Comparative Study on The Analysis and Utilization of Citrus Peels Essential Oil and Pectin

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Abstract- The work aimed to analyse and utilised citrus peels essential oil and pectin from three citrus fruits peels; Orange (Citrus sinensis L.Osbeck), Lemon (Citrus limon L.Osbeck) and Lime (Citrus aurantifolia L.Osbeck).Then, a comparative study of each of the peels are carried out to ascertain the best raw material over what has been existent. These peels are treated using steam distillation for extraction the oil and the pectin is extracted by acid extraction method from the remaining peels. The physiochemical properties of the essential oil and pectin derived from the peels here determined. The properties of the oil ranged from 0.84 to 0.88 for specific gravity, 1.38 to 1.57 for refractive index @ 25℃, 122.74 to 158.42mgKOH/g for saponification, 6.96 to 12.93 mg/g for acid value and 41.05 to 84.42 mEq O_2/kg for peroxide value. The extracted oils were insoluble in water. Time determined the affect amount of oil extracted at a temperature of 100℃, this also presented that orange contains the highest amount of oil in the peels compared to Lemon and Lime. The qualitative and quantitative tests were carried out on the pectin produced from the three samples. The colour ranged from light brown to dark brown, moisture content from 3.2 to 4.4%, pH from 5.2 to 6.1 and ash content from 1.4 to 2.9%. The dry pectin derived from the three samples were insoluble in cold water and soluble in water boiled at 85℃ for 15 minutes. The result of essential oil derived from the peels were used in producing skin care oil, face wash and insecticide while pectin produced were used as binder in preparation of tablet and stabilizer in the production of yoghurt.

Indexed Terms- Citrus peels, Lemon, Lime, Orange, pectin, essential oil, skin care oil, face wash, insecticide

I. INTRODUCTION

Essential oils are products of natural extract comprising of volatile and complex compounds characterized by highly stimulating odor and are produced by aromatic plants as secondary metabolites which have been extracted and concentrated from different part of plants. They are rich sources of flavanoids, alkaloids, coumarins, limonoids, carotenoids, phenolic acid, and many polymethoxylated flavones, which are not mostly found in other plants. They are mostly obtained by steam or hydrodistillation and are known for their antiseptic (i.e. bactericidal, fungicidal, and virucidal) and medicinal properties and their fragrance since middle ages. They are used in food preservation, antimicrobial, perfumes, analgesic, sedative, anti-inflammatory, spasmolytic and medicine. Several plants have been extracted for their essential oil, plants like turmeric, garlic, coconut, palm fruit, olive etc. for their therapeutic, edible and aromatherapy purposes.

Citrus fruit processing produces many byproduct with significant value. These wastes could be used for the production of many phytochemical, pharmaceuticals, food products, essential oil, seed oil, pectin and dietary fibers. These by-products are considered to be rich source of edible and health promoting agents as polymethoxylated flavonoids, many of which are found exclusively in citrus peel (Putnik et al., 2017).
Citrus peel essential oil is produced from orange peel extraction using several extraction methods which include steam or hydro-distillation, and organic solvent extraction, cold pressing, and supercritical CO_2 (Palazzolo et al., 2013). Pectin is mostly used in the pharmaceutical and food industry. It is used to bind drugs, additives for food, etc. It is produced commercially in the form of white to light brown powder mainly extracted from orange fruits. They can be produced from orange peels using several methods which include microwave, ultrasound, high pressure,

subcritical water, enzyme utilization, electromagnetic induction heating, and combination of chelators like citric acid in the conventional process (Putnik et al., 2017). Generally, high methylated pectin is of commercial importance as the one obtained from citrus and orange fruits. This has been used instead of starch. As a drug consumption of pectin has been shown to reduce blood cholesterol levels. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health (Tang, 2011).

The objectives of the study are to; (a) Perform Steam Distillation of Citrus Peels (orange, lime and lemon) to obtain Essential oil from each

i) To determine the relationship between time and quantity of essential oil obtained from citrus peel (orange, lime and lemon).
ii) To compare quantitatively the composition of essential oil obtained from lime, lemon and orange peels.
iii) To determine the physico-chemical properties of the essential oil.
iv) To test the suitability of using essential oil for the production of skin care oil, Face wash, and insecticide.

- Extraction of Pectin

i) To extract pectin from citrus peel residue and determine its qualitative and quantitative properties.
ii) To test the suitability of using pectin for the production of yoghurt and Preparation of binders for tablets.

These peels can be harnessed to produce essential oil which can be used as pesticides as citrus contains a high level of D-limonene. Also, oil produced from lemon and oranges are used in confectionaries for cakes, ice creams, etc. They are also used in the production of household detergents, antiseptics and in cosmetics because of its refreshing odour. Pectin is also produced from the peels which is used in the pharmaceutical industry as binders for drugs and as drugs to reduce cholesterol.

II. LITERATURE REVIEW

The fruits are peeled or cut (for bitter rind avoidance) and eaten skinned, or processed to orange juice by extraction. The endocarp is rich in soluble sugar and contains significant amounts of vitamin C, pectin, fibres, different organic acids and potassium salt which give the fruits its characteristic "citrus flavour" (Ezejioforet, al., 2011). It constitutes about 45% juice, 15 to 30% albedo which is the white spongy layer below flavedo layer, and 8 to 10% flavedo which is the outer epidermis. The juice sacs are closely compacted, club shaped vesicles, which completely fill the segments and are attached to the walls with small hair-like papillae. Segments of most sweet orange varieties contain 1 to 3 or 4 seeds attached by means of placentae to the septum wall. The seeds are rich in oil (30 to 40%) and bitter limonoids.

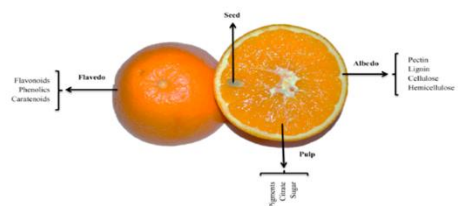

Figure 1: Picture showing the flavedo and albedo

As regards the interactions of the plant-pathogen, essential oils are recognised as defence compounds that confer protection against several natural enemies, but they also facilitate the reproduction of the vegetal species by attracting pollinators (Prins, et al., 2010; Marques et al., 2012). They are also used for cell regeneration. They are mostly found in certain organs of plants like the stems, roots, bark, seeds, leaves, wood and flowers.

Orange peel
↓
Separated flavodo layer
↓
Saturated in C_a (OH) Solution (1%, 8h)
↓
Removed and dried (45_0C)
↓
Powdered
↓
Steam distilled (80g powder + 500 ml
↓

Steam distilled (80g powder + 500 ml

↓

Distilled water + 10g NgC1)

↓

Oil

↓

Added N_gSO_4

↓

Filtered

↓

Added CH_2CL_2

↓

Added $N_{ag}SO_4$

↓

Flitered

↓

Added CH_2CL_2

↓

Concentrated (40^0C)

↓

Stored

Figure 2: Flow sheet for preparation of essential oil flavedo layer.

- Pharmacological Properties of Essential Oils

- Antiseptics:

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antibio-resistant strains. The most common sources of essential oils used as antiseptics are: Cinnamon, Thyme; Clover; Eucalyptus; Culin savory; Lavender. Citral, geraniol, linalool and thymol are much more potent than phenol.

- Expectorants and diuretics:

When used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anaesthetic action. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect.

- Spasmolytic and sedative:

Essential oils from the Umbellifereae family, Mentha 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.

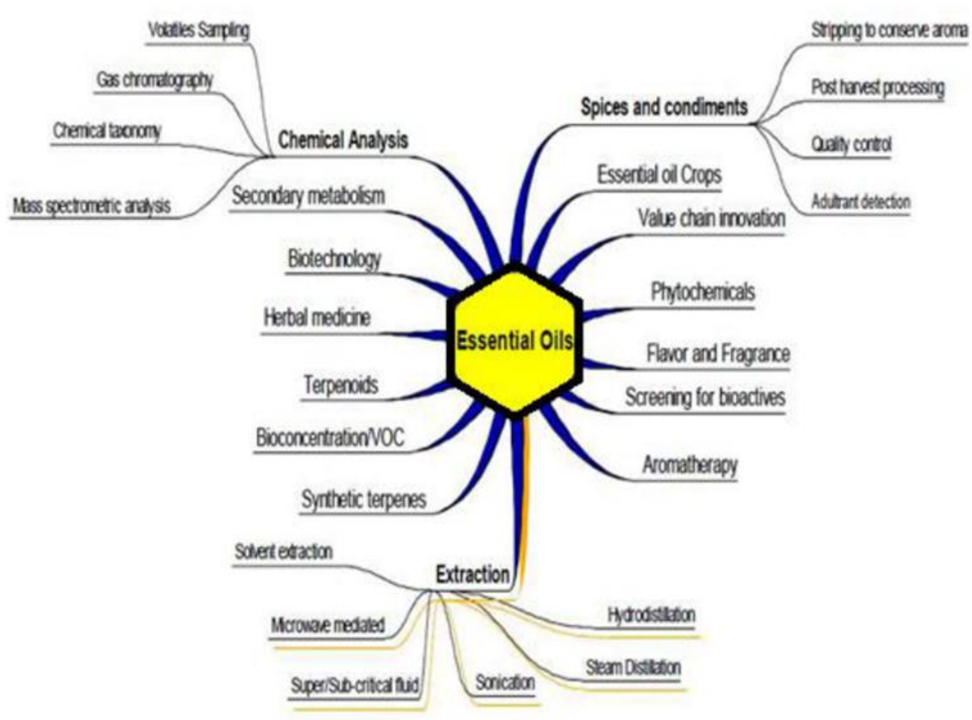

Figure 3: Picture showing the copious branching of specializations in the work of consequential oils.

- Extraction techniques

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 (Njoku and Evbuomwan 2014). The vast majority of essential oils are produced from plant material in which they occur by different kinds of distillation or by cold pressing in the case of the peel oils from citrus fruits.

- Classical and Conventional Methods

There are several methods of extraction behavior of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused in copious parts of the globe. Hydrodistillation (HD), Steam distillation (SD), Solvent extraction Hand pressing,and Maceration are the roughly traditional and generally used methods.

Hand pressing/Mechanical pressing: The hand pressing methods is mainly employed for laboratory purpose. It is also called cold pressing or mechanical pressing. It has been reported by some researchers (Vasek, et al., 2014). The hand pressing method involved manual pressing of the flavado layer to exposed oil sac. The oil can be collected in a brine solution and kept on ice. The extract can then be centrifuged at 6000 rpm for 20 minutes, and dried in anhydrous sodium sulphate. However, this method has not been widely used may due to lower yield as compared to the solvent, steam or hydro-distillation processes.

III. MATERIALS AND METHODS

- Equipment

Equipment required for the extraction of essential oil and pectin from citrus peels are Multipurpose pH meter, blender, muslin cloth, Microwave oven, litmus paper, test tube, density bottle, 25ml capacity dry bottle, hot plate/ magnetic stirrer, Radwig electronic top loading balance , conical flask, Burrete (50ml), 200 ml conical flask, Pipette (2ml), Measuring cylinder, Knife, Abb's refractometer, measuring cylinder (1000ml and 100ml), Separation funnel, 1000ml flat bottom flask as the distilling flask, 500ml round bottom flask as the receiver, condenser, glass aspirator, beakers, heating mantle, cotton wool, petri dish, crucible and desiccator. Auto sampler vials, 150 μL vial inserts, and crimp seals, Vial crimper and decrimper, 2.5mL airtight syringe or 3mL disposable hypodermic syringe, 10 micro-liter autosampler syringe, 30 m × 0.25 mm × 0.25 μm Elite-5MS column (Perkin Elmer, USA).

- Chemicals and Reagents

All chemicals and reagents are of analytical grade and are: Phenolphthalein indicator, acetic acid chloroform, potassium iodide, 0.1M sodium thiosulfate, starch indicator, ethanol,0.5M alcoholic potassium hydroxide, 40% citric acid, 0.5M hydrochloric acid, Orange, Lime, Lemon, distilled water, acetone, diethyl ether, anhydrous magnesium sulphate. Air
–Zero grade, Ultra-high purity (99.999%) helium, anhydrous sodium sulphate, internal standard

- Methods

A. Preparation of Samples

Orange, Lime and Lemon was bought from the Fruit market at D-line in Port Harcourt, River's state. These fruits were washed to remove dirt from the epicarp with distilled water. The fruits were peeled with knife to remove shell, pureed for steam distillation, sun dried for one to two hours in an oven. The dried peels were grounded to give consistent and fine particles.

B. Procedure for Steam Distillation: Extraction of Essential Oil.

150g of the prepared pureed peels was introduced into the distillation flask, which was connected to a round bottom flask containing water. The flask was connected to a condensing unit with its tubing. The essential oil was extracted with the distillation set-up using steam as it was percolating through the peels. The heat source was adjusted so that the distilling rate will drop 20 drops per second. Distilled water was always added as it boils to prevent water from going too low. At the respective minutes,60, 90, 120,150, and 180, the distillate was collected and transferred into a 250ml conical flask where 20ml of diethyl ether was added to separate the oil. The water layer was then removed using a separating funnel, the diethyl ether layer was collected and few drops of anhydrous magnesium sulphate was added to dry off water. The oil was then kept in a fume cupboard for any remaining diethyl ether to vapourize. The oil extracted was measured and recorded. The recovered mixture of oil and water was allowed to settle and the oil was withdrawn. After the steam distillation process, the product, which was a mixture of water and oil, was collected and separated using separating funnel. This was done separately for the essential oil of lime, lemon and orange.

- Determination of the Physio-Chemical Properties of Citrus Peels Essential Oil

The following are the determination of the physio-chemical properties of citrus peels essential oil

I. Determination of the Solubility of Essential Oil of Orange, Lime and Lemon in Water.

Three drops of oil were put into a test tube and six drops of water was added to it. A glass rod was used to stir it thoroughly. The two separate phases were observed. Measurement of pH was taken using litmus paper to determine if the essential oil was partially soluble in water or has altered the pH of the water. It was observed that there was no change in color, therefore, the oil is insoluble in water. This was done separately for the essential oil of lime, lemon and orange.

II. Determination of Saponification Value (SV) of Essential Oil of Orange, Lime and Lemon.

5g of the oil was weighed (W) into a 200ml conical flask, and 25 ml of 0.1M alcoholic potassium hydroxide(N) was added. This was refluxed for 1hr, followed by the addition of two drops of phenolphthalein indicator and was titrated with 0.5N hydrochloric acid (V_1) until the pink color disappears. A blank titration(V_2) was equally performed (Ezejiofor, et al., 2011). This was done separately for the essential oil of lime, lemon and orange.

The saponification values were calculated using the formular in equation 1

$$\text{Saponification Value} = \frac{(V2 - V1) \times N \times 56.1}{\text{Weight of oil (g)}} \quad (1)$$

III. Determination of Peroxide Value of Essential Oil

Thirty milliliters of acetic acid chloroform solution were measured into a flask containing 2 g of the oil sample. A 0.5ml saturated solution of potassium iodide was then added, followed closely by the addition of 30 ml of distilled water. The flask content was then titrated against 0.1M sodium thiosulfate(V_1) until the yellow color almost disappeared; 0.5 ml starch indicator was added and the titration continued until the end point (where the blue-black color just disappeared). A blank titration (V_2) was equally performed (Njoku and Evbuomwan, 2014). This was done separately for the essential oil of lime, lemon and orange.

$$\text{Peroxide Value} = \frac{(V2 - V1) \times N \times 1000}{\text{Weight of oil (g)}} \quad (2)$$

IV. Determination of Refractive Index of Essential Oil

The Abb's refractometer was used in determining the refractive index. It gives values up to four decimal places, the index is denoted by $^{n}D^{25}$

Where n= refractive index at 25 ̊C taken with sodium light.

The refractometer was standardized with distilled water which has a refractive index of $^{n}D^{29.5} = 1.3315$, then cleaned with acetone and dried with cotton. After this, a drop of oil was placed between the prisms of refractometer. 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 at room temperature. This was done separately for the essential oil of lime, lemon and orange.

V. Determination of Specific Gravity of Essential Oil

This was determined using the procedure described by Adepoju and Eyibio (2016). A specific gravity bottle of 25ml capacity was dried and weighed to give W_0. Then filled with water. It was weighed and recorded as W_1. The bottle was emptied and properly dried after which it was filled with the oil. It was also weighed and recorded as W_2. This was done separately for the essential oil of lime, lemon and orange.

$$\text{Specific gravity} = \frac{\text{weight of density bottle filled with water}}{\text{Weight of density bottle filled with oil}} \quad (3)$$

VI. Determination of Acid Value of Essential Oil
Twenty-five milliliters of diethyl ether and 25 ml of ethanol was mixed in a 250 ml beaker. The resulting mixture was added to 5 g of the oil in a 250 ml conical flask. Few drops of phenolphthalein were added to the mixture, and the mixture was titrated with 0.1 M potassium hydroxide and consistently shaken until a dark pink color will be observed. The volume of the 0.1 M potassium hydroxide was noted (Njoku and Evbuomwan 2014). This was done separately for the essential oil of lime, lemon and orange.

Acid value = (Titre value X Normality of KOH X 56.1) / Weight of Oil sample (4)

IV. RESULTS AND DISCUSSION

Physiochemical Properties of Orange, Lemon and Lime Peel essential oil

The physicochemical properties of essential oil are presented in Table 1.

Table 1: Physiochemical Properties of Orange, Lemon and Lime Peel essential oil

Properties	Orange	Lemon	Lime
Physical			
Specific gravity	0.88	0.86	0.84
Refractive index @ 25℃	1.38	1.57	1.45
Solubility in water	Insoluble	Insoluble	Insoluble
Chemical			
Saponification (mgKOH/g)	145.61	158.42	122.74
Acid value (mg/g)	6.96	8.68	12.93
Peroxide value (mEq O_2/kg)	84.42	53.76	41.05

The specific gravity of the Orange, Lemon, and Lime essential oil were 0.88, 0.86 and 0.84 respectively. The specific gravity determines the weight of the essential oil. It is also important in determining the quality and purity of essential oil. The extracted essential oils have a specific density less than 1 which implies that it is lighter than water and consequently will be insoluble in water. The specific gravity of Orange was higher than that of Lemon and Lime, the difference in their specific gravity is 0.02 which is minimal.

The refractive index @ 25℃ of the Orange, Lemon, and Lime essential oil were 1.38, 1.57 and 1.45 respectively. These values are consistent with those obtained by (Blazquez 2014), in studies with different varieties of orange, 1.4608 to 1.4714, consistent with the value obtained by (Kumar 2014). It measures the refraction of light rays as these pass through the oil. The refractive index is a unique number that designates how the oil responds to and bends light. Therefore, the refractive index obtained from the extracted essential oil shows that the oil is highly pure. The acid value of the Orange, Lemon, and Lime essential oil were 6.96 mg/g, 8.68 mg/g and 12.93 mg/g respectively. Acid Value is analytically used to detect the level of unesterified fatty acid in a lipid sample to define its quality. The low acid value of the extracted essential oil indicates that the oil has excellent storage life. Lime has the highest acid value at 12.93 mg/g.

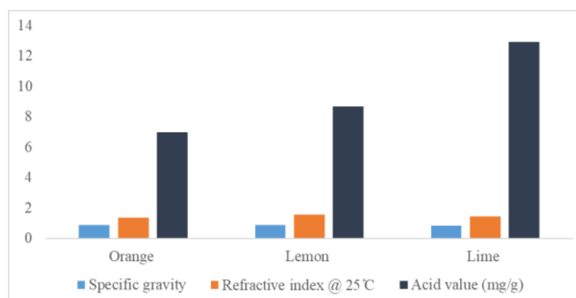

Figure 3: Comparison between Orange, Lemon and Lime for specific gravity, refractive index and acid value

From Figure 3, it is shown that the three oils fell within the same range and not much significant difference in their specific gravity (0.84-0.88) and refractive index (1.38-1.45). Also from Figure 3, Lime has the highest acid value at 12.93 mg/g, Orange the lowest at 6.96 mg/g, Lemon at 8.68 mg/g.

The saponification value of the Orange, Lemon, and Lime essential oil were 146 mg KOH/g, 158 mg

KOH/g and 123 mg KOH/g respectively. Saponification value is an indicator of the average molecular weight and hence chain length. Low molecular weight (short to medium chain) fatty acids have more glyceride molecules per gram of fat than high molecular weight acids.

The peroxide value of the Orange, Lemon, and Lime essential oil were 84.42 mEq O_2/kg, 53.76 mEq O_2/kg and 41.05 mEq O_2/kg respectively. Peroxide value gives a measure of the extent to which the oil has undergone primary oxidation. Detection of peroxide shows initial evidence of rancidity in unsaturated fats and oils.

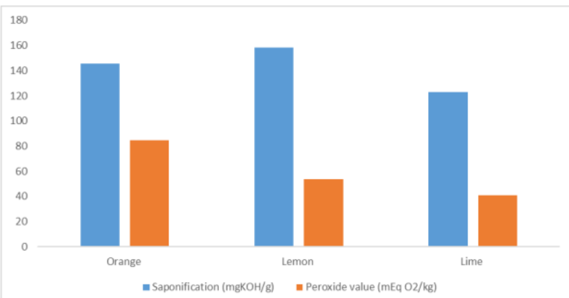

Figure 4: Comparison between Orange, Lemon and Lime for saponification and peroxide value

From Figure 4, it is shown that lemon has higher saponification value than Orange and Lime which indicates that higher molecular weight and longer carbon chain than the other two. Also from Figure 4, Orange has the highest peroxide value amongst Lime and Lemon which according to the International Olive Council (IOC) standard that any essential oil > 20 mEq O_2/kg oil is less stable oil with a shorter shelf life (Mailer, 2006).

Effect of Time on Oil Extracted from Orange, Lemon and Lime Peels Using Steam Distillation

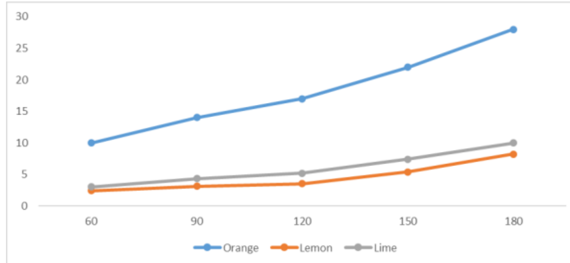

Figure 5: Effect of Time on oil extracted from Orange, Lemon and Lime Peels using Steam Distillation.

The essential oil extracted from Orange ranged from 10- 28ml, Lemon from 2.4-8.2ml and Lime from 3-10ml. In Fig. 5, increase in extraction time leads to a corresponding increase in amount of extracted oil at a constant temperature of 95℃. This agrees with the findings of (Giwa et al. 2018) who also obtained an increasing trend while working on essential oil extraction from orange peels using water distillation method. Oil extracted from Orange was higher than that of Lime and Lemon.

a. Qualitative and Quantitative Tests for Pectin from Orange, Lime and Lemon Peel

The qualitative and quantitative tests for Pectin from Orange, Lime and Lemon peel is shown below in table 2

Table 2: Qualitative and quantitative tests for Pectin from Orange, Lime and Lemon peel

Properties Qualitative tests	Orange	Lime	Lemon
Colour	Light Brown	Light Brown	Dark brown
Solubility of dry pectin in cold water	Insoluble	Insoluble	Insoluble
Solubility of dry pectin at 85-95℃ for 15 minutes	Soluble	Soluble	Soluble
Quantitative tests			
Moisture Content (%)	3.2	4.4	3.7
pH	5.3	6.1	5.2

Ash Content (%)	2.9	1.4	2.6

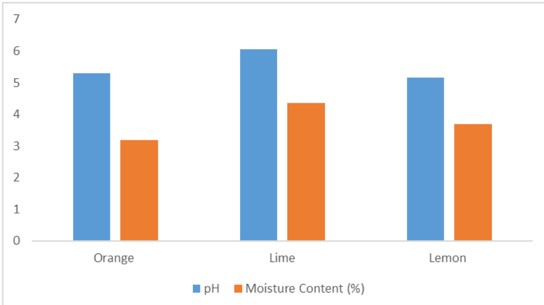

Figure 6: Moisture content and pH values of Pectin from Orange, Lime and Lemon peels.

The pH value of the Orange, Lemon, and Lime pectin were 5.3, 6.1 and 5.2 respectively. pH is considered as one of the more crucial parameters affecting the properties of extracted pectin. The presence of high concentration of hydrogen ions in the solvent has stimulated the hydrolysis of protopectin. At low pH, as the hydrogen ion concentration of the solution is increased, ionization of the carboxylate groups is repressed, i.e., the highly hydrated carboxylate group is converted into hydrated carboxylic acid groups. The loss of carboxylate groups is able to reduce the repulsion of the polysaccharide molecules which promotes the gelation properties of pectin giving more precipitated pectin at lower pH.

The moisture content value of pectin derived from Orange, Lemon, and Lime peels 3.2%, 4.4% and 3.7% respectively. Moisture content is actually a confirmatory check on the dryness of the oil or fat sample.

The colour of pectin obtained from the Orange and Lemon peel were light brown whereas Lime peel was dark brown. While pectin is usually light in colour, factors such as surface contamination or environmental factors might have contributed to the discrepancy in colour. This could also be due to the amount of alcohol used for precipitation or purification during the experiment not being enough.

In cold water (H_2O), the dry pectin from the three samples were insoluble, which dissolved (became soluble) when heated at 85-90°C for 15 min.

The ash content value of pectin derived from Orange, Lemon, and Lime peels 2.9%, 1.4% and 2.6%. This indicates that Orange and Lime have a high residue that can be used as supplements in feed for animals.

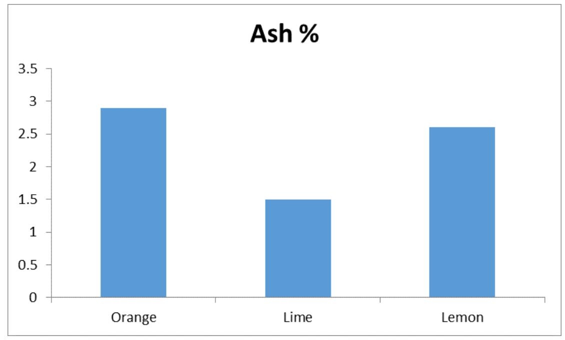

Figure 5: Ash content of Pectin from Orange, Lime and Lemon peels.

b. Composition of Essential Oil

The GC/MS analysis of the citrus peel essential oil was done using Agilent 5975 MSD with an Agilent 6890N. the results of the GC-MS analysis of the orange, lemon and lime peels essential oil are shown. The results showed that the orange peel. lemon peel and lime peel essential oil contains a total of 23 components each.

c. Utilization of Essential Oil and Pectin

1) Essential oil

The essential oil from Orange, Lime and Lemon peels were used in the preparation of skin care oil, face wash and insecticide.

Due to high quantity/quality of Limonene present in the Essential oil (59.12%,48.43% and 45.62%) extracted from Orange, Lime and Lemon respectively, the following were observed in the produce

i. The Limonene acted as a good carrier agent, it helped the other ingredients penetrate the surface of the skin more effectively (skin care oil and face wash)
ii. It also served as anti-inflammatory agent (active agent) in skin care oil and face wash
iii. It serves as an effective repellent in the insecticide produced.

2) Pectin

The pectin derived from Orange, Lime and Lemon peels were used in the preparation of tablet (paracetamol) using pectin as binder and yoghurt using pectin as stabilizer and this was achieved because it's gelling ability when dissolved in water at a temperature of 85^{0}C.

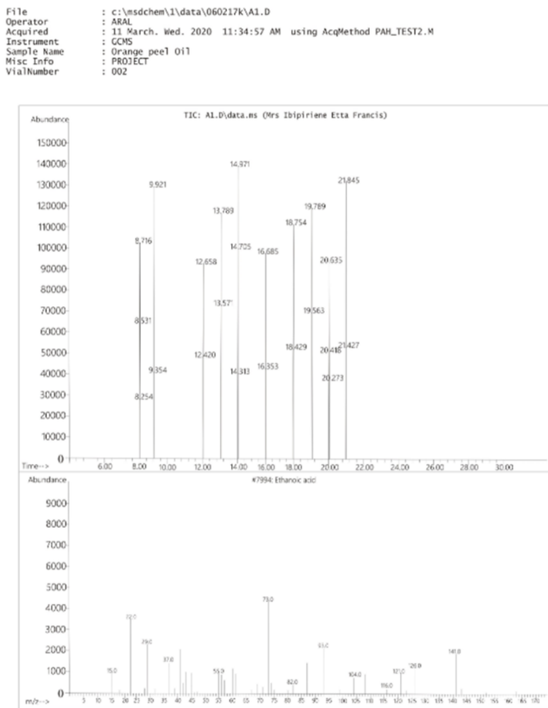

Figure 8: GC-MS Chromatogram showing the analysis of orange peel essential Oil

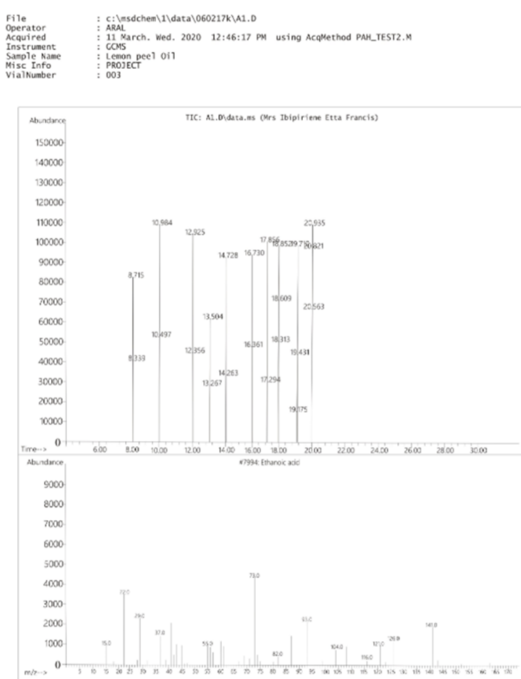

Figure 4.7: GC-MS Chromatogram showing the analysis of Lemon peel essential Oil

CONCLUSION

From the results obtained as shown in chapter four, the analysis carried out on the extraction of essential oil and pectin from Orange, Lime and Lemon peels showed that orange has a larger amount of oil produced under 180 minutes than lime and lemon. This further shows that orange peel is a great source of essential oil. Also, time was shown to have effect on the amount of oil produced at an interval of 30 minutes. The production of pectin from Orange, Lime and Lemon peels confirmed that they are a great source of pectin. Essential oil and pectin obtained from the three (3) samples were found to be insoluble in cold water but pectin was soluble in water heated at 85℃ for 15 minutes.

The essential oil obtained was used to produce face wash, skin care oil and insecticide as citrus peels contains high Limonene that is a key component. Also, pectin obtained was used as a binder in making paracetamol and a stabilizer in yoghurt. These results demonstrate the successful extraction of essential oil and pectin, providing potential benefits for industrial extraction of pectin from an economic and environmental point of view. The suitability of essential oil and pectin for different purposes is determined by their physicochemical properties which is highly important.

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