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# BIO-INSECTICIDES/REPELLENT FROM CITRUS PEELS EXTRACT AND ITS EFFICACY ON MOSQUITOES

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**Abstract:** *The study seeks to produce bio-insecticides/repellent from citrus peels extract and determine its efficacy on mosquitoes. The specific objectives were to; extract the essential oils - limonene from citrus peels of three citrus species and convert the fruits waste to useful product. Two extraction methods were adopted for study the extraction of the essential oils of the citrus peels: cold method of extraction and steam distillation (Clevenger-type apparatus). It has been observed (in this study) that yield of the orange oil was higher in steam distillation (Clevenger-type apparatus) and also higher in other citrus species than that of the Cold method. Steam Distillation, produced orange oil of almost two times more than cold method and also showed that the orange oil yield was five times more than the oil extracted by Cold method. It was also inferred that more oil was extracted from C. sinensis to C. lemon and C. aurantifolia. The distillate was colourless and cloudy in nature. The study recommended that there is need to use biotechnology tools to come up with methods that will extract higher quantity/higher percentage yield of the citrus essential oil so as to make economical valuable.*

***Keywords:*** *Bio-Insecticides/Repellent, Citrus Peels Extract, Efficacy on Mosquitoes*

## INTRODUCTION

Citrus is a term commonly used for the genus of flowering plants in the family Rutaceae originating in tropical and sub-tropical regions of the world. The taxonomy and systematics of the genus is complex, and the precise number of natural species is unclear (Ellis *et al.,* 1985). The fruits are botanically classified as berries and could be termed as a hesperidium. Citrus fruits have rough, robust and bright (green to yellow) coloured skin. They are usually 4 to 30 cm long and 4 to 20 cm in diameter, with a leathery surrounding rind or skin known as epicarp (or flavedo) that covers the fruits and protects it from damages. Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids contained in the rind (Manthey, 2004). The endocarp is rich in soluble sugars and contains significant amounts of vitamin C, pectin, fibers, different organic acids and potassium salt which give the fruits its characteristics citrus flavor (Roger, 2002). Citrus juice also contains a high quantity of organic acids such as citric, malic, acetic and formic acids (Rogers, 2002). In many parts of the world, citrus fruits particularly those of the class *Citrus sinensis* (sweet orange), *citrus limon* (lemon) and lime (*C. aurantifolia*) have always remained part of human diet for many years. In recent times, however, they have assumed greater importance in diets of both urban and rural dwellers. The increased interest in their consumption is not only due to their sweet refreshing properties but also as a result of increased knowledge of their nutritional and medicinal values.

### 1.2 Statement of the Problem

Citrus fruits have been part of human diet for ages due to its nutritional and medicinal values. But consumption of these fruits including post-harvest waste of citrus fruits generates citrus peel wastes that could bring about environmental pollution if not properly handled. Processing of citrus peels into essential oils is a sure way of transforming these wastes with great potential for environmental pollution into a resource with great potential for economic prosperity, and also for securing

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the public health impacts of safer and healthier environment, likely to be obtained from the indirect waste management option so offered. This study therefore, aims to lessen the waste disposal of citrus peels and to propose an alternative insecticide/repellent that is less harmful than the synthetic chemical products, but conforms to the standards of the market.

### 1.3 Objectives of This Research

The broad objective of this research is to produce bio-insecticides/repellent from citrus peels extract and determine its efficacy on mosquitoes. The specific objectives were to;

*i.* extract the essential oils - limonene from citrus peels of three citrus species
ii. convert the fruits waste to useful product

## Literature Review

### 2.1 The Family Rutaceae

The Rutaceae is distributed in warm and tropical areas of both hemispheres, with its centres of speciation in South Africa and Australia. There are 150 genera and some 900 species. The most important genus commercially is *Citrus.*

### 2.1.1 The Genus *Citrus*

*Citrus* species are small, often spiny shrubs and trees of the tropics and subtropics native to Asia and Malaysia. A number are cultivated for their edible fruit: citron, orange, sweet orange, lemon, lime, pomelo, grapefruit, mandarin orange and tangerine.

*Citrus* is one of the most important fruit crops known by humans since antiquity and is a good source of vitamin "C" with high antioxidant potential (Gorinstein *et al*., 2001). Citrus originated from south-eastern Asia, China and the east of Indian Archipelago from at least 2000 BC. The fruit has been introduced to the new world *via* the great trade routes of Africa to the eastern Mediterranean basin by the Arab traders while the crusaders brought the fruit to Italy, Spain and Portugal around 1000 AD (Scora, 1975). The fruit was introduced further to the western hemisphere by Columbus on his second voyage in 1493 (Samson, 1980) and the planting material to the Cape in South Africa by a Dutch merchant in 1654 (Oberholzer, 1969). Currently, citrus is cultivated in the subtropical and tropical regions of the world between $40^0$ north and south latitude in over 137 countries on six continents and generates about 105 billion US dollar per year in the world fruit market (Ismail and Zhang, 2004).

### 2.2.2 Importance of Essential Oil (Citrus Oil)

An essential oil is a concentrated 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 the "oil of" the plant material from which they were extracted, such as oil of clove. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them is fairly stable and contains natural antioxidants, repellants and natural antimicrobial agent as on citrus fruits (Somesh *et al*., 2015). Essential oils are usually colorless, particularly when fresh. Nevertheless, with time essential oil may oxidize resulting the color from becoming darker. Therefore, essential oil needs to be stored in a cool, dry place tightly knotted at the end and preferably fill in an amber glass container.

Essential oil is used in perfumery, aromatherapy, cosmetics, incense, medicine, household cleaning products and for flavoring food and drink. They are valuable commodities in the fragrance and food industries. A number of few countries produce different kinds of essential oils. India ranks second in the world trade of essential oils (Rao *et al.*, 2005). Essential oils are derived from various sections of plants. An essential oil is usually separated from the aqueous phase by a physical method that does not lead to significant change in its chemical composition. These essential oils could be subjected to an appropriate further treatment after extraction. Essential oils are oily aromatic liquids extracted from aromatic plant materials. They could be biosynthesized in different plant organs as secondary metabolites (El Asbhanni *et al*., 2015).

Citrus oil which is one of the essential pure oil, produced by gland found in the rinds of species of the fruit is well-known for its powerful antiseptic and solvent properties. Citrus oil is considered to be relatively safe, very effective and environmentally friendly. The liquid which comes in the packet of orange flavored soft drink concentrates is sometimes this oil. The main components of this oil are Alpha Pinene, Citronelial, Geranial, Sabinene, myrcene, Limonene, Linalool and Neral. Essential oil of orange has a wide variety of domestic, industrial and medicinal applications. Domestically, it is used to add orange flavor to beverages, desserts and sweetmeats. Industrially, it is used in soaps, body lotions, creams, anti-mark and wrinkle lifting applications, concentrates for soft beverages, room fresheners, sprays, deodorants, biscuits, chocolates, confectionary and bakery items and many such.

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Citrus oil is an excellent addictive to synthetic furniture and wood care products. It is gentle yet it powerfully protects wood from insect damage and easily cleans fingerprints and grime off of the furniture without damaging it. For penetration beneath the wood surface and as protective against insect damage, it is mixed with linseed oil. Nothing is more gentle or protective for wooden furniture and instruments than these 3 essential oils which is orange, Swiss-pine and cypress mixed injojoba oil.

### 2.3.1 Extraction of the Essential Oils from Citrus Peels

Essential oils are used in a wide variety of consumer goods such as detergents, 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 and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential oils are obtained from plant raw material by several extraction methods (Dick and Starmans, 1996).

Citrus juice is one of the most widely-consumed beverages today and as such, approximately50-60% of the processed fruits is transformed into citrus peel, which is composed of peels, seeds and membrane residues. With the increase in production of processed fruit wastes generated is increasing enormously. Large amounts of these wastes pose the problem of disposal without causing environmental pollution. These wastes can be effectively disposed by manufacturing useful by products from them.

Citrus peels oil has been chosen for extraction because it provides great potential for further commercial form. Peels of citrus fruit has numerous glands that contain oil that is typically recovered as major by product. Each citrus fruit has its own unique characteristic set of compounds that comprise the oil which are responsible for its flavor and aroma to products such as carbonated drinks, ice-creams, cakes, air-fresheners and perfumes (Braun & Cohen 2007).

Recently, developed extraction methods like supercritical fluid extraction, microwave assisted extraction and Soxhlet method has been used for oil extraction. The basic parameters influencing the quality of an extract are the solvent used for extraction, the manufacturing process (extraction technology) used with the type of equipments employed. The use of appropriate extraction technology, plant material, manufacturing equipment, extraction method and solvent with adherence to good manufacturing practices certainly helps to produce a good quality extract.

Nowadays, many novel techniques for the extraction of essential oils that could lead to more compact, safer, more efficient, energy saving, and sustainable extraction processes, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) have become relatively mature and widely accepted by industries (Chen, 2011).

**Classical and Conventional Methods**

There are several methods of extraction of essential oils. The old technologies about essential oils processing are of abundant significance and are still in use in some parts of the globe. Hydro-distillation (HD), Steam distillation (SD), Solvent extraction, Enfleurage, Cohobation, and Maceration are the roughly traditional and generally used methods.

**Hydro-Distillation (HD)**

Hydro-distillation is a traditional method for extraction of essential oils. Water or hydro-distillation is one of the oldest and easiest methods (Meyer and Warnod, 1984). Hydro-distillation normally used to isolate essential oils from the aromatic and medicinal plant. Conventional method of extracting essential oils by hydro-distillation (HD), involves the evaporation of essential oils by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser.

**Steam Distillation:**

Steam distillation is a type of extraction technique (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation is still important in certain industrial sectors (Fahlbusch *et al*., 2003). This method of extraction of essential oil is one of ancient and official approved methods for isolation of essential oils from plant materials.

**Solvent Extraction:**

Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a method used to separate a compound based on the solubility of its parts. In the Solvent-Extraction method of Essential Oils recovery, the extracting unit is loaded with

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perforated trays of essential oil plant material and repeatedly washed with the solvent. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel.

**Soxhlet Extraction:**

A Soxhlet extractor is a piece of laboratory apparatus (Harwood, Laurence M. and Moody, Christopher J, 1989) invented in 1879 by Franz von Soxhlet (Soxhlet, 1879). Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase.

**Cold Pressing Method:**

The term cold pressed theoretically means that the oil expeller is pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method. This method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils (Arnould and Tylor, 1981).

**Other Innovative Techniques Of Essential Oils Extraction includes; (Non-Traditional):**

Supercritical Fluid Extraction (SFE)
Microwave-Assisted Hydro-distillation (MAHD)
Ultrasound-assisted extraction (UAE)
Solvent-free microwave extraction (SFME)
Microwave hydro diffusion and gravity (MHG) etc.

## 3.0 Materials and Methods

### 3.1 Materials

Steam distillation apparatus (Clevenger-type), citrus peels (orange, lemon and lime), Anopheles mosquitoes (adult and larvae) round-bottom flask, weighing balance, pasture pipette, petri dish, graduated cylinder, spray bottle, plastic container etc.

### 3.2 Sample Collection

Three types of fresh citrus peels: Orange *(Citrus Sinensis*), Lemon *(Citrus Limon)*, and Lime (*Citrus Aurantifolia*) used for this study were acquired from different vendors in New market, Ogbete and Oye Emene, in Enugu State, Nigeria. After the peels were obtained, they were air dried for 5 days. The dried peels were then pulverized (those used for steam distillation only) before being used.

### 3.3 Essential Oil Extraction Methods

#### 3.3.1 Conventional Method: Cold Extract Method

The peels were gathered without drying, cut into tiny pieces and blended with distilled water to obtain a homogenous mixture of the peels. The homogenized mixture were transferred into a separate clean container and left in the refrigerator for 5 hours so as to let the essential oil to separate on the surface of the water (due to immiscibility of the oil and water at room temperature for essential oil is lighter than water). This was done for all the different species of citrus used in this study respectively.

#### 3.3.2 Steam Distillation Procedure

Approximately 200g of the prepared peels (orange, lemon, and lime) were introduced into steam distillation using a Clevenger-type apparatus, and the oil was extracted until there was no significant increase in the volume of the oil collected. Steam distillation flask was connected to a round bottom flask containing water. The flask was connected to a condensing unit with its tubing. The set-up of the distillation unit has a heating mantle.

The essential oil was extracted with the distillation set-up using steam as it was percolating through the peels. The recovered mixture of oil and water were allowed to settle and the oil was decanted. After the steam distillation process, the product, which was a mixture of water and oil, was collected and separated using separating funnel. The essential oil settled on the top layer and the water was in the bottom layer of the funnel.

The mixture was separated until negligible amount of water was left with the oil.

#### 3.3.3 Separation of Essential Oil from Water

In the separation funnel, the essential oil floated on top of the hydrosol (distilled water component) also called floral water was decanted off.

Water layer was carefully run out from the bottom of the funnel by opening the tap leaving the oil. (the water left together with the essential oil, was removed using pasture pipette). The

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essential oil was stored in a glass/container at room temperature for further analysis.

### 3.4 Characterization of the Essential Oil

After the essential oil was extracted, it was characterized in order to be sure that the extracted liquid was actually the oil. Outlined below are the tests carried out on the oil.

#### 3.4.1 Sensory/Physical Analysis of the Essential Oil

Sensory analysis was carried out on the oil to determine its physical properties. This involved sense of sight, taste, smell and touch (the sense of sight was done to check impurities, ie if the oil is pure by eyes, taste with the tongue, smell was done by inhaling the oil in a container and touch by physical contact with the hand).

#### 3.4.2 Determination of Solubility of the Essential Oil in Water

Few drops of the oil were 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.

#### 3.4.3 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 (1) (Saad, 2015).

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

#### 3.4.4. Iodine Test

This test was performed to check the presence of unsaturated bonds in a molecule. A few drops of the citrus oil were taken in a test tube. A few crystals of iodine were added and shaken well.

#### 3.4.5 pH Test

The pH was measured using pH meter. Approximately 5 mls of the citrus oil were taken for pH analysis

#### 3.4.6 UV-Spectrophotomter

Approximately 3 ml of the extracted essential oil were added in a cuvette and placed in the spectrophotometer for analysis and to determine the absorbance and wavelength. The absorbance and wavelength of the three citrus essential oils were determined as the spectrophotometer scans the extracted oil.

### 4.0 Results

### 4.1 Conventional Method: Cold Extraction

#### 4.1.1 Variation of Volumes of Essential Oil Extracted From Citrus Peel: (Cold Extraction)

The yeild of extracted essential oil from the three different citrus fruit peels using conventional/ cold method is 6 ml for orange, 4 ml for lemon and 1 ml for lime. The three different citrus peels were exposed/subjected to the same temperature, time, and volume of water and weight of peel. The result obtained is as shown in Table 3

### 4.2 Steam Distillation

Essential oil which was extracted from the three different types of citrus peels had three different oil yields of 20 ml for orange, 15 ml for lemon and 11ml for lime. And the process exposed to the same temperature, time volume of water and weight of peel. The result is as shown in Table 4

### 4.3 Sensory/ Physical Analysis of the Essential Oil

The extracted essential oil was analyzed to determine its physical and some chemical properties. Given in Tables 5 is the results obtained from the analyses for the physical and some chemical properties of the extracted essential oil respectively. The table shows the data representation of the colour, taste, density etc. of the extract respectively.

**Table 3. Variation of Volumes of Essential Oil Extracted from Citrus Peel using Cold Extract/Conventional method**

| Citrus Peel Used | Weight Of Peel (Kg) | Volume Of Water (ml) | Time Of Refrigerating (Hour) | Temperature ($^0$C) | Volume Of Oil Extracted (ml) |
|---|---|---|---|---|---|
| Orange | 1 | 400 | 5 | 4 | 6 |
| Lemon | 1 | 400 | 5 | 4 | 4 |
| Lime | 1 | 400 | 5 | 4 | 1 |

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**Table 4. Variation of Volumes of Essential Oil Extracted from Citrus Peel using Steam Distillation method**

| Citrus Peel Used | Temperature ($^0$ C) | Weight Of Peel (gm) | Volume Of Water (ml) | Time Of Heating (Mins) | Volume Of Oil Extracted (ml) |
|---|---|---|---|---|---|
| Orange | 100 | 200 | 350 | 50 | 12 |
| | 120 | 200 | 350 | 60 | 8 |
| Lemon | 100 | 200 | 350 | 50 | 10 |
| | 120 | 200 | 350 | 60 | 5 |
| Lime | 100 | 200 | 350 | 50 | 7 |
| | 120 | 200 | 350 | 60 | 4 |

**Table 5. Physical Properties of Essential Oil Extracted from Orange, Lemon and Lime Peels.**

| Citrus oil | Colour | Odour | Taste | Water Solubility | Specific Gravity |
|---|---|---|---|---|---|
| Orange | Yellow to orange | Fresh to tangy smell | Bitter | Insoluble in water | 0.843 |
| Lemon | Translucent yellow. | Fresh Pungent smell of citrus | Bitter | Insoluble in water | 0.863 |
| Lime | Translucent yellow-green. | Tart, sour citrus | Bitter | Insoluble in water | 0.854 |

### 4.4 Characterization/Chemical Properties of Citrus Oil

The citrus essential oil so obtained from the peels of different citrus species was analyzed for the presence of limonene as it is the major component of citrus oil**.** About 97% of citrus oil is limonene. Hence, this compound was chosen to characterize the orange oil. For analyses, the citrus oil extracted was subjected to iodine test, pH test and UV-spectrophotometer.

#### 4.4.1 Iodine Test

Iodine test was the characterization (chemical) test for the presence of any unsaturated compound in a test sample. On addition of iodine, the colorless orange oil sample extracted turned into brown color. The presence of limonene and other aromatic compounds in the sample was confirmed.

The brown color is due to the reaction of iodine with limonene and other unsaturated compounds like α-pinene, β-pinene, myrcene, limonene and linalool.

#### 4.4.2 pH Test

The oil samples after distillation of the pre-heated peels were collected and subjected for pH analysis using a pH meter. The pH value of *C. sinensis, C. limon, C. aurantifolia and* was found to be 6.67, 4.98 and 4.67 respectively, which are very much acidic in nature

#### 4.4.3 UV-Spectrophotometer

The wavelength and absorbance of the citrus essential oils were 346, 1.6062., 346, 1.0883 for orange and lemon respectively as shown in figures 7and 8 below.

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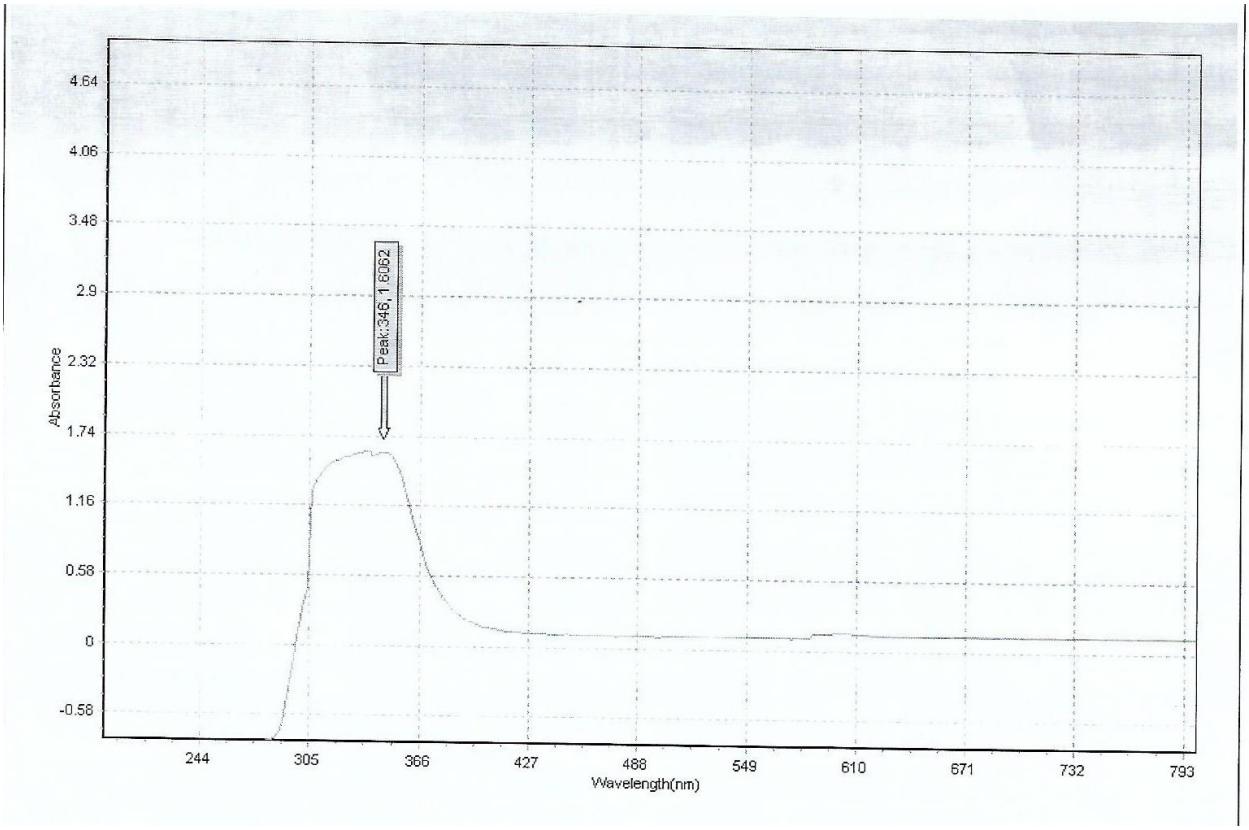


**Fig 6**. Absorbance and wavelength scan of orange essential oil

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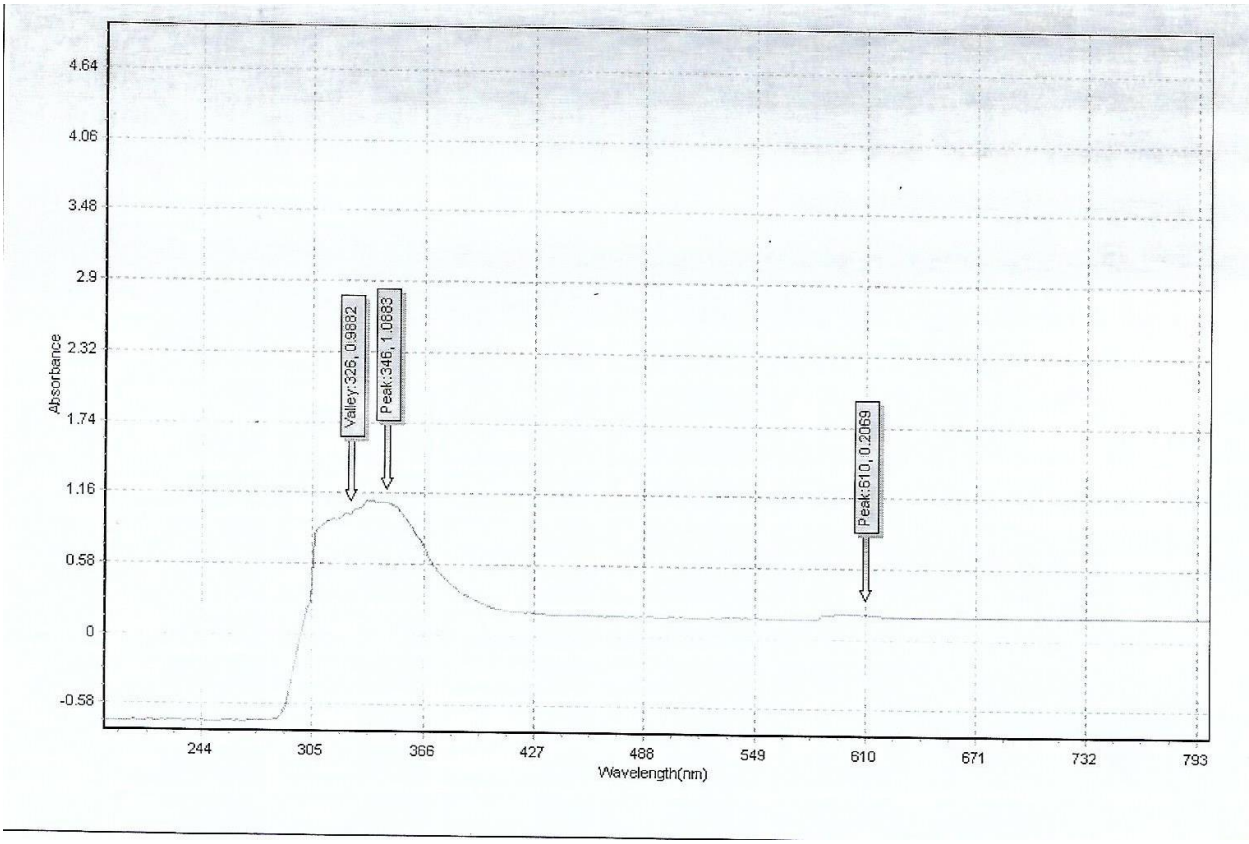


**Fig 7**. Absorbance and wavelength scan of lemon essential oil

**DISCUSSION**

In this study, two extraction methods were adopted for the extraction of the essential oils of the citrus peels: cold method of extraction and steam distillation (Clevenger-type apparatus). The cold extraction method did not yield much oil (6, 4 and 1ml for orange, lemon and lime respectively) as it contains some impurities. Though the distillation procedure allowed only the separation of volatile compounds (essential oils), which, to a greater or lesser extent, are transformed under the influence of the elevated temperature, the absence of light and air during extraction reduced the risk of degradation reactions (Zoran P. Zeković *et al.*, 2009). It has been observed (in this study) that yield of the orange oil was higher in steam distillation (Clevenger-type apparatus) and also higher in other citrus species than that of the Cold method. Steam Distillation, produced orange oil of almost two times more than cold method and also showed that the orange oil yield was five times more than the oil extracted by Cold method. It was also inferred that more oil was extracted from *C. sinensis* to *C. limon and C. aurantifolia*. The distillate was colourless and cloudy in nature. It was soft and had a very pleasant, aromatic and lemon taste. Essential oils are mixtures of organic compounds that are dominated by the terpenes and the terpenoids which are the oxygen containing derivatives of the terpenes (Villen *et al.*, 1998). The oil turned to slight orange colour after few hours of extraction.

Steam distillation method can be one of the promising techniques for the extraction of essential Oils from plants as this process will preserve the original qualities of the plant. Sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point can be prevented by using steam distillation method at low

pressure. Sahoo *et al.*, (2010) observed the increase in volume of essential oil with increase in temperature by keeping time of heating constant. They also found that the volume of essential oil increased with increase in the time of heating (temperature being constant).

Thus oil was extracted using both conventional (cold method) and steam distillation (Clevenger-type apparatus). It was found that steam distillation produced more oil than the conventional method. Limonene was qualitatively characterized. This can be considered as a green method of extraction as it is less energy intensive process. The distillate containing water and essential oil can be used as such for further applications and this method can also be used for the extraction of essential oils from other fruits and flowers. This study can be further optimized for the temperature and time.

**Conclusion and Recommendation**

According to the results obtained from this study, the following conclusions are obtained:

1- The two extraction methods adopted in this study shows that steam distillation yields more oil than conventional and the extracts of conventional methods contains some impurities. So it would be better to use steam distillation for more yield and purity.

2- It was noticed that the volume of essential oil/limonene in orange is higher than that of lemon and lime. Therefore, orange contains more limonene than other citrus peels used in this study.

**Recommendation**

1. There is need to use biotechnology tools to come up with methods that will extract higher quantity/higher percentage yield of the citrus essential oil so as to make economical valuable
2. Further studies should be done on the antimicrobial activities of citrus peels essential oil for production of antibiotics against specific pathogens.

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