes technologies non conventionnelles in proceeding of the intern Congr. Arom. Medicinal plants and essential oils, benjilali B, ettalibi M, ismaili-Alaoui M,zrira S (eds).Actes editions,rabat,morocco, 115-120, (1997) .

43.Chiasson H, Belanger A, Bostanian N, *et al.* Acaridial properties of *Atermisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction(2001).

44. Ghoulami, S., Oumzil, H., Rhajaoui, M., *et al*.. Biologie ET. Kenitra, Morocco, 49.(2001).

45. Safir, O., Fkih-Tetouani, S., Soufiaoui, M., *et al*. Microwave extraction of the aerial parts of *Zygopyllum gaetulum. Rivista Italian,* ***25****:3*-10. (1998).

46. Keravis, G. Spectrometrie de masse et chromatographie dans l'analyse des plantes aromatiques et huilies essentielles, 375-384(1997).

47.Buchbauer, G., Jager ,W., Jirovet, L., *et al.* Therapeutic properties of essential oils and fragrances in bioactive volatile compounds from plants, teranishi R , bettery R, Sugisawzb H (eds) , 161,(1993).

48. Koo, B., Ha, J, Lim J., *et al.* Inhibitory effect of the fragrance inhalation of essential oil from Acorus gramineus on central nervous system. Acorus gramineus on central nervous system. *Bio Pharm Bull***, 26**: 978-982, (2003).

49. Koo, B., Lee, S., Ha, J., *et al* Inhibitory effects of essential oil for SuHeXiang Wan on central nervous system. *Bio Pharm Bull,* **27**: 515-519,(2004).

50.Hossain, S., Aoshima, H., Koda ,H., *et al.* Fragrances in oolong tea that enhances the *Biotechnol Biochem,* **68**:1842-1848.(2004).

. 51. Kagawa, D., Jokura, H., Ochiai R, *et al.* The sedative effects and mechanism of action cendrol inhalation within behavioural pharmacological evaluation. *Planta Med*, 69: 637-641,(2003).

52. Yamada, K., Mimaki, Y., Sashida, Y. Effects of inhaling the vapour of Lavandula burnati superderived essential oil and linalool on plasma adrenocorticotropic hormone (ACTH), catecholamine and gonadotropin levels in experimental menopausal female rats. *Biol Pharm Bul*, **l 28**: 378-383, (2005).

53. Barocelli, E., Calcina, F. Chiavarini, M. *et al.* Anticipative and gastroprotective effects of inhaled and orally administered lavandulla hybrid reverchon gross essential oil. *Life Sci*, **76**: 213-223,(2004).

54.Jirovetz, L., Buchbauer, G., Denkova, Z., *et al.* Antimicrobial testing and gas chromatographic analysis of pure oxygenated monoterpenes 1,8cineol, alpha-terpineol, terpene-4-ol and camphor as well as target compounds in essential oils of pine (Pinus pinaster) rosemary (Rosmarinus officinalis) and tea tree (Melaleuca alternifolia). *Sci Pharm***, 73**: 27-39,(*2005*).

55 .Burt, S. Essential Oils: their antibacterial properties and potential applications in food-a review. *Inter. J food microbial* **, 94:** 223-253,(2004).

56. Dadalioglu, I., Evrendile K.G., Chemical composition and antibacterial effect of essential oils of Turkish oregano (Oreganium minutiflorum) bay laurel (Laurus nobilis), Spanish lavender (Lavandula stoechas L.) and fennel (Foeniculum vulgare) on common food burn pathogens. *J Agric Food chem*, **52**: 8255-8260, (2004).

57.Ngufack, J., Budde ,B., Jakobsen, M. Five essential oils from aromatic plants of Cameroon: their antibacterial activity and ability to permeabilize the cytoplasmic membrane of Listeria innocua examined by flow cytometry. *Lett Appl Microbiol*, **39**: 395-450,(2004).

58. Hulin ,V., Mathot, A., Mafart ,P. Les proprieties anti-microbiennes des huiles essentielles et composes d'aromes. *Sci. Ailments* , **18:** 563-582,(1998).

59. Penalver, P., Huerta, B., Astorga ,R., *et al*. Antimicrobal activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS, **133**: 1-6.(2005).

60. Gaysinsky, S., Davidson, P., Bruce, D.*et al.* Growth inhibition of *Escherichia coli* 0157:H7 and Listeria monocytogenes by carvacol and eugenol encapsulated in surfactant micelles. *J Food Prot*, **68**: 2559-2566,(2005).

61. Ultee, A., Bennik, M., Moezelaar, R.The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Appl Environ Microbiol, 68: 1561-1568,(2006).

62. Nevas. M., Korhonen, A., Lindstrom M., *et al.* Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. *J. Food Prot,* **67**: 199-202,(2004).

63.Skocibusic M, Bezic, N., Dunkic ,V., *et al.* Antibacterial activity of *Achillea clavennae* essential oil against respiratory tract pathogens. *Fitoterapia,* **75**: 733-736,(2004)

64. Yengopal V .The use of essential oil mouthwash as preprocedural rinses for infection control. *SADJ,* **59**: 247-248, 250,(2004).

65. Yengopal V. Essential oils: some lesser known uses and properties for improved oral health *SADJ*,**59**: 381-382, 384.(2004) .

66.Yengopal V. Essential oils and interdental hygiene. *SADJ* **, 59**: 155, 157- 170. (2004).

67. Yengopal V. Preventive dentistry: essential oil and malodour. *SADJ,* **59:** 204 - 206,(2004).

68. Hennessy ,B., Joyce, A.A. survey of preprocedural antiseptic mouth rinse use in Army dental clinics. *Mil Med* , **169**: 600-603,(2004).

69. Ouhayoun J.P. Penetrating the plaque biofilm: impact of essential oil as mouthwash. *J Clin Periodontol,* **30** (S5): 10-12 ,(2003).

70.Seymour R. Addition properties and uses of essential oils. *J Clin Periodontol* **30** (S5): 19-21,(2003).

71.Alviano W, Mendonca-Filho R, Alviano D *et al.* Antimicrobial activity of *Croton cajucara* Benth linalool-rich essential oil on artificial biofilms and planktonic microorganisms. *Oral Microbial Immunol,* **20**: 101-105,(2005).

72. Baqui, A., Kelley, J., Jabra-Rizk, M. *et al. In vitro* effects of oral antiseptic human immunedeficiency virus-1 and herpes simplex virus type 1. *J Clin periodontal* **28**: 610-616,(2001).

73. Wagstaff, A., Faulds, D., Gona, K. 1994. Acyclovir: a reappraisal of its antiviral activity. Pharmacokinetic properties and therapeutic efficacy. Drugs, 47: 153-205,(1994).

74. Sinico, C., De. Logub, A., Laia ,F., *et al*. Liposomal incorporation of Artemisia Arborescens L. Essential oil and *in vitro* antiviral activity. *Eur J Pharm Biopharm,* **59**: 161-168.(2005).

75.Allahverdiyev, A., Durgran, N., Ozguven M *et al.* Antiviral activity of volatile oils of Melissa L. Against herpes simplex virus type-2 Phytomedicine , 11: 657-661.(2004).

76. Bammi J, Khelifia R, Remmal A. *et al*. Medicinal plants and essential oils,502,(1997).

77. Bordia A. Effect of garlic on blood lipids in patients with coronary heart disease. *Am J CliNutr,* **34**: 2100-2103 ,(1981).

78. Mathew B, Daniel R, Augusti K. Hypolipidermic effect of garlic protein substituted for casein in the diet of rats compared to those of garlic oil. *Indian J*

79. Lahlou S, Interaminense F, Leal-cardoso J. *et al*. Cardiovascular effects of the essential oil of Ocimum gratissimum leaves in rats: role of the autonomic nervous system. *Clin Exp Pharmacol Physio,* **1**: 219-225,(2004).

80. Brophy, J., Davis, N., Southwell, I. *et al.* Gas chromatographic quality control for oil of *Mellaleuca* terpinene-4-ol type. *J Agric Food Chem,* **37**: 1330-1333,(1989).

81. Broadhurst C, Polanski M, Anderson R. Insulin-like biological activity of culinary and medicinal plant aqueous extracts *in vitro*. *J Agric Food Chem ,* **48**: 849-852,(2000).

82. Abdollahi M., Salehnia A., Mortazavi, S. *et al.* Antioxidant, antidiabetic, antihyperlipidermic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzesstanica* in rat *in vivo*: *Med Sci Monit ,* **9**: 331-335,(2003).

83. De Angelis L., Braintumours, *N. Engl J Med*, **344**: 114-123.(2001).

84. Tan P, Zhong, W., Cai, W. Clinical study on treatment of 40 cases of malignant brain tumour by elemene emulsion injection, 20: 645-648,(2000).

85. Loutrari, H., Hatziapostolou, M., Skouridou ,V., *et al.* Perillyl alcohol in an angiogenesis inhibitor. *J Pharmacol Exp Ther,* **311**: 568-575.(2004).

86. Rajesh D, Howard P. Perillyl alcohol mediated radio-sensitization via augumentation of the Fast pathway in prostate cancer cells *Prostate*, 57: 14-23 (2003).

87.Motakii, H., Hibasami, H., Yamada ,Y., *et al.* Specific induction of apoptosis by 1,8-cineole in two human leukemia cell lines, but not in human stomach cancer cell line. *Oncol Rep***,** **9**: 757-760,(2002).

88. De Sousa, A., Alviano, D., Blank, A., *et al Melissa officinalis* L. Essential oil: antitumor and antioxidant activities. *J Pharm Pharmaco,* **56**: 677-681,(2004).

89. Li, Y., Li M, Wang ,L., *et al.* Induction of apoptosis of cultured hepatocarcinoma cell by essential oil of *Artemisia annul sichuan* Da Xue Bao Yi Xue Ban , 35: 337-339,(2004).

90. Calcabrin, A., Stringaro, A., Toccacieli ,L., *et al*Terpinen-4-ol, the main component of *Melaleuca alternifolia* oil *in vitro* growth of human melanoma cells. *J Invest Dermatol,* **122**: 349-360,(2004).

91.Cheng, J., Chang, G., Wu WA controlled clinical study between hepatic arterial infusions with embolized Curcuma aromatic oil and chemical drugs in treating primary liver cancer, 21: 165-167,(2001).

92. Buhagiar, J., Podesta, M., Wilson A, *et al.* The induction of apoptosis in human melanoma, breast and ovarian cancer cell lines using an essential oil extract from the conifers. *Anticancer Res* , **19**: 5435-5443,(1999).

93. Sell, C.S. The Chemistry of Fragrance. From Perfumer to Consumer, 2nd ed.The Royal Society of Chemistry: Cambridge, UK, p. 329,(2006).

94. Vainstein, A.; Lewinsohn, E.; Pichersky, E., Weiss, D. Floral Fragrance. New Inroads into an Old Commodity. Plant Physiol, 27, 1383-1389,(2001).

95. Pophof, B.; Stange, G.; Abrell, L. Volatile Organic Compounds as Signals in a Plant—Herbivore System: Electrophysiological Responses in Olfactory Sensilla of the Moth Cactoblastis cactorum. Chem. Senses **30**, 51-68,(2005).

96. Modzelewska, A.; Sur, S.; Kumar, K.S.; Khan, S.R. Sesqui terpenes: Natural products that decrease cancer growth. Curr. Med. Chem. Anti-Cancer Agents, 54, 477-499,(2005).

97.Hussain, A.I.; Anwar, F.; Sherazi, S.T.H.; Przybylski, R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. *Food Chem.* **108**, 986–995,(2008).

98.Anwar, F.; Hussain., A.I.; Sherazi, S.T.H.; Bhanger, M.I. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (Foeniculum vulgare Mill.) fruit at different stages of maturity. *J. Herbs Spices Med. Plants* **15**: 1-16,(2009).

99 .Cocking,T.T., Middleton, G. Improved method for the estimation of the essential oil content of drugs. *Q. J. Pharm. Pharmacol* **8:** 435–442,(1995).

100. Nickerson, G., Likens, S. Gas chromatographic evidence for the occurrence of hop oil components in *beer*. *J. Chromatogr*, **21**:1-5,(1996).