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Extraction Of Essential Oil From Orange Fruit Rind

(Citrus sinensis)

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Port Harcourt, Rivers.

In Partial Fulfilment Of The Requirement For The Award Of The Degree Of Bachelor of Science In The Department Of Pure And Industrial Chemistry

SEPTEMBER, 2018.

EXTRACTION OF ESSENTIAL OIL FROM ORANGE FRUIT RIND (*citrus sinensis)*

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DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY

FACULTY OF CHEMICAL SCIENCE

UNIVERSITY OF PORT HARCOURT

PORT HARCOURT, NIGERIA.

BEING A RESEARCH PROJECT SUBMITTED IN PARTIAL FUFILMENT FOR THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY.

SEPTEMBER, 2018

CERTIFICATION

This is to certify that this research project was carried out by Johnson, Emediong Ime with Matric. Number U2014/5581024 under the supervision of Prof. Gloria Obuzor and that it meets the standards approved by the Department of Pure and Industrial Chemistry, Faculty of Chemical Science. University of Port Harcourt

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DEDICATION

This work is dedicated to God Almighty, the one who saw me through the years of study and to all those who recognize the relevance of Chemistry in our modern society.

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I wish to appreciate my Head of Department, Prof. O. J. Abaye and all the lecturers who have contributed significantly during the course of my educational pursuit.

I want to profoundly appreciate my project supervisor Prof. Gloria Obuzor for her help and encouragement and for introducing me to the world of research. She has indeed been a blessing to me.

To my parents, Mr/Mrs Ime Johnson Udoh and my entire family I confess my complete indebtedness and gratitude for without them this work and many of my activities wouldn't have seen the light of the day.

To my fellow corrosives especially my project mates Boma, Michael, Ola and John; I say a big thank you.

ABSTRACT

Presently in Nigeria, orange peels are being discarded as waste after the consumption of the edible parts of the orange fruits. However, the country depends on imported essential oils for use in industries for the manufacture of products ranging from food to beverage, cosmetics and pharmaceuticals. This research work was carried out to demonstrate the utilization of orange rind in the production of essential oils by employing the cold pressing method. From the result obtained, it was discovered that orange peel gave a yield of 5.05% when cold pressing method was employed. The constituent of the extracted oil analysed by Gas Chromatography-Mass Spectroscopy gave twenty-eight compounds with eleven major compounds such as D-limonene (14.14%), ergosta-5, 7-dien-3-ol (13.30%), 9-octadecenoic acid (12.66%), n-hexadecanoic acid (12.34%), di(1-phenylpropyl)ester, adipic acid (6.91%), 5,6,7-trimethoxy-2-(4-methoxyphenyl) 4H-1-benzopyran-4-one (6.73%), octadecanoic acid (4.50%), 2,7-dimethyl-1,3,7-octatriene(4.29%), n-hexadecanoic acid (3.53%), cholest-5-en-3-ol,acetate (3.06%), and 4-hydroxy-3-methylacetophenone (2.04%) together with eighteen minor constituents namely 1,2,3-cyclopentanetriol (1.57%), bicyclo [4.1.0]heptanes-3-cyclopropyl,7carbomethoxy (1.29%), 2,3-dihydrobenzofuran (1.20%), 3,5-dimethyl-2-pyrazoline-1-carboxamide (1.88%), 2,2,2-trifluoroethylester (1.64%), 4,4-dimethyl-cholesta-6,22,24-triene (1.46%), 2-methyltrans-3-methyl-2-n-quinazoline (1.06%), 3-methyl-2-phenylimino-perhydro-1,3-oxazine (0.90), 2-methyl-2,3-hexadiene (0.77%), (1-methylethyl)-benzene (0.75%), 5,7-dimethoxy-2H-1-benzopyran-2-one (0.69%), 4-hydroxy-4-methyl-2-pentanone (0.67%), tetradecanoic acid (0.60%), 2,3,5,6-tetrafluoroanisole (0.54%), trans-1-methyl-4-(1-methylethenyl)-2-cyclohexen-1-ol (0.53%), (-)-cis-isopiperitenol (0.48%) and benzoic acid (0.46%) corresponding to 99.99% of the total oil. The characterization of the extracted oil gave its physical properties values that indicated that it could be used for the production of other valuable products in different process industries. Hence, it was utilized in the production of liquid air freshener.

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CHAPTER ONE

INTRODUCTION

Oil is any non-polar chemical substance that is a vicious liquid at ambient temperature and is both hydrophobic ("water fearing") and lipophilic ("fat loving"). Oils have a high carbon and hydrogen content and are usually flammable and surface active. Oils may be animal, vegetable or petrochemical in origin and may be volatile or non- volatile (Igwe, 2010)

TYPES OF OIL

MINERAL OILS: Mineral oils which are basically petrochemicals consist of crude oil or petroleum and its refined component and are crucial resources in the modern economy. The name mineral oil is a misnomer in that minerals are not the source of the oil- ancient plants and animals are. Mineral oil is organic although not named under organic oils because its organic origin is remote and was unknown at the time of it discovery and is obtained in the vicinity of rocks, underground traps and sands.

Organic oils: Organic oils are oils produced by plants, animals and other organisms through natural metabolic processes. There are the overall mixture of chemicals other than lipids, which includes proteins, waxes and alkaloids (Mahmood, 2005)

ESSENTIAL OIL: Essential oil which is classified under organic oils is plant-based volatile oils with strong aromatic components that are made up of different chemical compounds. Alcohols, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components of essential oil. Essential oils are also hydrophobic liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as oil of the plant material from which they are extracted such as oil of orange.

The advantages of essential oil are their flavour concentration and their similarity to their corresponding sources. The majority of them are fairly stable and contain natural anti-oxidant and anti-microbial agent (Ezejiofor*et al.,* 2015). Essential oils are usually colourless particularly when fresh. Nevertheless, with age, essential oil may oxidize which causes the colour to become darker. They need to be stored in a cool, dry place, tightly stoppered and preferably in amber glass containers. Essential oils are used in a variety of consumer goods such as detergents, soaps, toilet product, cosmetics, pharmaceuticals, perfumes, confectionary food products, soft drinks, distilled alcoholic beverages (hard drink) and insecticides. The world's production and consumption of essential oils and perfumes are increasing very fast (Ezejiofor*et al.,* 2011)

HISTORICAL BACKGROUND OF *CITRUS SINENSIS* (SWEET ORANGE) OIL

The orange is the fruit of the citrus species called *citrus sinensis* in the family Rutaceae. It is also called sweet orange to differentiate it from bitter orange. Sweet oranges were mentioned in Chinese literature in 314 BC (Mahmood, 2005). As of 1987, orange trees were found to be the most cultivated fruit tree in the world. Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit of the orange tree can be eaten fresh or processed for its juice or fragrant peel. As of 2012, sweet oranges accounted for approximately 70% of citrus production. In 2014, 70.9 million tonnes of oranges were grown worldwide, with Brazil producing 24% of the world total followed by China and India.

Citrus peels consist of two layers called flavedo and albedo. Citrus fruits have a rough, robust and bright (green to yellow) coloured skin. They are usually 4 to 30cm long and 4 to 20cm in diameter with a leathery surrounding skin called flavedo that covers the fruit and prevents it from damages. They are notable for their fragrance partly due to the flavonoids and limonoids contained in the rind (Galadima, 2004). The endocarp is rich in soluble sugars and contains significant amounts of Vitamin C, pectin, fibres, different organic acids and potassium salt which gives the fruits its characteristics citrus flavour (Hesham*et al.,* 2016). Like all other citrus fruits, the sweet orange is non-climacteric. Citrus essential oils are obtained by cold pressing sometimes called scarification method or distillation from fruit rind.

STATEMENT OF PROBLEM

The current annual world production of citrus fruits is approximately 10 million tons. In Nigeria, about 930,000 tons of citrus fruits are produced annually from an estimate of 3 million hectares. In 1993, the world sale value of fragrance and flavours was 19 Billion USD. Out of this amount, Nigeria didn't earn anything but spent about $14 million on importation of flavours, fragrance and essential oils between June and December 1994. (Njoku, 2014)

Currently, Nigeria's local production of essential oil is insignificant, so nearly 100% of the essential oils used by our industries are imported. Research statistics from the Raw Materials Research and Development Council (RMRDC) indicates a local demand of over 10,000kg annually; a figure that could be met through local production efforts (Muhammad, 2004). Generally, the waste disposal problem from juice producing industries and fruits such as orange peels causes environmental pollution. In order to reduce this problem, the waste (peels of citrus fruits) can serve as raw materials for the extraction of essential oils needed for various domestic and industrial usages.

OBJECTIVES

The objective of this research is extract the essential oil from citrus peel using cold pressing method and to determine the chemical composition of this essential oil then further utilize the essential oil for the production of liquid air freshener.

SCOPE OF STUDY

This research is aimed at extracting essential oils from the rind of orange fruits using the cold pressing method. The extracted essential oil will then be utilized for the production of finished products. The extracted oil will then be analysed using the GC-MS to identify the various components present in the oil and their relative amounts.

SIGNIFICANCE OF THIS STUDY

The research into essential oil is becoming serious owing to the usefulness of the oil. In order to contribute our quota to this area of research, this work has been carried out to investigate the essential oil from *citrus sinensis* rind which is currently discarded as waste in Nigeria using the cold pressing method which is one of the best methods of extracting the essential oils.

Presently in Nigeria, orange peels are discarded as waste after the consumption of the edible parts of the orange fruit. However, the country depends on imported essential oil for use in industries for the manufacture of products ranging from food to beverages, cosmetics and pharmaceuticals. Thus, exploring essential oil is an additional way of evaluating the underlying economic value of citrus due to their usefulness as food nutrient and flavour. Processing of citrus rind into essential oils is a sure way of transforming this waste with great potential for environment pollution into resource with great potential for economic prosperity and also for securing the public health impact for a safer and healthier environment. This is likely to be obtained by the indirect waste management so offered (waste to wealth).

CHAPTER TWO

LITERATURE REVIEW

Essential oils have been used in folk medicine throughout history. The earliest recorded mention of the techniques and methods used to prepare essential oils is believed to be that Ibn al-Baitar (1188-1248), a Physician, Pharmacist and Chemist (Bajpa, 2007). Rather than refer to essential oils themselves, modern works typically discuss specific chemical compounds of which the essential oils are composed. For example methyl salicylate rather than "Oil of Wintergreen" (Gilman, 2005).Interest in essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that uses essential oils and other aromatic compounds. Oils are volatilized, diluted in carrier oil and used in massage, diffused in a nebulizer, heated over candle flame or burned as incense (Gilman, 2005). Medical applications proposed by those who sell medicinal oils range from skin treatments to remedy for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatment of cancers in particular are now subject to regulations in most countries.

Most citrus peel oils are expressed mechanically or cold pressed. Due to relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus fruits oils are cheaper than most other essential oils. Sweet orange oils are obtained as by-products of the citrus industries. Before the discovery of distillation, all essential oils were extracted by pressing (Bajpa, 2007)

Estimates of total production of essential oils are difficult to obtain. One estimate compiled from data in 1989, 1990 and 1994 from various sources gave the following total production in tonnes of essential oils (Table 2.1) for which more than 1000 tonnes were produced (Igwe, 2015).

TABLE 2.1: ESTIMATES OF TOTAL PRODUCTION OF ESSENTIAL OIL IN TONNES BETWEEN 1989, 1990 AND 1994 (Igwe, 2015)

OIL	TONNES
SWEET ORANGE	12,000
MENTHA ARVENSIS	4,800
PEPPERMINT	3,200
CEDARWOOD	2,600
LEMON	2,300
EUCALYPTUS GLOBULUS	2,070
LITSEA CUBEDA	2000
CLOVE (LEAF)	2000
SPEARMINT	1300

When taken by mouth essential oils can be dangerous in high concentration. Typical effect begins with a burning feeling followed by salivation. In the stomach, the effect is carminative relaxing the gastric sphincter and encouraging eructation (belching). Further down the gut, the effect typically is antispasmodic (Sawamura*et al.,* 2004). Aromatherapy is a form of alternative medicine in which healing effects are ascribed to the aromatic compounds in essential oils and other plant extracts. Aromatherapy may be useful to induce relaxation but there is not sufficient evidence that essential oils can effectively treat any condition (Igwe, 2015).

USE AS PESTICIDE

Research has shown that essential oils have potential as natural pesticides. In case studies, certain oils have been shown to have a variety of deterring effects on pests specifically insects and selected arthropods. The effect may include repelling, inhibiting digestion and stunted growth decreasing the rate of production of pests that consume the oil (Aadhithiya*et al.,* 2015). However, the molecules within the oils that cause these effects are normally non-toxic for mammals. These specific actions of the molecules allow for widespread use of these green pesticides without harmful effect to anything other than pests (Marvin*et al.,* 2012). Using essential oils as green pesticides rather than synthetic pesticides have ecological benefits such as decreased residual actions in addition, increased use of essential oils as pest control could have not only ecological but economical benefit as the essential oil market diversities. Some essential oils, including many of the citrus peel oils are photo sensitizers increasing the skin's vulnerability to sunlight. Industrial users of essential oils should consult the safety data sheets (SDS) to determine the hazards and handling requirement of particular oils. Even certain therapeutic grade oils can pose serious threat to individuals with epilepsy or pregnant women. Essential oils used in children can pose a danger when misused because of their thin skin and immature livers. This might cause them to be more susceptible to toxic effects than adults (Tiwari, 2012).

Essential oil is usually obtained from the peel of sweet orange. The orange is the fruit of the citrus species *citrus sinensis* in the family *Rutaceae.*It is also called sweet orange to distinguish it from the related *citrus aurantium* referred to as bitter orange. Sweet orange was mentioned in Chinese literature in 314BC. As of 1987, orange trees were found to be the most cultivated fruit tree in the world. Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit of the orange tree can be eaten fresh or processed for its juice or fragrant peel. As of 2012, sweet orange accounted for approximately 70% of citrus production (Muhammad, 2017). In 2014, 70.9 million tonnes of oranges were grown worldwide with Brazil producing 24% of the world total followed by China and India. The taste of orange is determined mainly by the relative ratios of sugars and acids whereas orange's aroma is derived from volatile organic compounds including alcohols, aldehydes, ketones, terpenes and esters (Kabuba, 2009)

Bitter limonoid compounds, such as limonin, decrease gradually during development whereas volatile aroma compounds tend to peak in mid-to-late season development. Taste quality tends to improve later in harvest when there is a higher sugar/acid ratio with less bitterness. As a citrus fruit, the orange is acidic with pH levels ranging from 2.9 to 4.0. Sensory qualities vary according to genetic background, environmental conditions during development, and ripeness at harvest, post-harvest condition and storage condition (Saad, 2015)

Table 2.2: MINERALS AND THEIR QUANTITIES IN ORANGE (Igwe, 2005)

Minerals	**QUANTITY**
Calcium	40 mg
Iron	0.1 mg
Magnesium	10 mg
Manganese	0.025 mg
Phosphorus	14 mg
Potassium	181 mg
Zinc	0.07 mg

BENEFITS OF CITRUS ESSENTIAL OILS

Citrus oils are among the favourites in any essential oils collection. They are versatile and effective, providing many uses and benefits. Citrus essential oils offer a variety of benefits for health and wellness. Some of these benefits include:

1. **Boosting Mood:**

   Citrus oils are a wonderful way to enhance mood and emotional balance. They work on the brain's chemicals and hormones resulting in an improved mood.

2. **Antioxidant Protection:**

   Citrus oils contain antioxidant properties that help neutralize free radicals which are responsible for damage to cells and tissues. Antioxidants also help to relieve stress on the immune system.

3. **Germ elimination:**

   Citrus oils are well known for killing germs and fighting against pathogens. The use of citrus oils on homemade cleaning products to enjoy all natural germ elimination

4. **Immune System Support:**

   Citrus oils are known to effectively boost the immune system.

5. **Superb Air Freshener**:

   It can be used to remove odours throughout the house and the work place.

6. **Sanitize surfaces and produce:**

   It helps in the sanitization of the surface in kitchen, bathroom and the rest of the home.

7. **Ease Anxiety and Irritability:**

   Several citrus oils can help reduce feelings or anxiety and irritability as well as other mood disorders.

8. **Boost Energy:**

   Citrus oils help to boost physical and mental energy levels. Especially great oils to use in a diffuser at home or work place

9. **Remove toxins:**

Some citrus oils help to remove toxins and impurities from cells.

10.Helpful To Skin:

Applying citrus oils to skin issues like warts, bunions, corns or calluses may eliminate these bothersome problems

11.Flavour Food:

Citrus oils are a wonderful way to add flavour to food and drinks. It should be labelled for internal use as not all essential oils are safe for consumption.

12.Versatility:

Citrus oils can be used aromatically, topically and internally.

ESSENTIAL OIL EXTRACTION METHODS

Essential oils are used in a wide variety of consumer goods such as detergent, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils are increasing very fast. Production technology is necessary to improve the overall yield and quality of essential oil. Essential oils are obtained from various plant raw materials by several extraction methods (Mahmood, 2015) The methods are classified into two:

- Innovative technique of essential oil extraction (Non-traditional)
- Classical and conventional methods

NON- TRADITIONAL METHOD

Due to technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations such as the production of costly essential oils in a natural state without alterations of their thermo sensitive components or the extraction of essential oils for micro-analysis. Some of the non-traditional method includes:

- **Supercritical Fluid Extraction (SFE):**
  SFE is a process of separating one component (the extractant) from another (the matrix) using supercritical fluids example CO_2 as the extracting solvent. Extraction is usually from a solid matrix but can also be from liquids. This technique is very expensive due to high price of the equipment and it is not easily handled.

- **Microwave-Assisted Hydrodistillation (MAHD):**
  It is an advanced hydrodistillation technique utilizing microwave oven in the extraction process. Golmakani*et al.*, 2008 reported some recently published stuffs have successfully utilized a microwave oven for the extraction of active components from plants. The efficiency of Microwave-Assisted Hydrodistillation (MAHD) is strongly dependent on the dielectric constant of water and the sample (Brachet*et al.*, 2002)

- **Ultrasound-Assisted Extraction (UAE):**
  UAE is a good process to achieve high yield valuable compounds and could lead to an increase in some food by-product when used as source of natural compounds or plant material (Bhaskaracharya*et al.*, 2009).

- **Solvent-Free Microwave Extraction (SFME):**
  This is an extraction procedure that occurs without adding any solvent.

- **Microwave Hydrodiffusion and Gravity (MHG**):
  It is a new green technique for the extraction of essential oils. It is not only economic and efficient; it is also environment friendly, does not require solvent or water and requires less energy (Chemat*et al.*, 2004)

CONVENTIONAL AND CLASSICAL METHOD

This method includes hydrodistillation, steam distillation, solvent extraction, soxhlet extraction and scarification which are the traditional methods generally used.

- **Hydrodistillation:**
  It is one of the oldest and easiest methods. It is a multilateral process that can be utilized for small or large industries. Here, plant material is soaked for some time in water after which the mixture is heated and volatile materials are carried away in the steam, condensed and separated. It is a common extraction method for separating phytochemical compounds from plant materials. It is a variant of steam distillation.

- **Steam Distillation:**
  This method is used for temperature-sensitive plants such as natural aromatic compounds. It can also be carried under pressure depending on the essential oil extraction difficulty.

- **Solvent Extraction:**
  It is also known as liquid-liquid extraction or partitioning. It is used to separate compounds based on their solubility. It is done using two immiscible liquids

- **Soxhlet Extraction:**
  Soxhlet extractor is a piece of apparatus invented in 1879 by Franz von Soxhlet (Soxhlet *et al.*, 2010). It was originally designed for the extraction of a lipid from a solid material. It is used when the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent.

- **Cold pressing method:**
  Here, the oil is expeller-pressed at low temperature and pressure. It is one of the best methods to extract essential oils. It is a mechanical method where heat is reduced and minimized throughout the batching of the raw material (Meyer, 2015).

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS

The analysis of the component of the essential oil was carried out using the GC-MS technique. GC/MS is an instrumental technique comprising of a gas chromatograph (GC) coupled to a mass spectrometer (MS) by which complex mixtures of chemicals may be separated, identified and quantified. This makes it ideal for the analysis of the hundreds of the hundreds of relatively low molecular weight compounds found in environmental materials. It is used for analysing volatile compounds in complex samples.

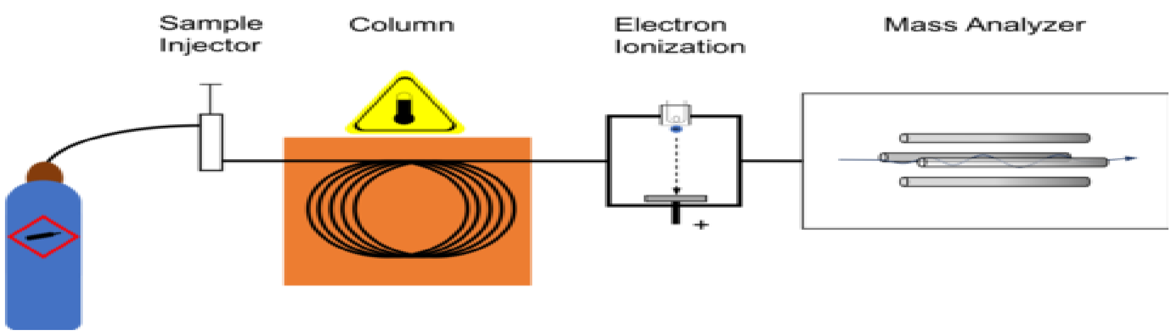
The sample is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (Helium) the sample flows through the column and the compound comprising the mixture of interest are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase). The mobile phase passes through a heated transfer line and ends at the entrance to the ion source where compounds eluting from the columns are converted to ions. When the resulting peak from this ion is seen in the mass spectrum, it gives the molecular weight of the compound.

GC separates the chemical mixture into pulses of pure chemicals while the MS identifies and quantifies the chemicals

WORKING PRINCIPLE OF GAS CHROMATOGRAPHY

A gas chromatogram is an analytical instrument that measures the content of various components in a sample. The analysis is called gas chromatography. The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column" (Helium or Nitrogen is used as the carrier gas). The various components are separated inside the column. The detector measures the quantity of the components that exit the column.

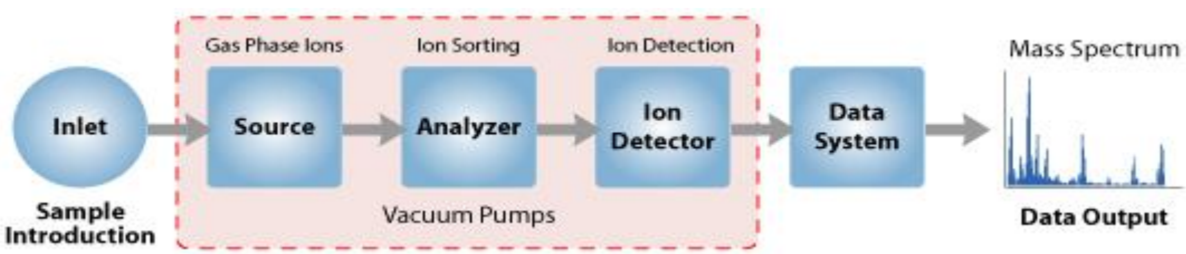
Fig 2.1: A SCHEMATIC DIAGRAM OF A GAS CHROMATOGRAM (Aadhithiya, 2015)



WORKING PRINCIPLE OF MASS SPECTROSCOPY

MS is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample and elucidate the structure and chemical properties of different molecule. A mass spectrometer generates multiple ions from the sample under investigation, it the separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type

Fig. 2.2: A SCHEMATIC DIAGRAM OF A MASS SPECTROMETER (Aadhithiya, 2015)



CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: SAMPLE COLLECTION

Fully ripened fresh fruits of sweet orange *(citrus sinensis)* were collected in July 2018 from the local market in Choba, Port Harcourt, Rivers State, Nigeria and were taken to the laboratory of the Department of Pure and Industrial Chemistry, University of Port Harcourt. The orange fruits were washed carefully with tap water to remove dust and foreign materials.

3.2: SAMPLE PREPARATION

The rinds of fifty oranges were collect, oven dried and grinded to give 1980g (1.98kg) sample.

3.3: MATERIALS

3.3.1 Chemical Apparatus

- Round bottom flask
- Beaker
- Water bath
- Spatula
- Separating funnel
- Plastic foil
- Tripod stand
- Whatmann filter paper
- Weighing balance
- Oven
- Sample holder/container
- Measuring cylinder
- Grater
- Glass container

3.3.2: CHEMICALS/REAGENTS

- Orange (*citrus sinensis*) rind
- Ethanol (absolute)
- Menthol
- Industrial camphor

- Isopropyl alcohol
- Distilled water
- Colouring agent
- Production ethanol

3.4: ESSENTIAL OIL EXTRACTION

To 1980g (1.98kg) sample in a 5liter glass container was added 1liter ethanol, sealed and kept in a cool, dark corner for 2weeks with daily stirring. The mixture was filtered with a filter paper and filtrate was then transferred into a separating funnel where the mixture separated with the oil on the upper layer and ethanol at the lower layer. Solvent in the upper layer was allowed to evaporate leaving behind jelly-like oil (100g, 5.05%) which was transferred to a glass vial with a rubber cap (see appendix I for calculation).

3.5: ESSENTIAL OIL ANALYSIS.

The oil was analysed for the various composition of its essential oil using Gas Chromatography-Mass Spectroscopy (see appendix II) at BGI Laboratory, Aba road, opposite Shell RA.

GC-MS condition for analysis of oil

Gas Chromatography analysis was accomplished with HP 6890 Powered by HP Chem Station Rev. A 09.01 [1206] Software and HP 5MS capillary columns (30m x 0.25mm x 0.25µm film thickness). The program temperature is 60 ^{O}C per 3min, 8 ^{O}C/min to 140 ^{O}C. Injector and detector temperature were maintained at 230 ^{O}C and 275 ^{O}C respectively; the carrier gas is hydrogen (1.0ml/min), detector dual, FID. Volume injected was 0.5µl. Identification of components was obtained by comparison of their retention time with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons (see appendix I).

Sensory analysis of the essential oil:

Sensory analysis was carried out on the oil to determine its physical properties. This involved the sense of sight, smell and touch. It had a citrus fragrance, had a pale yellowish colour and was liquid at room temperature.

Determination of solubility of the essential oil in water:

A few drops of oil was added to a test tube containing little amount of water. The test tube was stirred thoroughly with a stirring rod. Two separate phases were observed. The insolubility of the oil in water was inferred from that operation.

Determination of specific gravity of the oil:

A clean and dry bottle was weighed using a weighing balance. Distilled water was poured into the bottle and weighed. In the same manner, the same volume of oil was poured into the same bottle and weighed. The specific gravity was calculated as the ratio of weight of oil to that of water as given in equation (2)

$$\text{Oil specific gravity} = \frac{\text{Weight particular volume of oil extracted}}{\text{Weight of equal volume of water}}$$

3.6: PRODUCTION OF LIQUID AIR FRESHENER.

To 200ml water in a 500ml beaker was added 150ml ethanol, 3g industrial camphor and dissolved in the mixture. Menthol (3g) was also dissolved in the mixture with an addition of 50ml isopropyl alcohol with stirring. The extracted orange essential oil (100ml) was added as fragrance. The mixture was stirred continuously in order

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to obtain a homogeneous mixture. Orange color was added to make the mixture aesthetically appealing. The air freshener was poured into spray cans and ready for use.

CHAPTER FOUR

RESULTS AND DISCUSSION

RESULTS

Table 4.1: Constituents of *citrus sinensis* rind extracted oil.

S/n	Compound	Retention Time	Yield	Molecular Weight	Molecular Formula	Class Of compound
1	4-hydroxy-4-methyl-2-pentanone	4.96	0.67	116	$C_6H_{12}O_2$	Ketone
2	1,2,3-Cyclopentanetriol	5.89	1.57	118	$C_5H_{10}O_3$	Alcohol
3	1-methylethyl benzene	6.03	0.75	120	C_9H_{12}	Aromatic
4	D-Limonene	7.27	14.14	136	$C_{10}H_{16}$	Monoterpene
5	Trans-1-methyl-4-(1-methylethenyl)-2-cyclohexen-1-ol	8.22	0.53	154	$C_{10}H_{18}O$	Oxygenated monoterpene
6	2-methyl trans-3-methyl-2-n-propyl Quinazoline	8.49	1.06	175	$C_9H_9N_3O$	Aromatic heterocycle

7	Benzoic acid	8.79	0.46	112	$C_7H_6O_2$	Monocyclic terpene
8	2 methyl-2,3-hexadiene	8.85	0.77	96	C_7H_{12}	Branched alkene
9	(-)-cis-isopiperittenol	9.16	0.48	152	$C_{10}H_{16}$	Oxygenated monoterpene
10	2,3-dihydrobenzofuran	9.20	1.20	120	C_8H_8O	Heterocyclic compound
11	Bicyclo [4.1.0]heptane,3-cyclopropyl,7-carbomethoxy	9.25	1.29	194	$C_{12}H_{18}O_2$	Monocyclic terpene
12	4-hydroxy-3-methylacetophenone	10.20	2.04	150	$C_9H_{12}O_2$	Ketone

13	2,3,5,6-tetrafluoroanisole	13.30	0.54	180	$C_7H_4F_4O$	Methoxy benzene
14	Tetradecanoicacid	15.55	0.60	652	$C_{38}H_{68}O_8$	Ester
15	n-hexadecanoic acid	17.80	3.53	256	$C_{16}H_{32}O_2$	Ester
16	n-hexadecanoic acid	17.90	12.34	256	$C_{16}H_{32}O_2$	Ester
17	5,7-dimethoxy-2H-1-benzopyran-2-one	18.28	0.69	206	$C_{11}H_{10}0_4$	Ketone
18	9-octadecenoic acid	19.70	12.66	282	$C_{18}H_{34}O_2$	Ester
19	Octadecanoic acid	19.90	4.50	242	$C_{15}H_{30}O_2$	Ester
20	3-methyl-2-phenylimino-perhydro-1,3-oxazine	20.80	0.90	190	$C_{11}H_{14}N_2O$	Heterocyclic compound

21	2,2,2-trifluoroethyl ester	28.08	1.64	280	$C_8H_6F_6O_4$	Ester
22	Cholesta-5-en-3-ol, acetate	28.26	3.06	678	$C_{47}H_{82}O_2$	Polyterpene
23	3,5-dimethyl-2-pyrazoline-1-carboxamide	28.35	1.88	396	$C_{29}H_{48}$	Amide
24	2,7-dimethyl-1,3,7-octatriene	28.53	4.29	138	$C_{10}H_{16}$	Monoterpene
25	4,4-dimethyl-cholesta-6,22,24-triene	28.71	1.46	398	$C_{28}H_{46}O$	Aliphatic compound
26	Di(1-phenylpropyl)ester, adipic acid	28.85	6.91	236	$C_{13}H_{16}O_4$	Ester
27	Ergosta-5,7-dien-3-ol	29.03	13.30	398	$C_{28}H_{46}O$	Alcohol

28	5,6,7-trimethoxy-2-(4-methoxyphenyl) 4H-1-benzopyran-4-one	29.70	6.73	342	$C_{19}H_{18}O_6$	Ketone

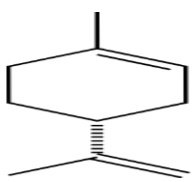
Table 4.2: Physical properties of oil from orange rind

PARAMETER	**VALUE**
Colour	Yellow to orange
Odour	Fresh to tangy smell
Solubility	Insoluble in water
Density(g/cm^3)	0.86
Specific Gravity	0.843

The GC/MS analysis result of orange rind extracted oil is shown in Table 4.1.while the physical properties of the oil from orange rind is presented in Table 4.2.

DISCUSSION

The volume of the extracted from 4.98kg orange rind is 30ml giving 0.6% extraction success rate. The GC/MS analysis of the orange rind oil (Table 4.1) gave twenty-eight (28) compounds corresponding to 99.99% of the total oil of the *citrus sinensis.* The twenty-eight (28) compounds are made up of Methoxy benzene (1), heterocyclic compound (1), polyterpene (1), Amide (1), Aliphatic compound (1), Ester (7), Amine (1), Ketones (4), Alcohol (2), Aromatic (2), Monoterpene (5) and Alkene (2) eleven (11) major constituents (2% yield and above) were observed together with eighteen (18) minor components (<2%yield) at different retention time.

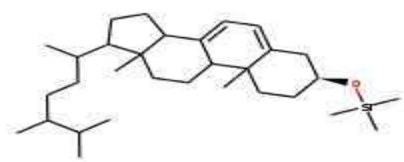


d-limonone

D-Limoene: It is a cyclic monoterpene and is the major component of the *citrus sinensis* oil. It has an IUPAC name 1-Methyl-4-(prop-1-en-2-yl) cyclohex-1-ene. It has the chemical formula $C_{10}H_{16}$ with molar mass 136.24g/mol. It constituted 14.14% of the total essential oil making it the most abundant compound in the oil (Table 4.1).This oil is used as: a dietary supplement, used in food manufacturing, a relieve gallstones, reflux disease and heartburn, flavouring to mask the bitter taste of alkaloids, a fragrance in perfumery, aftershave lotions, bath products and other personal care products, in organic herbicides, It is added to cleaning products for its ability to dissolve oils, Limonene has been considered as a biofuel as it is combustible, as a solvent for cleaning purposes such as the removal of oil from machine parts and for 3D printing (Hesham, 2016)

Ergosta-5,7-dien-3-ol: It is also called Brassicasterol is an alcohol. It is frequently used as a biomarker for the presence of marine algae matter in the environment (Hesham, 2016). It is a white solid with chemical formula $C_{28}H_{46}0$ with molar mass 398.68g/mol. It constitutes 13.30% of the total oil making it the second most abundant compound in the extracted oil (Table 4.1).

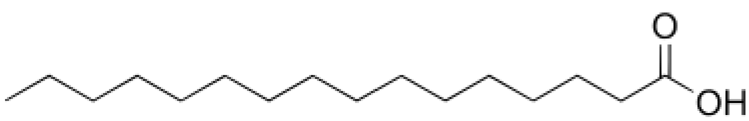
STRUCTURE:



Elaidic acid: Elaidic acid also called (E)-octadec-9-enoic acid. It has the chemical formula $C_{18}H_{34}O_2$ and a molar mass of 282.46g. It is a *trans* isomer of oleic acid. It is an unsaturated *trans* fatty acid which is colourless and oily. It is an organic compound which constitutes 12.66% of the total oil making it the third most abundant compound in *citrus sinensis* essential oil (Table 4.1). The structure is given below. It is used as: an emulsifying agent in soap production, it is used in pharmaceuticals, it is a component in food in the form of its triglyceride, it is used as an emollient and it is also used in a research for the treatment of underdeveloped lungs in new born (Ezejiofor*et al.*, 2011).

Palmitic Acid: It is called hexadecanoic acid in IUPAC nomenclature with chemical formula $C_{16}H_{32}O_2$ and molecular weight 256.43g/mol. It is a common saturated fatty acid found in animals, plants and microorganisms. It constitutes 12.34% of the total oil making it the fourth most abundant compound in the oil (Table 4.1). It is used as: a thickening agent for napalm used in military action, it is a permitted additive in organic products, and it is used in the production of soaps, cosmetics and as industrial mould release agent (Hesham, 2016).

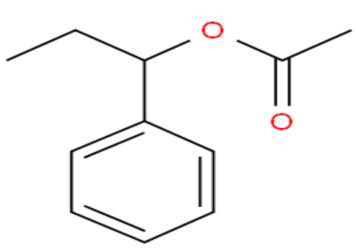
STRUCTURE:



DI (1-PHENYLPROPYL) ESTER, ADIPIC ACID

It has the chemical formula $C_{11}H_{14}O_2$ with the molar mass 178g/mol. It is an ester attached to a phenyl group. It is the most important carboxylic acid. It is present in essential oil in 6.91% as seen in (Table 4.1). Its usage includes: production of polymer, it is used as a plasticizer for PVC production; small amount is used in food ingredient as flavourant and gelling aid and it is used in nylon production (Hesham, 2016).

STRUCTURE:



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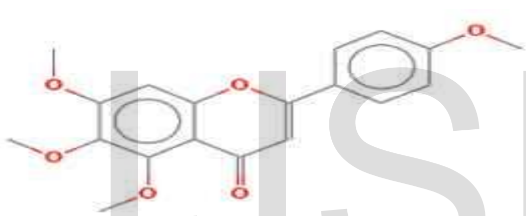
5,6,7-TRIMETHOXY-2-(4-METHOXYPHENYL)4H-1-BENZOPYRAN-4-ONE

It has the formula $C_{19}H_{18}O_6$ with molecular weight 342. It is also called flavones. It is a ketone. It is common in food supply mainly used in food spices. It is also used in metabolizing drugs in the body (Galadima, 2004). It constitutes 6.73% of the total essential oil as shown in (Table 4.1).

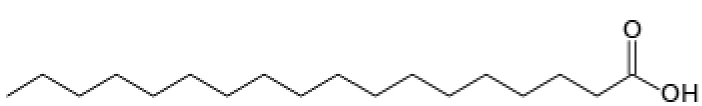
STRUCTURE:



OCTADECANOIC ACID

It is also called stearic acid. It is a saturated fatty acid. A waxy solid with the molecular formula $C_{17}H_{35}CO_2H$ It is one of the most common fatty acids found in nature. It constitutes 4.50% of the total essential oil from (Table 4.1). Its usage includes: the production of soaps, cosmetics, detergents, shaving cream products. It is also used as a lubricant for machine parts. It is used for preparing softeners in textile sizing and in candle making (Galadima, 2004)

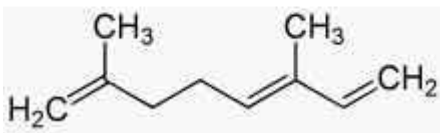
STRUCTURE:



2,7-DIMETHYL-1,3,7-OCTATRIENE

It has the formula $C_{10}H_{16}$ with the molecular weight 136g/mol. It is a monoterpene. It constitutes 4.29% of the total essential oil as shown in (Table 4.1). It forms aerosols that are proposed to serve as cloud condensation nuclei which can increase brightness of cloud and cool the climate; it is also used in flavours such as menthol (Ezejiofor*et al.*, 2011)

STRUCTURE:



HEXADECANOIC ACID

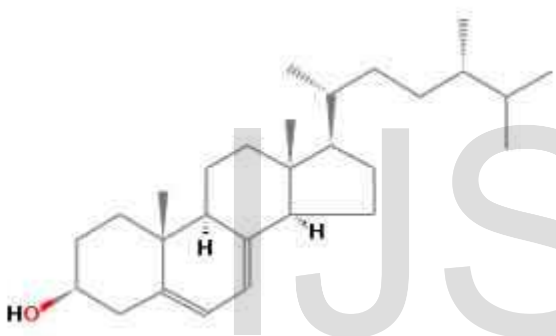
It is also called palmitic acid. It is the most common saturated fatty acid found in animal, plants and micro-organisms. It has the molecular formula 256g/mol. It constitutes 3.53% of the total essential oil as shown in (Table 4.1). It is used in the production of soaps and cosmetics; it is also used as an industrial mould release agent. The hydrogenation of palmitic acid yields cetyl alcohol used to produce detergents (Galadima, 2004)

STRUCTURE:

Cholest-5-en-3-ol, acetate

It has the chemical formula $C_{29}H_{48}O_2$ with molecular weight 428g/mol. It is also called cholesterol acetate. It is present in food. It is a major transport and storage form of cholesterol in lipoprotein particles and most cell types. It catalyzes fat and vitamins adsorption. It also helps in lipid transport and metabolism (Galadima, 2004). It constitutes 3.06% of the total essential oil as shown in (Table 4.1).

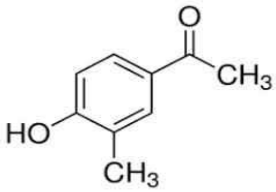
STRUCTURE:



4-HYDROXY-3-METHYLACETOPHENONE

It has the chemical formula with molecular weight 150g/mol. It is used as a precursor for the production of resin. It is an important ingredient in fragrance which is also used in chewing gums. It is also used for the synthesis of pharmaceuticals (Hesham, 2016). It constitutes 2.04% of the total oil as shown in (Table 4.1).

STRUCTURE:



The result of the sensory analysis (Table 4.2) showed that the extracted oil was dark orange in colour, had a sweet, fresh, tangy smell and was liquid in viscosity at room temperature. The physiochemical test showed that it was insoluble in water and had a specific gravity of 0.84 and a density of 0.86g/cm^3.

CONCLUSION

The results obtained from the extraction and characterization of the essential oil from orange rinds have shown that the maximum yield of essential oil obtained from the orange rind used in this work was 5.05% using cold pressing method. The physical properties obtained from the characterization of the oil revealed that it could be utilized in different process industries for the production of other valuable products for instances the liquid air freshener. It was also deduced from the value of the oil yield obtained given the quantity of orange rinds that large amount of the raw materials which was a waste would be required for large scale extraction of the oil thus making this process an advantage for the environment and waste management sector of the community.

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