EXTRACTION AND CHARACTERIZATION OF CITRUS PEEL OIL FOR SUSTAINABLE MOSQUITO REPELLANT APPLICATIONS

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ABSTRACT

Poor disposal of organic waste poses significant risks to human health and the environment. In line with sustainable laboratory practices, this study explores the extraction of limonene from orange peels for use as an eco-friendly mosquito repellant. Orange (*Citrus sinesis*) peels were locally sourced and processed through steam distillation, with trials conducted on both fresh and one-day-old samples. The extracted oils were characterized by determining the Brix value and refractive index using a handheld refractometer, in which a few drops of the sample were placed on the prism surface for direct readings under ambient conditions. Functional groups were identified through Fourier Transform Infrared Spectroscopy (FTIR), scanning the oils over the 4000–400 cm^{-1} range to match absorption peaks with standard reference data. Results showed that the FTIR spectrum fell within the 3200–3500 cm^{-1} range, indicating alcohol functional groups consistent with limonene's volatility. The refractive index (1.39) and Brix value (38.5%) closely matched standard limonene parameters. These findings demonstrate that citrus waste can be effectively repurposed into valuable bio-products through efficient laboratory processes, thereby reducing environmental waste. Adoption of such innovations within science laboratories can enhance productivity, encourage resource-efficient practices, and align with policy frameworks promoting sustainability in the profession.

INTRODUCTION

Citrus fruits of the family Rutaceae are among the most common fruits in the world. They include oranges, lemons, limes, grapefruits, and pomelos that are extensively grown in parts of Asia, Southeast Asia, East Asia, Australia, and other tropical or subtropical regions of the world (Langgut, 2017). Apart from their high nutritional value due to their vitamin C content, citrus fruits are known to contain bioactive compounds like flavonoids, limonoids, organic acids, pectin, and terpenes in their peels and essential oils. These compounds have diverse biological properties and wide-ranging industrial uses (Dong *et al*., 2012).

Terpenes, particularly monoterpenes, represent a major component of citrus essential oils. Terpenes refer to naturally occurring organic compounds that are usually synthesized from multiple units of isoprene $(C5H8)n$ (Davies *et* al., 2000). They play an essential role in the aroma and biological properties of plants. Being volatile and having a strong smell, monoterpenes, consisting of two isoprene units, represent one of the most important groups of terpenes. Due to the unique properties of these substances, monoterpenes have diverse applications in the pharmaceutical, cosmetic, agricultural, and food industries. Namely, they exhibit antibacterial, antifungal, antioxidant, anti-inflammatory, insecticidal, antiviral, and antiparasitic effects (Saeidnia and Gohari, 2012).

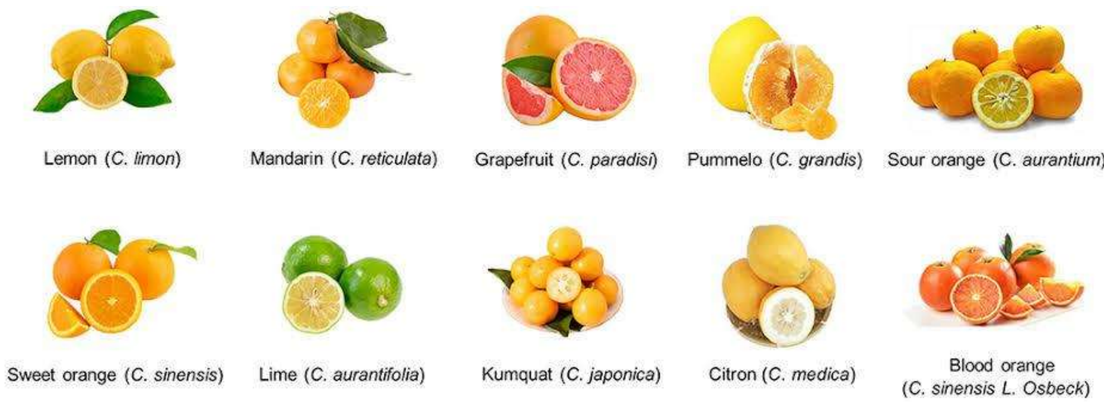

Figure 1.0: Pictorial Representation of the Rutaceae Family (Source: Liu *et al*., 2022).

One of the monoterpenes abundantly present in citrus peel oil is limonene. It is a cyclic monoterpene widely distributed in various kinds of oranges and other citrus fruits. Limonene usually occurs in two isomers – D-limonene and L-limonene. The first form, representing the main compound in orange peels, gives the aroma typical for citrus fruits (Nikfar and Behboudi, 2014). Limonene received significant interest in scientific research due to its wide range of applications. The substance is widely used as a food flavoring agent, perfume, detergent, drug, and insect repellent (Mursiti *et* al., 2019). It has also shown antimicrobial, anti-inflammatory, antioxidant, and anticarcinogenic properties (Zhang *et al*., 2014). Limonene represents a promising replacement for synthetic insecticides due to its ability to repel insects without posing a risk of serious health problems in people and the environment (Berliocchi *et al*., 2011).

However, despite its numerous applications and economic value, vast amounts of citrus peel waste generated by households, markets, juice industries, and food production facilities tend to be disposed of randomly. In fact, improper fruit waste management is one of the key causes of pollution in developing countries where waste disposal is inefficient (Hawkins *et al*., 1954). Poor waste disposal practices are common in Nigeria, resulting in increasing environmental issues related to municipal and organic waste generation. However, it should be noted that the peels of citrus fruits are characterized by a rich phytochemical composition and can be extracted to obtain valuable compounds, including essential oils. This idea is based on the principle of waste valorization or waste-to-wealth transformation to make agricultural waste useful (European Union, 2008).

There are many articles devoted to the chemistry, applications, and biological properties of limonene (Keiloff *et al*., 1996). Still, there is a necessity for further research on the extraction of limonene-rich oil from readily available orange peels. In particular, there is a lack of information on the analysis and characterization of orange peel oil obtained from orange peel waste. In addition, no significant effort was put into investigating functional groups contained in limonene-rich oil via refractive index determination, Brix analysis, and Fourier Transform Infrared Spectroscopy (FTIR).

This research will focus on the extraction and characterization of limonene-rich essential oil from orange peel waste. The main objectives will be to:

1. extract essential oil from orange peel waste through steam distillation.
2. analyze the refractive index and Brix value of the extracted oil; and
3. detect functional groups in the extracted oil using Fourier Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

The primary material used was orange (*Citrus sinesis*) waste and it is strongly dependent on the availability of this particular waste. In this case, there is an abundance of orange waste all around the country.

The orange waste (solid waste) used in this study was collected from different markets in Benin City, Edo State metropolis. The markets are Uselu and New Benin and from some local fruit vendors at Ugbowo.

Figure 2.1: Fresh Sweet Orange (*Citrus Sinensis*) Samples Collected From Markets And Local Fruit Vendors In Benin City, Edo State, Nigeria

Steam Distillation

Four hundred grams of orange zest was placed into a round-bottom flask. Then water was added to the flask underneath; about two-thirds of the flask was filled with water.

The process used for the extraction of limonene from the peels was steam distillation. This process was only possible because limonene is immiscible with water and as such this separation process was very effective. The heating mantle was placed and plugged in but not turned on. The flask containing water was placed on the heating mantle and then attached to the round-bottom flask containing the orange peels as seen in Figure 2.1. They were both connected with a clamp on the retort stand. The connecting adapter was then attached to the round-bottom flask by one end. On the other end, the condenser was connected to it and a separating funnel/flask. Once the boiling started, the water vaporized and was converted into steam. The steam passed into the round-bottom flask, then into the connecting adapter with the water and the essential oil, limonene.

As the steam (water and limonene) entered into the condenser, it liquefied because of the drop in temperature. The limonene and water were collected in the separating funnel/flask. The partition of the oil and the water was visibly seen. The two layers of immiscible liquid were observed, with limonene on top and water below because it has a lower density, hence it floats. The water was collected and placed in a beaker, while the limonene was also collected and placed in another beaker (Lisa, 2020).

Brix Value

Brix value is used to determine and measure the amount of dissolved solids in a liquid solution. In this case the amount of dissolved sugar in a hundred grams of solution. The higher a brix value, the sweeter the solution (Swapna *et* al., 2022). The brix value was collected by using a refractometer, as seen in Figure 2.3.1. A few droplets of the essential oil were placed on the sensitive part (silver coating at the top) of the device and the readings were obtained.

Figure 2.2.1: Handheld Digital Refractometer Used To Determine The Brix Value And Refractive Index Of The Extracted Citrus Peel Oil

Refraction

Refraction is simply the ability of a light ray travelling on a plane to bend downwards from the incident. Refractive index on the other hand is the value obtained from the ratio of speed of light in a vacuum to the density of the material (Feynman *et al*., 2011). The refractive index was obtained using a device called the refractometer as seen in Figure 2.3.1. The sample of the essential oil was placed on the device and the readings were obtained.

Generally, it is used to find raw and unknown facts especially the functional group in a compound. It converts this data into an infrared spectrum which is read and the active functional group(s) is determined in a given solid, liquid or gas (Griffiths and Haseth, 2007).

FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is employed to analyze the functional groups of the essential oil sample. FTIR spectrometry uses the principle of introducing the sample into infrared radiation and examining how the absorption of infrared light occurs at different wavenumbers. This absorption is displayed by peaks on the infrared spectrum that can help deduce functional groups such as hydroxyl group, carbonyl group, alkene group, alkane group, ester group, ether group, and aromatic groups.

For this FTIR, a small amount of the essential oil sample was placed onto the sample holder or the ATR crystal of the FTIR spectrophotometer. The spectrum was then measured within the mid-infrared range, typically ranging from 4000 to 400 cm^{-1}. The peaks obtained from the test were compared with the infrared absorption values for determining the functional groups that may have been present in the essential oil. Thus, the FTIR experiment was conducted to determine the functional groups in the oil sample.

Figure 2.2.2: Fourier Transform Infrared (FTIR) Spectrophotometer Used For Functional-Group Analysis Of The Extracted Citrus Peel Oil

RESULTS

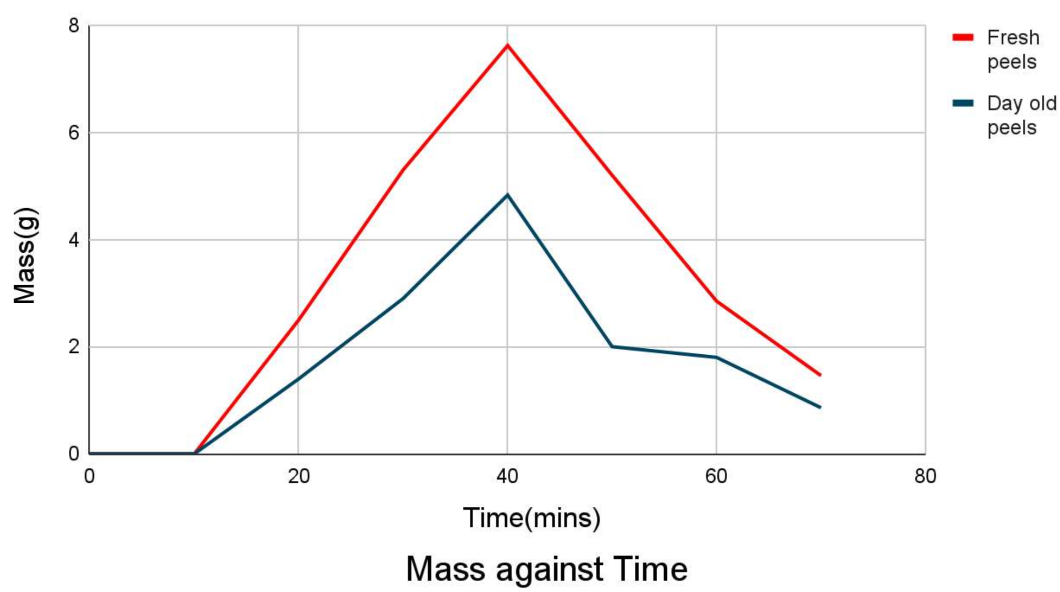

Figure 3.1: A plot of mass against time for the yield of the essential oils

The yield of the essential oil was presented in Figure 3.1. The result obtained from fresh peels was compared to the one obtained from day-old peels.

The brix value was obtained as 38.5%. The refractive index was obtained as 1.39690.

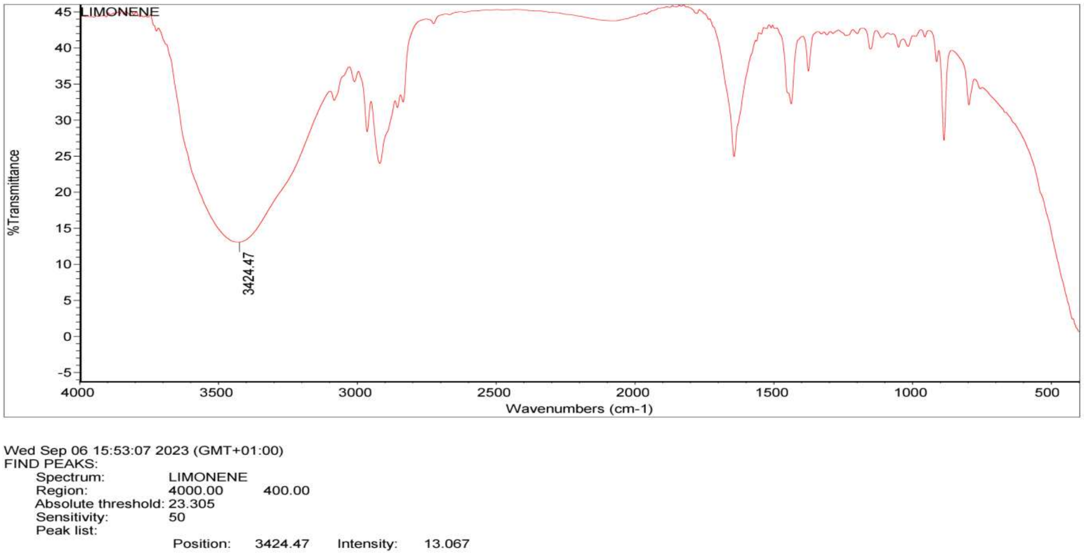

Figure 3.2: FTIR Spectrum Of The Extracted Citrus Peel Oil Showing The Characteristic Absorption Peaks Used For Functional-Group Identification

Table 3.1: FTIR Absorption Bands And Corresponding Functional-Group Assignments For The Extracted Citrus Peel Oil

Absorption (cm^{-1})	**Ranges (cm^{-1})**	**Compound Class**	**Functional Group**	**Appearance**
3424.47	3200–3500	Alcohol	O-H stretching	Strong, Broad

It was observed that fresher orange peels produced a better yield of oil in the experiment. As indicated by Figure 3.1 below, there was a notable difference in how well the freshness of the orange peels affected the quantity of limonene obtained.

The Brix value of the orange oil extract obtained indicates that the soluble solids content of the oil was moderate. This means that the sample was not particularly extremely sweet, however, there was a fairly high level of dissolved solids in it.

The refractive index value of the extracted oil was 1.39. It is clear that the refractive index value obtained indicates that light moved relatively slowly in the sample. It also shows that the optical property of the extracted sample was similar to the optical property expected of limonene or a limonene essential oil.

The FTIR result indicated the presence of organic substances. As shown in Figure 3.2 above, there were some peaks. These peaks were explained as shown in Table 3.1.

DISCUSSION

From the result of the extraction test, it can be seen that orange peels can be used as a natural resource for limonene extraction. Since there is a better yield from fresher peels, this means that the fresher orange peel samples contain a relatively higher quantity of volatile oil than the dried orange peels. Therefore, if limonene was to be extracted commercially, there will be need for many orange peels to get a considerable quantity of limonene.

From the Brix value test, the extract contains a fairly high quantity of dissolved solids. Although this is important for the oil, this is irrelevant when determining the quality of pure limonene since it is an essential oil and not made up of sugars.

From the result of the refractive index test, there is a good indication that the extracted sample was an essential oil similar to limonene. In addition, refractive index test can be used as a fast and effective way of testing the identity and purity of essential oils. With the result obtained, which is very similar to the refractive index of limonene, the extracted oil can be considered similar to limonene.

From the FTIR result, it can be seen that the extracted sample was similar to limonene and other organic compounds in orange essential oil. However, D-Limonene cannot be said to be a strong alcohol compound. Limonene is a terpene compound and not alcohol. In addition, the volatility of limonene is determined by its molecular structure.

CONCLUSION

From the results of this study, it was found out that orange peel waste serves as a viable natural source of limonene-rich essential oil. It was found that fresher peels yield higher oil, which implies that there is a relationship between the state of the peel and the amount of oil obtained. As a result, there could be the utilization of fresh orange peels before being disposed of as waste.

In terms of its properties, the result of the Brix value analysis shows that the extract contains a reasonable amount of dissolved solids, whereas the refractive index value close to 1.39 indicates that the oil has optical properties near that of limonene or limonene rich essential oil. Furthermore, according to the FTIR test, the sample contains functional groups found in organic compounds typical of the essential oil of citrus fruit. In effect, it is possible that the extracted oil is made up of limonene or related compounds.

In conclusion, orange peel waste serves as a valuable material both environmentally and economically. Orange peel waste can provide the natural essential oil, which is beneficial not only because of reducing organic waste but also due to its usefulness in various industries, such as insect repellents, cosmetic products, cleaning materials, and other relevant areas. However, additional tests, such as GC-MS, are necessary to validate the exact makeup of the extracted oil.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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