Sudan University of Science and Technology

College of Graduate Studies

Comparative Study of Volatile Oils from peels of Orange, Lemon and Grapefruit

دراسة مقارنة للزيوت الطيارة من قشرة البرتقال و الليمون والقريب فروت

A Thesis Submitted in Partial Fulfillment of the Requirements of M.Sc. Chemistry

By

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APROVAL PAGE

Dedication

To soul of my Father, to my Mother, Brothers and, Sisters

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Acknowledgement

I would like to thank Allah Almighty. I would like to thank my supervisor, Dr. Omer Adam Mohamed Gibla for his continuous support, guidance, and encouragement that lead to the completion of this work. My Great thank to Dr: Ashraf from Medical and essential plants center (UK) for his support. I would like to thank my colleagues for their encouragement and I would like to thank the members of my family for their great help, support.

ABSTRACT

Amount of Lemon oil extracted was 53ml compared to orange 45ml and grapefruit 43ml. Refractive index was 1.48 in lemon, 1.47 orange and 1.42 grapefruit.

Density values found to be 0.86 for lemon, 0.84 orange and 0.89 for grapefruit. P^H values were 3.3 for lemon, 2.2 orange and 3.6 grapefruit. Acid number values found in lemon, orange and grapefruit were 1.79, 1.99, and 2.23 respectively. Saponification values of lemon, orange and grapefruit were 16.75, 13.5, and 13.7. Peroxide number values in lemon oil were 13.67, orange 18.68 and grapefruit 9.59. GC.MS analysis showed presence of, 12,11and 24chemical constituents in lemon, orange and grapefruit volatile oils respectively. The three main components and their percentage compositions were, D- limonene(64%),2-cyclohe-xen-1-ol(6.21%) and pinene(3.822%)in lemon peel volatile oil D-limonene(54.151%) pulegone (11.653%) and L-carvone(2-cyclohexane-1-one) (5.457%)in orange peel volatile oil, and β – pinene(23.124%), D-limonene (17.07%) and α-pinene(10.399%)in grapefruit volatile oil. The main component found in the three samples is D-limonene.

الخلاصة

كمية الزيت المستخلص من قشرة الليمون كانت (٥٣مل) مقارنة مع قشرة البرتقال (٤٥مل) وقشرة القريب فروت(٤٣مل).وعند دراسة الخصائص الكيميائية والفيزيائية كانت كالاتي. .معامل الانكسارلزيت الليمون ١.٤٨ لزيت البرتقال١.٤٧ زيت القريب فروت١.٤٢ .الكثافة لزيت الليمون(٠.٨٦) وزيت البرتقال ٠.٨٤ و زيت القريب فروت ٠.٨٩ وجد ان رقم الهيدروجين لزيت الليمون ٣.٣ البرتقال ٢.٢ زيت القريب فروت٢.٢٣ . رقم الحموضة لزيت الليمون ١.٧٩البرتقال ١.٩٩ و زيت القريب فروت ٢.٢٣. رقم التصبن لزيت البرتقال١٦.٧٥ زيت الليمون١٣.٥ زيت القريب فروت١٣.٦٧. رقم البيروكسيد في زيت الليمون كان ١٣.٦٧، البرتقال ١٨.٦٨ وفي زيت القريب فروت(٩.٥٩). تحليل العينات بواسطة جهازGC-MS وضح وجود ١٢,١١و٢٤ مكون كيميائي في الليمون، البرتقال و القريب فروت علي الترتيب . المكونات الثلاثة الاساسية والنسب المئوية كانت كالاتي. زيت الليمون هناك ثلاثة مركبات اساسية وهي .مركب دي ليمونين(٦٤%) ومركب بيتابينين(٣.٨٢٢) ومركب ٢سيكلوهكسين-١-ول(٦.٢١%) زيت البرتقال المكونات الثلاثة الاساسية هي مركب دي ليمونين بنسبة (٥٤.١٥١%)ومركب البوليقون (١١.٦٥٢%)ومركب ٢سيكلوهكسين-١-ون(٥.٤٥٧%) بالنسبة لزيت القريب فروت الثلاثة مكونات الاساسية هي مركب دي ليمونين(١٧.٥٧%) ومركب بيتا بينين(٢٣.١٢٤%) ومركب الفا بينين(١٠.٣٩٩%).وجد ان هناك مركب مشترك موجود في العينات الثلاثة وهو مركب دي ليمونين .

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CHAPTER ONE

INTRODUCTION

1.INTRODUCTION

1.1 Volatile Oils

Volatile oils are concentrated aromatic compounds produced by plants. Their easy evaporation gives plants their wonderful scents. Each of these complex precious liquids is extracted from a particular time of plant. Each plant species originates in certain region of the world, with particular environmental conditions neighboring fauna and flora (Sheng-Min,et al,2012).Essential oils are frequently referred to as life force of plants (Sheng-mine et al 2012). Unlike fatty oils, these essential oils are volatile, highly concentrated substances, extracted from flowers, leaves, stems, roots, seeds, barks, or fruit rinds. The amount of essential oils found in these plants can be anywhere from 1-95 percent of total. That is why tons of plants material are required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents, these oils are often used for their flavor and their therapeutic properties, in a wide selection of products such as foods, medicines, and cosmetics, beware of imitations. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate(Maria et al,2012).

1.2 Occurrence of Volatile Oils

Volatile oils can be obtained from the flowers, leaves, roots, seeds, and bark of many plants. Oil of lavender for example, is derived from flowers, oil of patchouli from leaves, and oil of orange from the fruits (verzera 2004). The oils are formed in the green part of the plants (chlorophyll-bearing) and with plant maturity are transported to other tissues, particularly to the flowering shoots (Verzera et al, 2004). Essential oils are found in the vegetable structures to which they give their characteristic odors are intimately connected with the vital processes that take place in plants. In plants, they may be formed by the hydrolysis of certain glycoside or directly by

protoplasm or by decomposition of the resinogenous layer of the cell wall. Inside the vegetable cell, the essential oils are contained in the "vacuoles" cavities of roundish from bound by a single membrane. The tonoplast, and containing an aqueous solution full of a juice The vacuolar juice, vacuole is a cellular organelle, probably originating from the endoplasmic reticulum, into which the "secondary products" or the products of refusal of the metabolism are poured (Gamarra et al,2006).

The function of the essential oils in plants is not well understood. Odors of flowers probably aid in natural selection by acting as attractants for certain region in seeds, Leaf, wood, and roots oils, may serve to protect a against plant parasites or degradation by animals (Gamarra et al,2006).Oleo resinous exudations that appear when the trunk of tree is injured to prevent loss of sap and act as a protective seal against parasites and disease organism. Few essential oils are involved in plants metabolism, and some investigators maintain that many of these materials are simply waste products of plant biosynthesis (Rapisararda, 1999).

1.3 Volatile Oils from citrus fruit

Volatile oils from citrus fruits contain various type of natural flavors and fragrances, which are popularly used in food industries, daily chemical products and health care field (Sheng-min et al, 2012). Citrus species are potential sources of variable oils which might be utilized for edible and other industrial applications (maria et al,2012).Humankind used plants for healing many thousands of years, and it's from this tradition the use of aromatic plant compounds in medicine was begun. Oils were used in the embalming process, in medicine and in purification rituals. There are also over 200 references to aromatic, incense, and ointments in the old and new Testaments(Frankincense,

Myrrh, Galbanum, Cinnamon, Cassia, Rosemary, Hyssop and Spikenard) are noted for being used anointing rituals and healing of the sick.Research confirmed centuries of practical use of essential oils, and we now know that the fragrant pharmacy contains compounds with an extremely broad range of biochemical effect (Sheng-min et al,2012).Essential oils are broadly used as pharmaceutical components, in nutritious supplements and for cosmetic industry and aromatherapy (Maria et al, 2012).Guenther (1955) also stated that the oil is also employed in perfumes,toilet water, beaux cologne, and in cosmetics to, which it impacts a refreshing top note. There are about three hundred essential oils in general use today by professional practitioners .With continual bombardment of viral, bacterial, parasitic and fungal contamination in our world. Essential oils are a great benefit to help protect our bodies and health from sickness. Essential oils are products obtained from vegetable raw materials(Berger2007). They are complex mixtures their composition may include volatile terpenic compounds, which have the formula $(C_5H_8)_n$. where the compounds are monoterpenes if n=2, sesquiterpenes when n=3, diterpenes if n=4,etc(Smith et al,2001). These are secondary metabolites in plants (Mazen, 2002) and responsible for the characteristic aroma on the fruit. Immune system needs support and essential oils give that, because of the enormous amount of raw product used to make wholly natural essential oils, lots of products on the market have been polluted with lower quality, commercial grade oils or contain other chemical substances to reduce the cost or increase the profit margin a fact that not usually revealed on the label. This

is why it is important to study the chemical composition of the volatile fraction once the essential oil is extracted. This fraction is characterized by the complexity in the separation of its components, which belong to various classes of compounds and which are present in wide range of concentrations. Therefore it is complicated to establish a composition profile of essential oils. Lemon essential oils are complex mixture of chemical compounds like limonene, Y-terpinene, citral, linalool and β-caryophylene among other, which can be represented by the main classes,namely terpenes, oxygenates, and sesquiterpenes (Benvenuiti et al,2001).The most significant flavor compound is citral, while linalool possess highly distinctive organoliptic characteristic. In addition, limonene, octanol, and Y-terpene among other contribute with high aroma flavor of lemon oil (Benvenuiti et al, 2001). The composition of orange oil varies for several reasons. Region and seasonal change, as well as, the method used for extraction oil leads to this variation (Gamarra et al,2006).The quality of essential oil depends on different factors, among them are the chemo type and biotype of the plant, the climatic conditions ,as well as, the extractive process, several compounds have been identified in orange oil with gas-chromatograph-mass-spectrometry(Verzera et al 2004). Most of the substances are part of the terpene group(limonene,α-pinene,sabinene,β-pinene, myrcene and (α-3-carene)with limonene being the dominant one. Long chain aliphatic hydrocarbons alcohols and aldehydes like octanol, decanal, and octanal are second important groups of substances (Verzera et al,2004).Citrus fruits have a rough, robust, and bright(green to yellow)color skin they are

usually 4- 30cm long and 4-20cm in diameter with peel surrounding known as (epicarp)that covered the fruit and protect it from damages. Citrus fruits are notable for their fragrance partly due to flavanoids and lemonoids contained in the rind(Manthey,2004).The endocarp is rich in soluble sugar and contains significant amounts of vitamin c, pectin, different organic acids and potassium salt which give the fruits its characteristic citrus flavor (Ezejiofor et al,2011).Citrus juice also contains a high quality of organic acids(citric,malic,acetic,and formic acids).In many parts of the world ,citrus (sweet orange) are cheaply available ,thus serves as a major source of vitamins in diets. Orange fruits and its juice have several beneficial nutritive and health properties (Okwu,Emenike,2006).They are rich in vitamins especially ascorbic and folic acids. Over the last decades, many other virtues and medicinal benefits of orange fruits have been discovered besides their antis curvy property(Rapisararda,1999).There is convincing epidemiological evidence that the consumption of orange fruit is beneficial to health and contributes to the prevention of degenerative process, particularly lowering incidence of degenerative process,cardio and cerebio-vascular diseases (Rapisararda,1999).The protection that orange fruit provide against these diseases has been attributed to the various antioxidant phytonutrients contained in citrus species (Okwu and Emennike,2006 ,Rapisararda,1999).The current world production of citrus fruits is approximately 110million tons, of which oranges constitute about 80 million tons(USDA,2013).The gas chromatography method (GC) is exclusively used for the qualitative analysis

of volatiles. The analysis of essential oils was developed in parallel with the technological development in GC, such as stationary phases, detection devices etc(Manthey 2004).However, advances in instrumentation were not the only important factor in the development of analytical methods for essential oils in the plants. Sample extraction and amount also improved. The most outstanding improvements in the determination of the composition of essential oils came from the introduction of tandem techniques involving prior/further chromatography or spectroscopy. The great information on the application of GC field(Emenike,2006,Rapisararda,1999).

1.4 Commercial importance

Essential oils are generally expensive, with prices from several thousand USD per kilogram. The high price of the natural oils coupled with their limited availability has encouraged a search for substituents.Great progress has been made in the synthesis of individual's components such as geraniol. Citral, linalyl, acetate, and the like. These synthetics have been combined with natural oils to extend supplies, and they have also been blended together in an attempt to duplicate the oils themselves. Such reconstituted oils usually lack certain of the odor. Notes of the natural products, because of the absence of the trace ingredients, often unidentified, that may be present in the natural oils (Rapisararda 1999). They also tend to have a more chemical odor because of trace impurities in the synthetics that are different from the components of natural oils.

1.5 Function of the essential oils in the plants

Essential oils are extracted from oil sacs, in flowers, seeds, leaves, roots, wood and bark. They differ significantly from the well-known vegetable, nut

and seeds oils which are made up of various fatty acids, essential oils are used by the plants in somewhat the same way by humans,they fight infections, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. Despite their foliar origins however, essential oils have similar structure to some compounds found in blood and tissues, allowing them to be compatible with our physiology(GUENTHER,1955).

1.6 How uses of Volatile oils

The most effective way to use essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oil include" body oils", compresses, cosmetic lotion, baths, hair rinses, inhalation by steam, perfumes and room sprays(TOIA RF,1985). Essential oils are very potent some will cause skin irritation or have other harmful effects if not used properly. Unless specifically noted, it is best to dilute all essential oils in a carrier of base oil like Almond, Jojoba or Apricot Kernel before applying to the skin-appropriate dilution is usually only 1-10% essential oil in carrier. For inhalation, a diffuser or oil lamp is effective for releasing essential oils into your environment-a very pleasant way of creating a particular atmosphere(Raymond p.W.Scott,University of London).

1.7 Uses of essential Oils

1.7.1 Essential oils are used in flavoring, perfumes, Aromatherapy, as insect and animal repellents, in pharmaceutical preparations, as anti-microbial agents and in many other ways.

1.7.2 Orange can be used to assist with:-*cardiovascular, lymphatic, palpitation, malignant breast sores, stimulates lymphatic, and lymphatic cleaner.

1.7.3 Circulation, muscle, joints, soothes inflammation, soothes painful muscle and joints, increases circulation muscular spamgms, water retention.

1.7.4 Digestive system. Calms nervous, upset stomach, aids digestion restores the appetite (especially if lost due to surgery), regulates the bowels, encourages elimination of waste, assist gall bladder, help raise low blood sugar, constipation anorexia, mouth ulcers, constipation, gingivitis obesity, ant parasitic, diarrhea, fluid retention, intestinal spasms, digestive tonic.

1.7.5 Emotional. Assist with right brain function, promotes warm, happy feeling, and balances the emotion combats insomnia, depression, anxiety, nervous conditions stress, boredom, lethargy, physical and mental nervous energy.

1.7.6 Genitor-urinary system. Strengthens, relives some of the symptoms of chronic fatigue syndrome, cools a fever, warm a chill, and fights infections, antiseptic, antibacterial, antifungal.

1.7.7 Nervous system. Calms.

1.7.8 Respiratory system. Coughs, colds, bronchitis flu.

1.7.9 Skin care. Aids in releasing toxin from the skin, promotes production of collagen, hydrates dry skin, balance dry or oily skin, softens thick and callused skin, strengthens, antiseptic, dull and oily complexion, cellulite, psoriasis, eczema.

1.8 Methods for using essential oils

Essential oils are very powerful components of plants-they have the capability of being harmful if improperly used. Essential oils can be very helpful for some cases, supportive in other, and have little effect in others. There are three traditional uses of essential oils in aromatherapy.

1.8.1 Inhalation

Inhalation is often effective for mood-altering effects,Rosemary for mental "stimulation"lavender for relaxation,etc. this is the direct effect of essential oils components on the limbic system. One may certainly blend essential oils in diffuser or burner, adding a couple drops of each oil desired. Often anice result can be had from mixing a brighter or sweeter oil(Rosemary,Basil,Orange)with one more earthy and grounding (Patchouli, Frankincense, Cedar).

1.8.2 Topical Application

Perhaps even more common than inhalation, topical application is the preferred for many essential oils.Most essential oil require significant dilution as they can cause skin irritation. Lavender oil and chamomile oil are two essential oils that can be applied without dilution, others, such as cinnamon oil and oregano oil should not be applied topically in most cases.They may be applied as very dilute solution to the bottom of the feet. Avery small amount should be tested first, because essential oils tend to pass through the skin fairly readily,as they are liopotropic (fat soluble)and their molecular structure is fairly small. Essential oils can pass into the bloodstream and surrounding tissues.

1.8.3. Ingestion

Some essential oils may be ingested, usually either in water or in capsules,but this technique is rare, and not really considered effective in most cases.

TABLE(1-1).Essential oils derivation from various parts of plants:

Leaves	**flowers**	**Peel**	**Seeds**	**wood**
Basil	chamomile	Bergamot	Almond	camphor
Bay leaf	Clary stage	Grapefruit	Celery	cedar
Cinnamon	Clove	Lemon	Anise	rosewood
Eucalyptus	geranium	Lime	Cumin	sandalwood
Lemon grass	Hyssop	Orange	Nutmeg oil	
Melaleuca	jasmine	Tangerine		
Oraegano	lavender			
Patchouli	manuka			
Peppermint	marjoram			
Pine	Orange			
Rosemary	Rose			
Spearmint	Ylang-ylang			
Tea Tree				
Wintergreen				
Thyme				
Berries	**Bark**	**Resins**	**Rhizome**	**Root**
Allspice Juniper	Cassia Cinnamon	Frankincense	Ginger	Valerian

1-9. Aromatherapy

The treatment of anxiety or minor medical conditions by rubbing pleasant smelling natural oils into the skin or breathing in their smell.It is the use of aromatic essential oils to benefit the Body- in emotional and physical health and beauty. Science has discovered that our sense of smell plays a significant role in our overall health(GAMARRA,2006).Many common essential oils have medicinal properties that have been applied in medicine since ancient times and are still widely used today, e.g. many essential oils have antiseptic properties, though some are stronger than the others. In addition, many have

an uplifting effect on the mind, though different essential oils have different properties (MANTHEY,2004).

1.9.1 Historical background of Aromatherapy

The first day distillation of essential oils was performed by the Persian philosopher Avicenna (980-1037 A.D.) whoextracted the essence of rose petals through the(effleurage) process. His discovery and subsequent use of a wonderful perfume substance eventually lead him to write a book on the healing properties of essential oils of Rose,Early in the 20century the French chemist, (RENE-Maurice Gattefosse) began studying what he called (Aromatherapy).After several burning his arm in a laboratory accident, he thrust the arm into the nearest liquid, which happened to be tub of lavender oil. Surprised by the quick healing that followed, Dr.Gattefosse spent the remainder of his life researching the value of essential oils. His success made aromatherapy popular, and it became well-known in Europe(Guenther1995).

1.9.2 How essential oil works in Aromatherapy

An essential oil is inhaled and direct aromatherapy by the olfactory to the limbic system of brain. In true, the brain responds to particular scent affecting our emotions and chemical balance. Essential oil also absorbed by the skin and carried throughout the body via the circulatory system to reach all internal organs.By carefully choosing one or more oils, you can experience beneficial effects promoting overall health-and even specific tangents. Benefits depend upon the unique nature of each person response to an aromatic stimulus.

1.10 Pharmacological properties of essential oils

1.10.1 Antiseptics

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antiriot-resistant strains, moreover, they are also known to be active against fungi and yeasts(Candida). The most common sources of

essential oils used as antiseptic are: cinnamon, thyme Clover, Eucalyptus, Culin SAVORY, Lavender, CITRAL, geranoil, linalool, and thymol are much more potent than phenol(Manthey,2004).

1.10.2 Expectorants and diuret

When used externally, essential oils like(L essence de terebenthine) increase microcirculation and provide a slight local anesthetic action. Till now, essential oils are used in number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other Particular pains. Oral administration of essential oil like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilatation and consequences bring about a diuretic effect (Rapisararda,1999).

1.10.3 Spasmolytic and sedative.

Essential oils from the umbellifereae family, menthe species and verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia.

1.10.4 Others Cholagogue,anti-inflammatory, cicatrizing.

1.11 Chemical constituents of essential oils

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences can be classified into two groups;

1.11.1 Volatile fractions

Essential oils constituting 90-95%of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

1.11.2 Nonvolatile residues

That comprises 1-10% of the oil, containing hydrocarbons, fatty acids, sterol, carotenoids, waxes, and flavonoids.

1.11.3 **Hydrocarbons**

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic hydrocarbons found in plants are (Isoprene) having the following structure(CH_3-CH_2-C=CH-CH_2)

1.11.4 Terpenes

These are generally have names ended by ”ene” eg.limonene, pinene, camphene, piperene, etc. Terpene are anti- Inflammable, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the hydrocarbons heading, when two of these isoprene unit join head to tail, the result is a monoterpene, when three join, it is a sesquiterpene and four linked isoprene units are diterpene.

1.11.4.1 Monoterpene{$C_{10}H_{16}$}.

This terpenes are,analgestic Bactericidal, Expectorant, and Stimulant. Monoterpene are naturally occurring compounds, the majotity being unsaturated hydrocarbons (C_{10}). But some of their derivatives such as alcohols, ketones, and carboxlic acids known as monoterpene like (limonene, menthol).The branched-chain C10 hydrocarbons comprise of two isoprene units and is widely distributed in nature with more than 400 naturlly occurring monoterpene identified. Moreover, besides being linear derivatives Geraniol, Citronellol, the monoterpene can be cyclic molecules (menthol-monocyclic, Camphor- bicyclic; Pinenes, pine genera as well. Thujone (a monoterpene) is toxic agent found in Artemisia absinthium(wormwood) from which the absinthe is made. Borneol and camphor are two commonmonoterpenes.

Borneol, derived from pine oil, is used as disinfectant and deodorant. Camphor is used as counterirritant, anesthetic, expectorant, and antipruritic,among many other uses.e.g Camphene and pinene in cypress oil.Camphene, pinene,andthujhene in black pepper.

1.11.4.2 Sesquiterpenes They are anti-inflammatory, anti-septic, analgestic, anti-allergic. And in structure may be linear, monocyclic, or bicyclical Sesquiterpene are biogenetically derived from farensylpyrophosphate**.** They constitute a very large group of secondary metabolites, some have been shown to be stress compounds formed as a result of disease or injury.

1.11.4.3 Sesquiterpene lactones Over 500 compounds of this group are known; they are particularly characteristics of the composite but do occur sporadically in other families. Not only they have proved to be of interest from chemical and chemotaxonomic points of view, but also possess many antitumor, anti-leukemia, cyto toxic and antimicrobial activities. They may be responsible for skin allergies in human and they can also act as insect detergents. Chemically the compounds can be classified according to their carboxylic skeleton, thus, from the germacranolidesguaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides,etc can be derived. A structural feature of all these compounds, which appears to be associated with much of the biological activity, is the alpha- beta-unsaturated-Gama-lactones.e.g franesene in chamomile and lavender.beta-caryophyllene in basil and black pepper.

1.11.4.4 Diterpenes. These are Anti-fungal, expectorant, hormonal balancers, hypertensive. Diterpene are made up of four isoprene units. These molecules are too heavy to allow for evaporation with steam in distillation process. So they are rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C20 Skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plants hormones Gibberellins and phytol occurring as aside chain on cholorophyll

are diterpenic derivatives. The biosynthesis occur in plastid and interestingly mixtures of monoterpenes and diterpenes are amajor constituents of plant resins. In asimilar manner to monoterpenes, diterpenes arise from metabolism of gernalygernalypyrophpsphate GGPP Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs)as well as in antispasmodics and antoxiolytics.e.g.sclareol in clary sage is an example of a diterpene alcohol.

1.11.5 Alcohols These are Anti-septic, anti-viral, bactericidal and germicidal. Alcohols exist naturally, either as free compounds, or combined with terpene or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is amonoterpene, the resulting alcohol is called monoterpenol. Alcohols have very low or totally absent toxic reaction in the body or on the skintherefore, they are considered safe to use.e,g. linalool found in ylang-ylang and lavender,geraniol in geranium and rose. nerol in neroli.

1.11.6 Aldehydes These are Anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, sedative,medicinally, essential oils containing aldehydes are effective in treating candida and other fungal infections.e.g.citral in lemon, lemongrass and lemon balm,citronellal in lemongrass, lemon balm and citrus eucalyptus.

1.11.7 Acids Anti-infalmmatory, Organic acids in their free state are generally founds in very small quantities within essential oils. Plant acids act as components or buffer system to control acidity.e.g.cinnamic and benzoic acids in benzoin.citric and lactic in essential oil lemongrass and lemon balm,citronellal in lemongrass, lemon balm, and citrus eucalyptus.

1.11.8 Esters Essential oil containing esters are used for their thooting, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and

sedative, with a balancing action on the nervous system, they are generally free from precautions with the exception of methyl slicylate found in birch and wintergreen which is toxic within the ayatem.e.g., linyl acetate in bergamot and lavender, geranylformate in geranium.

1.11.9 Ketones Anti-catarrhal, cell proliferant, expectorant, vulnery.Ketones often are found in plants that are used for upper respiratory complaints, they assist the flow of mucus and ease congestion, essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue .ketones are usually very toxic, the most toxic ketone is thujone founds in mugwort, stage, tansy, thuja and wormwood oils, other toxic ketones found in essential oils are pulegone in pennyroyal, and pincocamphone in hyssop, some non-toxic ketones are jasmine in jasmine oil,fenchone in fennel oil, carvone in spearment and oil and menthone in peppermint oil.e.g.fenchone in fennel, carvone in speariment and dill, menthone in peppermint.

1.11.10 Lactones These are Anti-inflammatory, antiphlogistic,expectorant, febrifugethey are found to be particularly effective for their anti-inflammatory actions and they are have an even stronger expectorant action then ketones.

1.12 Methods of extracting Essential oils

Early efforts used alcohol fermentation process. New methods of essential oil extraction are entering the mainstream of aromatherapy. With the new label of carbon dioxide and super critical carbon dioxide, along with the traditional “steam “and “hydro” distillations, “Absolute”, and cold pressing, a little study for the aromatherapy enthusiast can go way in essential oils selection, Is one process better than other? Does one produce nicer smelling oil, or one with greater aroma therapeutic value? It turns out that essential oil productions, , is an art as well as a science, the way in which oils are extracted from plants is important because some processes use solvents that can destroy the

therapeutic properties . Some plants and particularly flowers do not lend themselves to steam distilling. They are too delicate, or their fragrance and therapeutic essences cannot be completely released by water alone. These oil will be produced as “absolutes” and while not technically considered essential oils they can still be of therapeutic value. Jasmine oil and rose oil in particular are delicate flowers whose oils are often found in “absolute” form. The value of the newer processing methods depends greatly on the experience of the distiller, as well as the intended application of the final product, each method is important, and has its place in the making of aromatherapy –grade essential oil. Some of the few methods are available for extraction oils are given below;

1.12.1 Maceration

Maceration actually creates more of an “influsedoil” rather than an ‘essential oils “the plants matter is soaked in vegetable oil. Heated and strained at which point it can be used for massage.

1.12.2 cold pressing . Cold pressing is used to extract the essential oil from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the simple pressing of the rind at about 120 degree F to extract the oil. The rinds are separated from the fruit, ground or chopped and are then pressed, the result is a watery mixture of essential oil and liquid which separate given time. Little, if any alteration from the oils original state occurs. These citrus oils retain their bright, fresh, uplifting aromas like that of smelling a wonderfully ripe fruit. It is important to note that oils extracted using this method have a relatively short shelf life. So make or purchase only what you will be using within the next six months.

1.12.3 Solvent extraction A hydrocarbon solvent is used is added to the plant material to help dissolve the essential oil. When the solution is a filtered and concentrated by distillation , a substance containing resin (resinoid), or a combination of a wax an essential oil (known as concrete) remains. From the

concentrate, pure alcohol is used to extract the oil. When the alcohol evaporates, the oil is left behind. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system.

1.12.4 Enfleurage An intensive and traditional way of extraction oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oil from the fat. The alcohol is then evaporated and the essential oil collected.

1.12.5 Supper critical CO_2 extraction The most modern technologies, carbon dioxide and super critical carbon dioxide extraction involve the use of carbon dioxide as the "solvent" which carries the essential oil away from the raw plant material. The lower pressure carbon dioxide extraction involves chilling carbon dioxide to between 35 and 55 degree, F, and pumping it through the plant martial at about 1000psi; the carbon dioxide in this condition is condensed to a liquid. Super critical carbon dioxide extraction (CO_2) involves carbon dioxide heated to 87 degree F and pumped through the plant material at around 8,000 psi, under these conditions; the carbon dioxide is likened to "densefog" or vapor. With release of the pressure in either process, the carbon dioxide escape in its gaseous form, leaving the essential oil behind. The usual method of extraction is through steam distillation, after extraction, the properties of good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a good essential oil extracted is through low pressure and low temperature processing, high temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance.

1.12.6 Hydro distillation Some process becomes absolute to carry out extraction process like hydro distillation which often used in primitive countries. The risk is that the still can run dry, or be overheated, burning, the aromatics and resulting in an essential oil with a burnt smell. Hydro

distillation seems to work best for powders (i.e. spice powders, ground wood…etc)and very tough material like roots, wood or ,nuts.

1.12.7 Turbo distillation extraction Turbo distillation extraction is suitable for hard-to-extract or coarse plant material, such as bark, roots, and seeds. In this process, the plants soak in water and steam is circulated through this plant and water mixture. Throughout the entire process, the same water is continually recycled through the plant material. This method allows faster extraction of essential oils from hard-to-extract plant materials.

1.12.8 Steam distillation Most commonly, the essence is extracted from the plant using a technique called distillation. One type of distillation place the plants or flowers on screen. Steam is passed through the area and becomes" charged" with the essence. The steam then passes through an area where it cools and condenses. This mixture of the water and essential oil is separated and bottled. Since plants contain such as a small amount of this precious oil, several hundred pounds may need to produce a single ounce.

1.13 Extraction using steam distillation method

Essential oil can be extracted using a variety of methods, although some are not commonly used today. Nowadays, a reputable distiller will try to preserve the original qualities of the plant, but the final therapeutic result is often not formed until after the extraction process. During extraction, the qualities of the essential oil change to give it more value. For example, chamazulene(characteristic of the pure blue color of German chamomile) is formed during the steam distillation process. Currently, the most popular method for extraction is steam distillation. Many old-time distillers favor this method for most oils, and say that none of the newer methods produces better quality oils. Steam distillation is Special type of separation process for temperature sensitive materials like oils, resins, hydrocarbons,etc. which are insoluble in water and may decompose at their boiling point, thefundamental

nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at temperature substantially below that of the boiling point(s) of the individual constituent(s) essential oils contain substances with boiling point up to 200^0c or higher temperature. In the presence of steam or boiling water, however, these substances are volatilized at temperature close to 100^0C at atmospheric pressure.Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and steam is allows to pass through the herb material under pressure which softens the cells and allows the essential oil to Volatile in vapor form. The temperature of the steam must be high enough to vaporize the oil present, without destroying the plants or burns the essential oils. As they are released, the tiny droplet of essential oils evaporate and together with the steam molecules, travel through a tube into the still's condensation chamber.as the steam cools, it condenses into water, the essential oil forms a film on the surface of the water. To separate the essential oil from water, the film is then decanted or skimmed off the top. The remaining water, a byproduct of distillation, is called floral water, distillate, or hydrosol.It retains many of the therapeutic properties of the plants, making it valuable in skin care for facial mists and toners. In certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or achild,or when a more diluted treatment is required. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma.Essential oil isolated by steam distillation are different in composition to those naturally occurring in the oil bearing glands of plants , since the steam distillation conditions cause chemical reactions to occur which result in the formation of certain artificial chemicals, called artificate, some of these are considered beneficial e.g. the formation of chamazulene during the steam distillation chamomile oils. Whilst others may not be, e.g. the hydrolysis of linalyl acetate during the distillation of clary sage. Few , if any, essential oils are unscathed by the thermal conditions of steam distillation, but some

distillation techniques can, in certain instances, be a measure less damaging than others(e.g. hydro diffusion-a sort of inverted steam distillation where steam is the introduced at the top of the vegetable material-packed container , and oil and condensate issue from the bottom-can produce oils with higher ester contents ,i.e. less thermally induced hydrolysis).A number of factors determine the final quality of steam distilled essential oils. Aside from the plant material itself, most important are time, temperature and pressure, and the quality of the distillation equipment, essential oils are very complex products. Each is made up of many. Sometime hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties, some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from along, slow 'cooking' process. It is possible that longer distillation times may give more complete oil. It is also possible however, that longer distillation time may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improving the odor, as sometime when materials that have a larger number of compounds are sniffed, the perception is often of slightly increased sophistication, added fullness character, and possibly, and extra pleasantness.

1.13.1 Advantages of steam distillation

The advantage of steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are well known. Newer methodology, such as sub critical water extraction, may well eventually replace steam distillation , but so far even contenders such as carbon dioxide extraction- although establishing a firm market niche –have not really threatened to take over as the major preparative technique.

1.14 Analytical methods

Chemical analysis of essential oils is generally done using GC gas chromatography (quantitative analysis) and GC.MS gas chromatography – mass spectrometer (qualitative analysis). Identification of the main components is carried out by the comparison of both the GCretention times and mass data against those of the reference standards with known source. Sometimes identification by GC.MS must be confirmed by retention indices(KOVATS INDICES)on two columns of different polarity and clams for the identification of new constituents should be supported by co-injecton with authentic compounds. Recently some 900Kovats indices of 400 individual compounds were summarized from the general literature.

The principle of GC is the differential distribution of the components between two phases (one stationary phase and the other is mobile phase). The mobile phase (carrier gas) usually is nitrogen. Depending on the nature of the mixture Ar, He, H_2, are also used. The stationary phase may be solid or liquid. Nowadays liquid stationary phase is more use. According to the nature of the stationary phase gas chromatography can be divided into two classes, if the stationary phase consists of silica, alumina, or carbon, the chromatography is termed as gas solid chromatography (GSC).and if the stationary phase is nonvolatile liquid held as a thin layer on a solid support, then the technique is known as gas liquid chromatography (GLC).The most common support used in GLC is diatomaceous earth or kiselguhr. Because of tailing caused by nonlinear adsorption isotherm in GSC,GLC has now become the most important and widely used technique. The availability of versatile and specific detectors and the possibility of coupling the gas chromatography to mass spectrometer or an inferred. Spectrophotometer further enhances the usefulness of gas chromatography. The main advantages of gas chromatography in analysis are.(1).The technique has strong separation powered event quite complex mixtures can be resolved into constituents.

(2).The sensitivity of the method is quite high, it is micro-method and only a few milligrams of the sample are enough for analysis, (3).The speed of the analysis is very fast, giving good precision and accuracy, it involves relatively simple instrumentation, operation of gas chromatography and related calculation do not require highly skilled personal and thus the technique is very suitable for routine analysis. The cost of equipment is relatively low and its life is generally long.

Aim of the study

1. To determine and identify the percentage of components present in the volatile oils.
2. To determine the physicochemical properties of the volatile oils.

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and Methods

2.1 Materials

2.1.1 Samples collection.

Orange, lemon, grapefruit samples used in this work were bought from Omdurman market Khartoum state.

2.1.2. Chemicals

Analytical grade chemicals and solvents were used

.2.1.3 Instruments

GC-MS analysis instrument Hewlett Packard 1800A with parameters-column capillary column HP-5(length30,1d0.25mm), carrier gas: Helium, flow rate.1ml min ,inlet temp:250^0c,Detector temp.280^0c with temperature programming 100^0c-3min-10-250^0c-30-280^0c.with mass detector and library search was carried out by HPchem software.

Abbe Refractometer A610 refractometer.

2.2 Methods

2.2.1 Samples preparation

Samples used in this work were washed with distilled water, peeled and then cut into small pieces and purred.

2.2.2 Extraction of oils

Air – dried peels of orange (lemon, grapefruit) were steam-distilled for 6 hours. The distillate was partitioned with diethyl ether (20ml x3), dried (anhydrous sodium sulphate) and the solvent was removed under reduced pressure to give the oil.

2.2.3. Determination of density

Density bottle was used for determining the density of the oil. Clean and dry density bottle (25ml) was weighed (w_0) and then filled with oil to the mark. It was stoppered inserted and reweighed to give (w_1). This was performed for lemon and grapefruit orange then the density of essential oil was calculated.

2.2.4 Determination of refractive index

The refractometer was standardized with distilled water, then it was cleaned with acetone and dried. Few a drops of orange peel essential oil was placed between the prisms of referactometer. The telescope was rotated to bring the border line of total refraction to the junction of cross-wire, in the telescope. The refractive index was recorded.

2.2.5. Determination of P^H value

The glass electrode was washed by distilled water, and dried. it was placed in a buffer solution p^H(4,7,9) in a clean and dry 50 ml beaker the electrode was then introduced in and the P^H was recorded.

2.2.6 Determination of peroxide value 30ml of acetic acid in chloroform (70ml) 30ml from the mixture was added to 5g of orange oil (10ml) of 0.5N saturated solution of potassium iodide was added Followed by closely by 30ml of distilled water. The solution was titrated against 0.1m potassium thiosulphate until the yellow color almost disappeared. (0.5ml) starch indicator was added and the titration continued until the end point (where the blue black color just disappeared) A blank titration was also performed. The above titration process was performed for lemon and grapefruit oils.

$$\text{PEROXIDE VALUE} = \frac{(S-B)x0.1x1000}{weghitofoil}$$

Where: S= volume of sample B= volume of blank

2.2.7 Determination of saponification value

(5.g) of orange peel were weighed into a conical flask. 25ml of 0.1N ethanol potassium hydroxide was added to the conical flask. The contents were stirred for 1 hour followed by reflux. Phenolphthalein indicator was then added to the conical flask and the solution was titrated against 0.5M HCL till the solution changes to colorless. The same procedure was repeated for the blank. The difference between the blank and test readings gives the number of milliliter of 0.5N KOH required to saponify the oil.

Weight of KOH= normality of KOH (equivalent weight value of KOH in liter.

Volume of KOH consumed by 5g =[blank-test] ml.

Saponification value=$\frac{(S-B)XNX56.1}{weightofsample}$

Where: s=volume of KOH B=Blank volume

2.2.8 Determination of acid value

25ml of ethanol and 25ml of diethyl ether was mixed in a 250ml beaker; the resulting mixture was added to 5.0g orange peel oil in 250ml conical flask, and then the flask contents was heated to dissolve the oil. Few drops of phenolphthalein were added to the mixture. The mixture was then titrated against 0.1M KOH(V).This steps was performed without oil as blank, the above experiment was repeated using 5.0g of lemon and 5.0g of grapefruit essential oil.

Acid value=$\frac{(s-b)XNX56.1}{weightofsample}$

s=KOH volume B= Blank volume N=polarity of KOH

CHAPTER THREE

RESULTS AND DISCUSSION

3. Results and discussion

The quantity of essential oil extracted from the citrus peels are presented in table 3-1, it was observed that the volume of essential oil extracted from the peels in lemon is grater than orange and grapefruit. The quantity and quality of essential oil depends on different factors. Among them are the chemotype and biotype of the plant, the climatic conditions as well as the extraction process.

Table3-1-1.amounts of the extracted oil.

Plant sample	Weight	Time	Oilquantity extracted
Orange peel	15 kg	6hours	45 ml
Grapefruit peel	15 kg	6hours	42 ml
Lemon peel	15 kg	6hours	53 ml

Table 3.2. Shows the refractive index of grapefruit oil in relatively low compared to that of orange and lemon oils which are almost of similar values. This difference may be affecting the density of the oils. The higher value of density of grapefruit oil may be due to its lower refractive index. P^H values shows that all the oil is acidic and orange oil has significantly higher acidity. The acid numbers are clearly different for the three oil samples, but the differences are not in the same order of the P^H values. Saponification number value of orange oil is higher than that of lemon and grapefruit which are approximately the same. Peroxide number values showed clear differences between the three oils. for grapefruit the value is nearly half that of orange sample and the lemon oil peroxide value in moderate .

Table 3.2. Shows the some Physical constants for the studded samples.

Property	Orange oil	Lemon oil	Grapefruit oil
Refractive index	1.47	1.48	1.42
Density	0.84	0.86	0.89
pH	2.2	3.2	3.67
Acid value(mgkoH/goil)	1.99	1.79	2.23
Saponification value(mgkoH/goil)	16.75	13.5	13.7
Peroxide value	18.68	13.67	9.59

Table 3.3 shows the constituents of the oil of orange, lemon, and grapefruit, and their percentage. The major components in orange oil are D-Limonene (54.15%), pulegone (11.56%) and L-carvone (2-cyclohexen-1-one)(5.46%).The major components in lemon essential oil are D-limonene(64%),2-cyclohexen-1-ol(6.21%) and β-pinene(3.82%). The three major components in grapefruit oil are β-pinene (23.12%),D-limonene(17.07%) and α-pinene(10.40%). The percentage of D-limonene and β-pinene is in agreement with Soumaya, et al(2012) findings. From this study it was concluded that the constituents of such oil may vary in the raw material, being influenced by plant health, growth stage, and climate as well as harvest time. However, from table 3-1-3,it is evident that lemon oil has highest percentage of D-limonene(64%)when compared to the other samples . it can also be seen from table (3.1) that lemon peel produced the highest volume of essential oil when compared to the other samples.

Component	orange%	lemon%	grapefruit%
1R-α-pinene	4.45	3.20	10.40
Cyclohexene	1.91	-	6.92
D-limonene	54.15	64.00	17.07
(Z)-3-Carene	3.71	-	-
1,3,8-p-Menthatriene	2.81	2.91	0.83
Pulegone	11.65	-	--
1-Methylcyclohexa-1,3-diene	4.35	-	-
1,3-Cycloheptadiene	3.98	-	-
2-Cyclohexen-1-ol	5.23	6.21	-
1,4-Cyclohexadiene	2.30	2.54	5.52
2-Cyclohexene-1-one(L-	5.46	3.27	-
β –phellandrene	-	3.52	-
β-pinene	-	3.82	23.12
1,4-Cyclohexadiene	-	1.96	-
Cis-p-Mentha-2,8-diene-1-ol	-	3.15	-
2-methyl-5-(1-methylethenyl)-	-	2.97	-
3-Cyclohexen-1-ol	-	2.46	4.96
α –phellandrene	-	-	1.33
Ocimene	-	-	0.73
Bicyclo(4.1.0)hept-2-ene	-	-	1.10

3-Carene	-	-	2.03
Component	orange%	lemon%	grapefruit%
Bicyclo(3.1.0)heptan-3-ol	-	-	1.32
3-Cyclohexene-1-methanol	-	-	3.61
E,Z-4Ethylidenecyclohexene	-	-	1.15
2-Cyclohexne-1-ol	-	-	1.12
2-Cyclohexen-1-one	-	-	0.81
2,6-Octadienal	-	-	1.32
1,3-Cyclohexadiene	-	-	1.01
1S-α-Pinene	-	-	0.79
1H-Cycloprop(e)azulene	-	-	1.24
Caryophyllene	-	-	4.80
Trans-α-Bergamotene	-	-	4.67
Bicyclo(3.1.1)hept-2-ene	-	-	2.48
Caryophyllene oxide	-	-	1.67

CONCLUSION

The obtained results showed that lemon peels contain large amount of D-limonene per unit volume of essential oil.It also contains more amount of essential oil than orange and grapefruit per unit mass of peel.It was concluded that lemon peel is a more economical source of volatile oil being very rich in D-limonene.

Suggestion for further studies

The shelf life of citrus peel essential oil have been established from literature to be greatly affected by its storage condition such as wrong storage containers,storage temperature and head space in storage containers. Thus, citrus essential oil should be stored in Anber bottles, and below room temperature, with the avoidance of headspace in storage containers.

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