بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Extraction and Analysis of Spearmint and Eucalyptus oils

إستخلاص وتحليل زيتي النعناع والكافور

A Thesis Submitted in Partial Fulfilment of the Requirement of Master Degree in Chemistry

By

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الإستهلال

قال تعالي:

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَنَزَّلْنَا مِنَ السَّمَاءِ مَاءً مُبَارَكًا فَأَنْبَتْنَا بِهِ جَنَّاتٍ وَحَبَّ الْحَصِيدِ (٩) وَالنَّخْلَ بَاسِقَاتٍ لَهَا طَلْعٌ نَضِيدٌ (١٠) رِزْقًا لِلْعِبَادِ وَأَحْيَيْنَا بِهِ بَلْدَةً مَيْتًا كَذَلِكَ الْخُرُوج(١١).

صدق الله العظيم

(سورة ق الآيات (9-11))

Dedication

To my father and the soul of my mother,

To my husband and sons,

My brother(Mohamed) and my sisters,

My lovely aunt (saadeia).

.

Acknowledgement

I would like to extend my endless thanks to Almighty Allah, the Merciful, for helping me to complete this work.

My appreciation and thanks would go to my supervisor Dr. Omer Adam Mohamed Gibla for his assistance and guidance during the performance of this work.

I would like to thank everybody who contributed in the success of this research.

Abstract

The aim of this study was the extraction and analysis of spearmint and eucalyptus volatile oils. The samples were collected from shambat farms (Khartoum north).

The results showed that the Saponification numbers were (24.41, 22.44), acid values were (0.5611, 0.8417), ester numbers were (23.85, 21.60) and peroxide numbers were (0.01, 0.035), Refractive index values were (1.483, 1.473), density (0.755, 0.66) g/cm^3 for spearmint and eucalyptus oils respectively.

The IR analysis results showed the presence of CH aromatic,CH_2,CH_3 stretching bond and C=O,C-C, C-O and substituted aromatic in spearmint oil, and presence of CH aromatic,CH_2,CH_3 stretching bond, C-H bending aromatic , and substituted aromatic in eucalyptus oil.

GC-MS results showed that the eucalyptus oil was composed of (70) components. The main compounds were found to be alpha phellandrene (26.35%), benzene, 1-methyl-3-(1- methyl ethyl) (18.48%), eucalyptol (12.74%), bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1- methyl ethyl) (5.26%).

المستخلص

الهدف من هذه الدراسة هو إستخلاص وتحليل زيتي النعناع والكافور . جمعت العينات من مزارع شمبات (الخرطوم بحري). تم تحديد بعض الخواص الكيميائية لكلا الزيتين وشملت رقم التصبن (24.41, 22.44) رقم الحموضة (0.5611 , 0.8417) رقم الاستر (23.85, 21.60) رقم البيروكسيد (0.01 ,0.035). معامل الانكسار(1.473, 1.483) الكثافة (0.755 , 0.66) جم/سم^{3} لزيتي النعناع والكافور على التوالي.

أظهرت نتائج جهاز مطيافية الأشعة تحت الحمراء لزيت النعناع وجود الزمر الوظيفية C–C، C=O ، C=O ، CH_2 ، CH_3 و CH أروماتي، بالاضافة الى وجود مستبدلات.

تحليل زيت الكافور أظهروجود الزمر الوظيفية C–H bending ، CH أروماتي ، CH_3 و CH_2.

أظهرت نتائج مطيافية الكتلة احتواء زيت الكافور على (70) مكون,وأن المركبات الرئيسية هي ألفا فلاندرين بنسبة (26.35%)، بنزين، 1ميثيل –3– (1– ميثيل ايثيل) بنسبة (18.48%)، ايكالبتول بنسبة (12.74%)، بايسايكلو [0.1.3] 2هيكسين2 ميثيل–5–(1– ميثيل ايثيل) بنسبة (5.26%).

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Chapter One

Introduction

1. Introduction

1.1 Essential oils

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it contains the "essence of" the plant's fragrance and the characteristic of the plant from which it is derived. The term essential used here, does not mean indispensable as with the terms essential amino acid or essential fatty acid, which are so called since they are nutritionally required by a given living organism. Essential oils are generally extracted by distillation, e.g. by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing (Reeds, 2000).

Most common essential oils can be extracted from raw plant materials, which may be flowers, leaves, wood, bark, roots, seeds, or peel, using steam distillation.

Most oils are distilled in a single process. One exception is ylang-ylang (Cananga odorata) which is purifed through fractional distillation. The recondensed water is referred to as a hydrosol, hydrolat, herbal distillate, or plant water essence, which may be sold as another fragrant product. Popular hydrosols include rose water, lavender water, lemon balm, clary sage, and orange blossom water. The use of herbal distillates in cosmetics is increasing (Ryman, 1984).

Some plant hydrosols have unpleasant smells and are therefore not sold. Most citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harve.st the raw materials, citrus-fruit oils are cheaper than most other

essential oils. Lemon or sweet orange oils are obtained as byproducts of the citrus industry. Before the discovery of distillation, all essential oils were extracted by pressing (Ryman, 1984).

1.2 Eucalyptus

Eucalyptus tree is known as one of the tallest plants on the planet. Most eucalyptus species are evergreen plants. Besides in the form of trees, some species of eucalyptus develop as shrubs. Most species of eucalyptus are native to Australia, New Guinea and Indonesia. Eucalyptus is very important and highly exploited plant because of its wood and oil. Eucalyptus is commercially grown in tropical and subtropical areas all over the world. Since it absorbs huge quantities of water from the ground, eucalyptus can be used for draining of marshes. It can also eradicate malaria by destroying wet habitats which are required for development of mosquito's egg (Soft schools2005).

Size of eucalyptus depends on the species. Eucalyptus tree can reach 33 to 200 feet in height. Leaves of the tree have lanceolate shape. They are positioned downwards to prevent direct exposure to sunlight and consequent loss of water. The Leaves are rich in oil that is used to alleviate symptoms of bronchitis, sore throat, and nasal congestion.

Koala's diet is, completely, based on the leaves of eucalyptus. Despite wide variety of available species, only few of them are on the koala's menu. Bark of the tree is, usually, brown in color. Most species of eucalyptus shed their bark once per year. Bark can be removed in long ribbons, spongy pieces, furrowed and rough pieces or small flakes. Some insects lay eggs and feed on the bark of eucalyptus tree. They leave specific marks on the bark that look like man-made scribbles (Eyles, et al. 2009).

Eucalyptus tree has unique strategy to survive fires that are usual for the Australian forests. Eucalyptus is also known as "gum tree" because of the sticky rubbery substance that flows from the injured bark. All parts of eucalyptus tree are used in the manufacture of dyes. Eucalyptus has white, yellow, pink or red flowers. Beautiful color of the flowers is result of huge number of stamens (male reproductive organs. they do not have petals. Insects, birds and small mammals (such as bats) pollinate eucalyptus flowers. They are attracted by delicious nectar that is produced in big quantity. Fruit of eucalyptus is called gum nut. Lifespan of eucalyptus depends on the species. Most eucalyptus trees can survive more than 250 years in the wild (soft schools, 2005).

1.2.1 Eucalyptus oil

Eucalyptus oil is a pure essential oil that has practical and industrial uses, as well as healing properties. It comes from a fast-growing evergreen tree native to Australia, with global eucalyptus oil production mainly from Eucalyptus globulus or "Blue Gum" (Angus and Robertson, 1990).

Eucalyptus oil is the distilled oil that comes from the dried leaves of eucalyptus – a colorless liquid with a strong woody and sweet smell. There are more than 700 different species of eucalyptus in the world, of which at least 500 produce a type of essential oil (Maiden, 1889).

Eucalyptus oils are made up of more than 100 different compounds. Its main chemical components are a-pinene, b-pinene, a-phellandrene, 1.8-cineole, limonene, terpinen-4-ol, aromadendrene, epiglobulol, piperitone and globulol

Eucalyptus oils in the trade are categorized into three broad types according to their composition and main end-use: medicinal, perfumery and industrial. The most prevalent is the standard cineole-based "oil of eucalyptus", a colorless mobile

liquid (yellow with age) with a penetrating, camphoraceous, woody-sweet scent (Lawless, 1995).

China produces about 75% of the world trade, but most of this is derived from the cineole fractions of camphor laurel rather than being true eucalyptus oil. Significant producers of true eucalyptus oil include South Africa, Portugal, Spain, Brazil, Australia, Chile and Swaziland (Ashurst, 1999).

Eucalyptus polybractea or Blue-leaf Mallee, a species yielding high quality eucalyptus oil.Global production is dominated by Eucalyptus globulus. However, Eucalyptus kochii and Eucalyptus polybractea have the highest cineole content, ranging from 80-95%.The British Pharmacopoeia states that the oil must have a minimum cineole content of 70% if it is pharmaceutical grade. Rectification is used to bring lower grade oils up to the high cineole standard required. In 1991, global annual production was estimated at 3,000 tonnes for the medicinal eucalyptus oil with another 150..0 tonnes for the main perfumery oil (produced from Eucalyptus citriodora).The eucalyptus genus also produces non-cineole oils, including piperitone, phellandrene, citral, methyl cinnamate and geranyl acetate (Ashurst, 1999).

1.2.2 Uses of Eucalyptus Oil

Australian Aboriginals use eucalyptus leaf infusions (which contain eucalyptus oil) as a traditional medicine for treating body pains, sinus congestion, fever, and colds.

Dennis Considen and John White, surgeons on the First Fleet, distilled eucalyptus oil from Eucalyptus piperita found growing on the shores of Port Jackson in 1788 to treat convicts and marines Eucalyptus oil was, subsequently, extracted by early colonists, but was not commercially exploited for some time (Angus and Robertson1990).

As early as the years1880s, surgeons were already using eucalyptus oil as an antiseptic during operations. Toward the end of the century; the oil was used in most hospitals in England to clean urinary catheters.

According to Medical News 2013 a diluted form of eucalyptus oil is taken orally for pain and inflammation of respiratory tract mucous membranes, coughs, asthma, bronchitis, sinus pain and inflammation, and respiratory infections. It is also used as an antiseptic, insect repellent, and treatment option for wounds, burns, and ulcer.

Eucalyptus oil is also popularly used as a fragrance in perfumes and cosmetics, and is found in mouthwashes, liniments and ointments, toothpastes, cough drops, and lozenges .One study examined the effect of eucalyptus oil on human monocytes, and specifically its ability to stimulate protective macrophage activity.The substance was also studied for its effect on the release of pro-inflammatory cytokines. Eucalyptus oil, significantly, induced macrophage activation and reduced the release of inflammatory cytokines (Sherril Sego, 2012).

Steroid-dependent asthma patients were studied by (Juergens, etal.2003) to determine the potential for steroid reduction when treated with an oral preparation of eucalyptus oil (Juergens, et al. 2003) after randomization, patients' daily steroid doses were reduced by 2.5 mg every three weeks. At the end of 12 weeks, 36% of the patients on the active eucalyptus-oil therapy tolerated an average of 3.5 mg/day reduction in oral steroid use while only 7% of the placebo-treated patients were able to decrease their daily dose with an average reduction of 0.91 mg/day. Eucalyptus oil has also been studied for its potential as an antimicrobial. A study of 56 respiratory isolates from 200 symptomatic patients showed definite antimicrobial activity with eucalyptus oil. Isolates included Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, and Haemophilus influenzae.

With the rise in antimicrobial resistance, a potential new method for treating these infections is welcome news (Sherril Sego, 2012).

A separate study by (Vigo, etal.2004) examining the use of eucalyptus oil in asthmatic patients explored the extract's effect on nitric oxide in respiratory cells. Previous studies have shown an increase in exhaled nitric oxide in cases of eosinophilic inflammation and other indices of asthma-related inflammation. If eucalyptus oil is found to mitigate inflammation in such patients, this could lead to significant nonsteroidal treatment options for asthma. This trial showed that the extract was highly effective at inhibiting the induction of nitric oxide and the concomitant pro-inflammatory effects, further suggesting that eucalyptus extract may be a clinically viable option for asthmatic patients (Sherril Sego, 2012).

The chemical composition of essential oils from three species of plants belonging to the eucalyptus genus was determined and their insecticidal effects on eggs, larva and adult phases of lutzomyia longipalpis were assessed · the insects were collected in the municipality of sobral in the state of ceará, Brazil. Five treatments with different concentrations were performced along with two negative controls, distilled water and Tween 80 (3%), and a positive control, Cypermethrin (0.196 mg/ml).The tests were carried out in plastic pots internally coated with sterile plaster and filled with a substrate made of rabbit feces and crushed cassava leaves .The eggs, larva and adults were sprayed with the oils. The hatched larvae were counted for 10 consecutive days and observed until pupation .Insect mortality was observed after 24, 48 and 72 h. E. staigeriana oil was the most effective on all three phases of the insect, followed by E. citriodora and E. globulus oils respectively. The major constituents of the oils were Z-citral and alpha-citral (E.staigeriana), citronellal, (E.citriodora) and 1.8-cineole (E. globulus). The eucalyptus essential oils constitute alternative natural products for the control of L.

longipalpis since the median effective concentration (EC50) values revealed relevant action as compared with other natural products, some of their insecticidal activity and these oils are produced in commercial scale in Brazil (M.V. Maciel , et al, 2010) .

A study on 'Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices' by Sadlon AE, Lamson DW (2010) Alternative Medicine says that, "Surprisingly, there are also immune-stimulatory, antioxidant, and spasmolytic effects. Of the white blood cells, monocytes and macrophages are most affected, especially with increased phagocytic activity. Application by either vapor inhalation or oral route provides benefit for both purulent and non-purulent respiratory problems."

Another study on 'Effect of Eucalyptus Oil Inhalation on Pain and Inflammatory Responses after Total Knee Replacement (2013), a Randomized Clinical Trial' by the Department of Basic Nursing Science, School of Nursing, Korea University have stated that, "Eucalyptus oil inhalation was effective in reducing patient's subjective pain and blood pressure after surgery. These findings suggest that the inhalation of eucalyptus oil might be a valuable nursing intervention for pain relief after TKR (Total Knee Replacement) (Ayuverdic, 2013).

'Effect of eucalyptus extract chewing gum on periodontal health (2008) a double-masked, randomized trial ,by the Department of Preventive Dentistry, Osaka, Japan witnessed the effectiveness of Eucalyptus oil by stating that "Eucalyptus extract chewing gum had a significant effect on Plaque accumulation (PLA), gingival index (GI), bleeding on probing (BOP), periodontal probing depth (PD). The use of eucalyptus extract chewing gum may promote periodontal health." (Ayuverdic, 2013).

1.2.3 Safety

Eucalyptus oil is unsafe when taken by mouth or applied directly to the skin without being diluted (Serafino, etal 2008).Fatal allergic reactions have occurred with ingestion of eucalyptus products. Even though eucalyptus use in asthma and bronchitis has been shown to be beneficial, bronchospasm can result with inhalation. Eucalyptus oil is not recommended for use in pregnant or nursing women or in infants. If using a topical formulation for the first time, a patch test is recommended. This involves applying the extract to the upper arm. If no rash occurs after 24 hours, it is typically safe to proceed. Clinicians considering the u.se of diluted oral eucalyptus should proceed with caution. There is little data regarding drug interactions (Sherril Sego, 2012).

1.3 Spearmint

Spearmint, or spear mint (Mentha spicata) ,also known as Mentha viridis, is a species of mint native to much of Europe and Asia (Middle East, Himalayas, China etc.), and naturalized in parts of northern and western Africa, North America, and South America, as well as various oceanic islands (Seidemann, Johannes, 2005).

It is herbaceous, rhizomatous, perennial plant growing to 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm broad, with a serrated margin. The stem is square-shaped, a trademark of the mint family of herbs. Spearmint produces flowers in slender spikes, each flower pink or white, 2.5–3 mm long, and broad (Huxley, 1992).

Spearmint has been grown in gardens since the 9^{th} century as an herb thought to possess a wide range of health benefits. Spearmint was introduced to the

Massachusetts by colony in 1628.Commerical cultivation of spearmint in the United States began in the 1790’s.

The production of spearmint spread west and now is primarily grown in Washington and Oregon. The majority of the world’s spearmint is grown in united states, with production totaling 2.93 million pound in 2013 (Lauren 2014).

1.3.1 Spearmintoil

Spearmint essential oil contains hydrocarbons, alcohols, esters, oxides, and ketones. Its main components include alpha-pinene, beta-pinene, cavone, cineole, caryophyllene, linalool, limonene, menthol, and myrcene(organic fact.net 2017).

Spearmint is most popularly used as a seasoning for food and beverages, and it is also used for its healing benefits. Many people prefer the less intense flavor and scent of spearmint over that of peppermint. Spearmint essential oil comes from the Mentha spicata plant, which has bright green leaves. This versatile oil has numerous health benefits (Sharon Therien, 2011).

Spearmint was grown across 24,500 acres, equating to approximately 119 pounds of spearmint oil per acre (Anon, 2014).

1.3.2 Uses of Spearmint Oil

The historical record shows that it was used extensively by ancient Greece. It was added to baths and used to help treat sexually transmitted diseases, whiten te.eth, and heal mouth sores (Ayuverdic2013).

Spearmint is used for its aromatic oil, referred to as oil of spearmint. The most abundant compound in spearmint oil is R- (–)-carvone, which gives spearmint its

distinctive smell. Spearmint oil also contains significant amounts of limonene, dihydro carvone, and 1.8-cineol (Hussain, et al, 2010).

Spearmint oil is often used in aromatherapy to help alleviate fatigue, headaches, migraines, nervousness, and even digestive problems. This essential oil is added to certain types of perfume. It is commonly mixed with other herbs like jasmine, lavender, bergamot, and sandalwood. Spearmint oil can help relieve muscle pain and even abdominal pain due to menstruation (Ayuverdic2013).

In a study carried by the Central Laboratory, Lotte Company Ltd, Urawa, Saitama, Japan (2001) proved the effectiveness of Spearmint essential oil in inhibiting the growth of pathogenic bacteria with its potential antibacterial agents. A 2011 study on 'Antifungal activity of Spearmint essential oil' talks about its effectiveness in treating fungal infections (AyurvedicOils.com 2017).

Spearmint has strong anti-bacterial properties, as most essential oils do. A H. Imai and colleagues (2001) found that spearmint essential oil blocked the growth of bacteria, even antibiotic-resistant bacteria, including H. pylori, Salmonella, E.coli and Methicillin Resistant Staphylococcus Aureus. Add 20 to 40 drops spearmint oil to 3.5 oz. water in a spray bottle to use as a cleaning solution. Spearmint was used in the past by Native Americans for digestive troubles and is still used today for the same purpose. This essential oil relieves problems such as indigestion, flatulence, diarrhea and a lack of appetite. Combine one drop of spearmint oil and a little bit of honey with one cup warm water and drink to ease your digestive troubles (Sharon Therien, 2011).

Jirovetz et al, (2002) studied the odor-active compound in native spearmint grown near ngaoundere, Cameroon .they used steam distillation to attain the spearmint oil from the plants, and extracted the native spearmint oil with

dichloromethane. Gas chromatography and Gas chromatography-mass spectrometry in combination with solid phase micro-extraction (SPME) were implemented in the identification of compounds in the native spearmint oil. To identify the odorants in native spearmint, they used a "GC-sniffing technique" more commonly known as GC-olfactometry (GC-O).The researches based the intensity of the individual odorants on the description of the aroma of the native spearmint oil given by perfumers. From the perfumers impression, the researches attested that the most potent odorants in the native spearmint oil were (-)-carvone, (-)-limonene, 1, 8-cineole .Other important compound were hexanal (green), linalool and linalyl acetate(floral),3-methylbutanal and 2-methylbutanal (fruity), and(Z)-β- ocimene and (E) -β-ocimene (spicy).

The composition of essential oils for two types of mint was analyzed, and the antimicrobial, antioxidant and anti-inflammatory activities of the two oils were compared. Peppermint and chocolate mint oils were obtained by steam distillation in a Clevenger-type apparatus, the chemical composition of the essential oils was determined by gas chromatography-mass spectrometry (GC/MS), the Minimal Inhibitory Concentration (MIC) of the essential oils were determined by broth dilution method, the antioxidant activities of the oils were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH)DPPH radical scavenging assay, β-Carotene-linoleic acid assay, and nitric oxide (NO) radical scavenging assay, The two essential oils contain high levels of alcohol (43.47-50.10%) and terpene(18.55-21.07%) with the major compound being menthol (28.19-30.35%). The antimicrobial activity (minimum inhibitory concentration, MIC) of peppermint oil against E. coli, S. aureus and P. aeruginosa (0.15, 0.08, 0.92 %v/v, respectively) was stronger than that of chocolate mint (0.23, 0.09, 1.22 %v/v, respectively). In the anti-oxidant test including DPPH and β- Carotenelinoleic acid assays,

peppermint oil showed superior antioxidant properties to chocolate mint oil (4.45 - 19.86 μl/mL),However, with regard to scavenging NO radical activity, chocolate mint oil exhibited higher activity than peppermint (0.31 and 0.42 μl/mL, respectively). Chocolate mint oil also exhibited higher anti-inflammatory activity than peppermint oil (0.03 and 0.08 μl/mL, respectively) (Mei-Lin Tsai12013).

The health benefits of spearmint has been described for years in numerous studies, but new research in mice suggests that antioxidants from spearmint made into an enhanced extract can improve learning and memory, potentially helping with age-related cognitive decline. Prof. Susan Farr, from Saint Louis University School of Medicine in Missouri, presented her early findings at Neuroscience 2013, a meeting hosted by the Society for Neuroscience. Also she observed reduced oxidative stress in the part of the mice brains that controls learning and memory, which she notes is a marker of age-related decline (Marie Ellis 2013).

Chapter Two

Materials and Methods

2. Materials and Method

2.1 Materials

2.1.1 Collection of sample

Spearmint whole plant and the leaves of eucalyptus tree were collected from shmbat farms. The samples were dried at room temperature.

2.1.2 Chemicals

-Ethanol Absolute ethyl alcohol, Product number: 4150

Danwongu ansansi gyung gido –Korea

-Potassium hydroxide Assay 85% Scharlau chemie S.A.Made in Barcelona, Spain

-Phenolphthalein indicator

PH (8.4-10), Hopkin &Williams Ltd.Chawell – Heath-Essex-England.

-Hydrochloric acid Assay: 35-38% .Mumbai-India. Product No.38507

-Diethyl ether Carloerba Reactifs –SDS, CE: 200-467-2

-Sodium thiosulphate (penta hydrate)

Assay 99% Loba Chemie PVT-LTD, Mumbai -India.

-Acetic acid glacial

Mwt 60.5, Hopkin &Williams Ltd.Chawell –Heath-Essex-England

-Potassium iodide Assay 99-100.5% S.d.FINE- CHEM Limited Mumbai-India

-Starch Acidity: o.8 ml N/1, Hopkin &Williams Ltd.

Chawell –Heath-Essex-England

-Chloroform Assay 99.5% S.d.FINE- CHEM Limited Mumbai-India

-S**odium hydroxide** Assay 98%

2.1.3 Instruments

-Gas chromatography-mass spectroscopy, model QP2010 Ultra Shimadzu - Japan, Carrier gas helium.

-Infrared Spectrophotometer, Shimadzu Corporation Kyoto - Japan.

Serial number: A21274501270LD

-Refractometer

Manufactured by Kruess, Hamburg – Germany

Model: D – 22297

2.2 Methods of analysis

2.2.1Oil Extraction

Steam distillation was used for volatile oil extraction. Three kilo grams of whole spearmint plant were used for spearmint oil extraction and one kilo gram of eucalyptus leaves was used for eucalyptus oil extraction. Each distillate was transferred to a separating funnel and the oil was isolated from the aqueous phase.

2.2.2 Determination of Refractive index.

Refractometer model D-22297was used for measuring refractive index of the oils samples.

2.2.3 Determination of density

Density was measured for each oil.

2.2.4 Determination of Ester number

2grams of oil were weighed in a 250 ml round bottom flask; 25 ml of 80%ethanol were added. In other 250 ml round bottom flask 25 ml of 80%ethanol were added (blank). 3 drops of phenolphthalein indicator were added to the two flasks and titrated against (0.1M) sodium hydroxide until end point .30 ml of (0.5M) alcoholic potassium hydroxide were added to the two flasks and were Placed in a water bath, a condenser was fixed above the two flasks for 30 minutes. After heating the two solutions were titrated against (0.5M) hydrochloric acid until the end point, and the results were recorded.

2.2.5 Determination of Acid value

2grams of oil were weighed in a 250 ml round bottom flask, 25 ml of diethyl ether and 25 ml of ethanol were added. In a second 250 ml round bottom flask 25 ml of diethyl ether and 25 ml of ethanol were added (blank). 3 Drops of phenolphthalein indicator were added to the two flasks and titrated against (0.1M) potassium hydroxide until the end point was reached. The results were recorded.

2.2.6 Determination of Peroxide value.

2grams of oil were weighed in a 250 ml round bottom flask, 15 ml of glacial acetic acid and 10 ml of chloroform were added. In a second 250 ml round bottom flask 15 ml of glacial acetic acid and 10 ml of chloroform were added (blank).1 ml of saturated potassium iodide solution and 1ml of starch were added to the two flasks, and they were kept in dark for 5 minutes. The two flasks contents were titrated against (0.1M) sodium thiosulphate up to end point and the results were recorded.

2.2.7 Determination of Saponification value

2grams of oil were weighed in a 250 ml conical flask.25ml of (0.5) alcoholic potassium hydroxide solution was added. 25ml of (0.5) alcoholic potassium hydroxide solution were transferred in to a conical flask (blank). Reflux condenser was attached to each flask and the flasks contents were heated on a water bath for one hour with occasional shaking. 3Drops of phenolphthalein indicator were added and the two solutions were titrated against (0.5M) hydrochloric acid until end point.

Chapter Three

Results and Discussion

3. Results and discussion

Table (3-1) shows that the yield percentage of spearmint oil is (1.25%) and the eucalyptus oil is (4.29%). The density of spearmint oil (0.755g/cm^3) was higher than the density of eucalyptus oil (0.66g/cm^3).

The refractive indexes of the two oils were nearly the same (1.483and1.473).

The acid value of eucalyptus oil (0.8417) is greater than that of spearmint oil (0.5611).

The peroxide numbers for the two oils were found to be very low (0.01) for the spearmint oil and (0.035) for eucalyptus oil.

The ester number of the spearmint (23.85) was found to be more than that of eucalyptus oil (21.60).

The Saponification values of the two oils were within the same range.

Table (3-1) Physicochemical properties of spearmint and eucalyptus oils

Property	Spearmint oil	Eucalyptus oil
Yield percentage	1.25%	4.29%
Density g/cm^3	0.755	0.66
Refractive index	1.483	1.473
Acid value	0.5611	0.8417
Peroxide value	0.01	0.035
Ester number	23.85	21.60
Saponification value	24.41	22.44

Table (3-2) shows that the major components of eucalyptus oil were alpha phellandrene (26.35%), benzene, 1-methyl-3(1-methylethyl) (18.48%) ,eucalyptol (12.74%),and bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methyl ethyl) (5.26%). Alpha – phellandrene was named after eucalyptus phellandra, now called eucalyptus radiata, from which it can be isolated (Jocobs1981).It is also a constituent of the essential oil of eucalyptus dives(Boland,etal1991).

The phellandrenes are used in fragrances because of their pleasing aromas.

The alpha – phellandrene isomer can form hazardous and explosive peroxides on contact with air at elevated temperature (Urben, Peter 2007).

Chemical formula $C_{10}H_{16}$

Molar mass: 136.24 g/mol.

Appearance: colorless oil.

Density: 0.846g/cm^3.

Boiling point: 171-172 ċ.

Solubility in water: insoluble.

-benzene, 1-methyl-3(1-methylethyl)

Formula:$C_{10}H_{14}$

Molecular weight: 134.2182.

Appearance: colorless to pale yellow clear liquid.

Assay: 95 to 100%.

Boiling point: 174 to 176 ċ at 760 mmHg.

Vapor pressure: 1.720000mm/Hg at 25 ̊c.

Flash point: 118.00 ̊F.Tcc (47-80 ̊c).

Solubility: soluble in alcohol water, 42.5 mg/l at 25 ̊c

(Cavalli, 2003).

-Eucalyptol is an organic compound which is a colorless liquid; it is acyclic ether and a monoterpene, eucalyptol is a natural constituent of a number of aromatic plants and their essential oil fraction. Eucalyptol was given GRAS (Generally Recognized As Safe) status by the flavor and Extract Manufacturer's Association FEMA, 1995and is approved by the food and drug administration for food .use.1, 8-Dihydroxy-10-carboxy-p-methane, 2-hydroxy-cineole and 3-hydroxy-cineole are the main metabolites of eucalyptol.

In a 1994 report released by five top cigarette companies, eucalyptol was listed as one of the 599 additives to cigarettes; it is added to improve the flavor.

Molecular formula:$C_{10}H_{18}O$

Molecular weight: 154.253g/mol.

Odor: camphor –like odor.

Boiling point: 349 to 351 ̊F at 760 mmHg, 176-177 ̊c.

Melting point: 34.7 ̊F, 1.5 ̊c.

Density: 0.921 to0.923 g/cm^3.

Solubility: insoluble in water, and soluble in alcohol, most fixed oils, glycerin, and propylene glycol; 1:5 in 60% alcohol.

Reactivity: highly inflammable.

Cineole is the main constituent of eucalyptus oil, and it is mainly used as a mucolytic agent in inflammatory airways diseases (http://pubchem).

Alpha-Thujene (Bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methylethyl).

Molecular formula:$C_{10}H_{16}$

Density: 9.36 g/cm^3

Boiling point: 159 ̊c.

Molecular weight: 136.2340.

Thujene is a natural flavoring obtained from eucalyptus, it is a natural organic compound classified as a monoterpene. It forms a major part of the constituents in essential oils of a variety of plants, and contributes a strong spicy flavor of certain herbs.

A study conducted in Iran stated that Thujene acts as an active anti-malarial activity of the chemical constituent a-Thujene was studied (Ayuverdic Oils.com2017).

Table (3.2) GC-MS result of eucalyptus oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.961	332994	0.06	1-Hexene, 5-methyl-
2	4.728	30400518	5.26	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-met
3	4.866	10460516	1.81	.alpha.-Pinene
4	5.057	705069	0.12	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-
5	5.599	3355562	0.58	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
6	5.681	1238110	0.21	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth
7	5.895	8314439	1.44	.beta.-Myrcene
8	6.255	152207341	26.35	.alpha.-Phellandrene
9	6.449	5467531	0.95	(+)-4-Carene
10	6.653	106738485	18.48	Benzene, 1-methyl-3-(1-methylethyl)-
11	6.741	47865938	8.29	.beta.-Phellandrene
12	6.799	73613448	12.74	Eucalyptol
13	7.050	470800	0.08	.beta.-Ocimene
14	7.311	7088573	1.23	.gamma.-Terpinene
15	7.530	993468	0.17	2-Nonenal, (E)-
16	7.940	5208978	0.90	(+)-2-Carene
17	8.158	3335198	0.58	1,6-Octadien-3-ol, 3,7-dimethyl-
18	8.232	408437	0.07	Butanoic acid, 3-methyl-, 3-methylbutyl es
19	8.296	355316	0.06	n-Amyl isovalerate
20	8.532	303374	0.05	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-
21	8.573	354503	0.06	Thujone
22	8.678	2404082	0.42	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet
23	9.065	1886481	0.33	p-Menth-8-en-1-ol, stereoisomer
24	9.695	1130908	0.20	1,3-Dimethyl-1-cyclohexene
25	9.761	1141480	0.20	Cyclopropane, 2-(1,1-dimethyl-2-pentenyl
26	9.890	13814965	2.39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylet
27	10.051	1109088	0.19	Benzenemethanol, .alpha.,.alpha.,4-trimet
28	10.104	1213301	0.21	2-Cyclohexen-1-one, 4-(1-methylethyl)-
29	10.171	1185455	0.21	.alpha.-Terpineol
30	10.268	526512	0.09	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylet
31	10.423	2733744	0.47	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1
32	10.479	302398	0.05	Acetic acid, octyl ester
33	10.525	935342	0.16	1-Methoxy-4,4-dimethyl-cyclohex-1-ene
34	10.758	996397	0.17	Fenchyl acetate
35	10.883	821718	0.14	2-Acetylcyclopentanone
36	11.175	987893	0.17	(E)-3(10)-Caren-4-ol
37	11.300	716061	0.12	(-)-Carvone
38	11.526	444646	0.08	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl
39	11.738	358063	0.06	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2
40	11.789	554676	0.10	2,6-Octadienal, 3,7-dimethyl-, (E)-
41	11.967	442561	0.08	1-Cyclohexene-1-carboxaldehyde, 4-(1-me
42	12.143	318341	0.06	Bornyl acetate
43	12.307	443630	0.08	1,3-Cyclopentadiene, 1,3-bis(1-methylethy
44	12.503	1745622	0.30	Thymol
45	12.833	801794	0.14	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimet
46	13.184	2896324	0.50	Cyclohexane, 1-ethenyl-1-methyl-2-(1-met
47	14.279	1312572	0.23	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-
48	14.473	5604833	0.97	Methyleugenol
49	15.095	951264	0.16	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4
50	15.260	9875297	1.71	Alloaromadendrene
51	15.643	3340021	0.58	Bicylo[4.1.0]heptane, 7-bicyclo[4.1.0]hept-
52	15.684	3924342	0.68	Aromandendrene
53	15.920	718235	0.12	Dibenzo[a,h]cyclotetradecene, 2,3,11,12-te
54	16.049	708634	0.12	1,6-Cyclodecadiene, 1-methyl-5-methylene
55	16.162	845027	0.15	Naphthalene, decahydro-4a-methyl-1-meth

Peak#	R.Time	Area	Area%	Name
56	16.202	975781	0.17	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22
57	16.344	9260137	1.60	.gamma.-Elemene
58	16.639	682046	0.12	.gamma.-Muurolene
59	17.526	1251847	0.22	Epiglobulol
60	17.680	655620	0.11	4aH-Cycloprop[e]azulen-4a-ol, decahydro
61	17.858	12265791	2.12	1H-Cycloprop[e]azulen-7-ol, decahydro-1,
62	17.976	11450344	1.98	Globulol
63	18.126	2562550	0.44	Viridiflorol
64	18.216	898299	0.16	(-)-Spathulenol
65	18.319	1136931	0.20	Ledol
66	18.424	376498	0.07	Lanceol, cis
67	18.488	360146	0.06	Cubenol
68	18.635	680531	0.12	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-o
69	18.900	2592859	0.45	.tau.-Cadinol
70	20.468	6077873	1.05	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-
		577637558	100.00	

Figure (3.1) showed the presence of CH aromatic,CH_2, CH_3 stretching bond and C=O,C-C, C-O and substituted aromatic in spearmint oil.

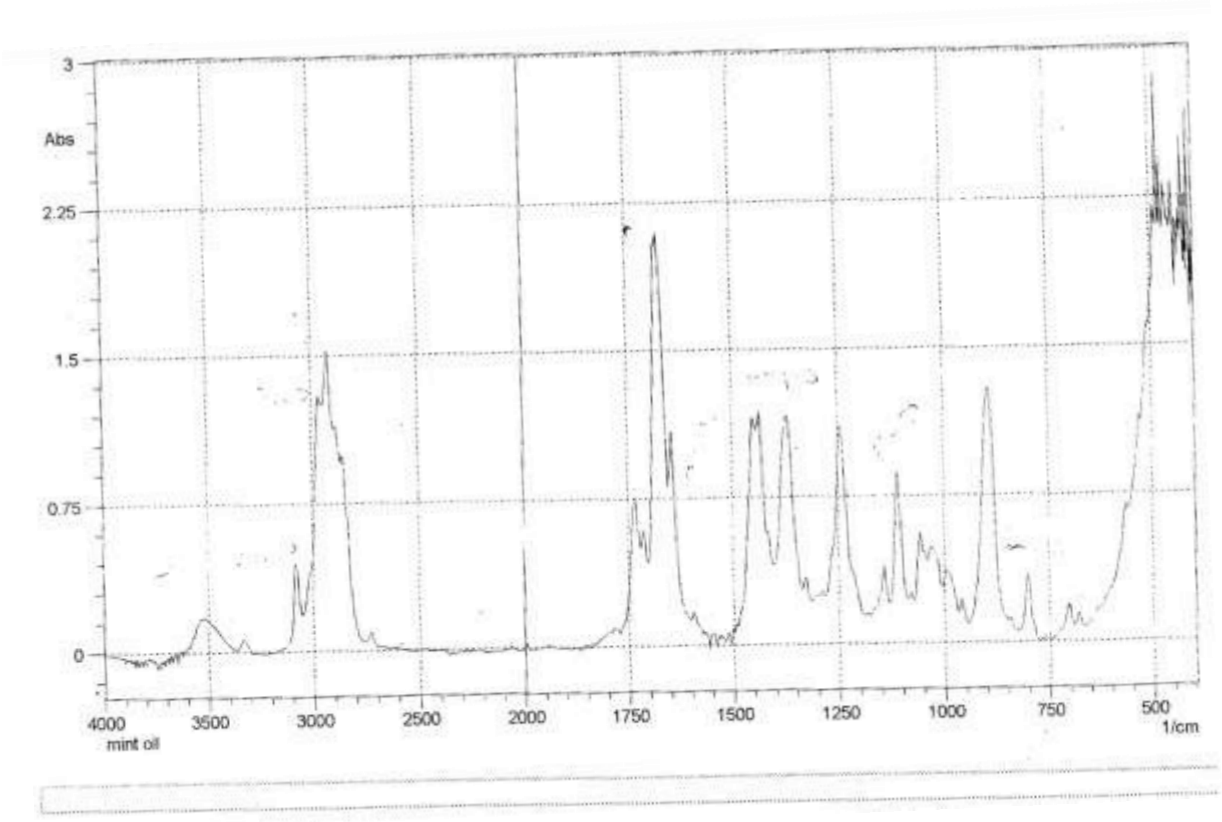

Figure (3.1) IR spectra of spearmint oils

Figure (3.2) showed the presence of CH aromatic,CH_2,CH_3 stretching bond, C-H bending aromatic , and substituted aromatic in eucalyptus oil.

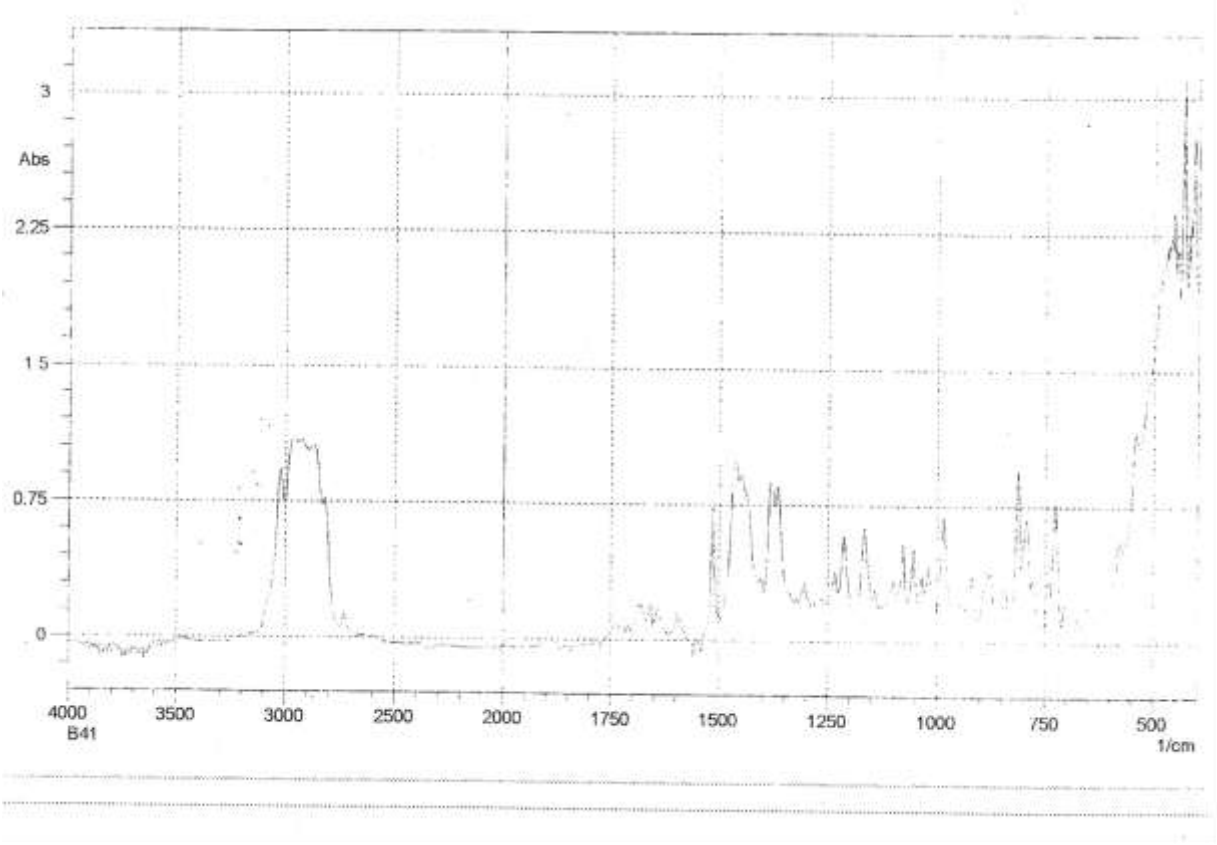

Figure (3.2) IR spectra of eucalyptus oils

Conclusions

The physicochemical properties of the two oils look to be similar. The extracted amount of oils is suitable because it exceed 50 ml for each extraction. The yield percentage was (1.25%) for spearmint oil and (4.29%) for eucalyptus oil.

Eucalyptus oil showed the presence of so many components when analyzed by GC-MS technique. Many fragments may be isolated and used separately.

Steam distillation may be used as a cheap available technique to extract many essential oils in commercial amounts. Spearmint and eucalyptus oils may be produced simply and rapidly without using any chemical reagents. The two oils find a wide range of medical uses .The two plants species may be cultivated in wide areas in Sudan. The two oils may be needed for various pharmaceutical and medicinal uses. They may also be used in cosmetics.

Recommendations

Further studies may be needed for more chemical characterization of the two types of oils. Side effects may also need to be investigated because the two plants and their oils are used by many people**.** The oil yield for each type of plants speciemay need to be evaluated by conducting more experiments**.**

GC-MS characterization may need to be carried for spearmint oil samples, to show if there are any differences in chemical constituents of the two volatile oils.

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