GC-FID and FT-IR Characterization of Lemongrass Oil Extracted With Soxhlet Extraction Apparatus Using Ethanol as Solvent

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Abstract: The extraction of lemongrass oil was performed with Soxhlet extractor apparatus using ethanol as solvents. The extraction was carried out with lemongrass sample with particle size 0.5cm, 1.0cm, 1.5cm, 2.0cm and 2.5cm and the respective oil yields were noted. The characteristic of the oil sample was done with Fourier’s Transform-Infrared Spectroscopy (FTIR) and Gas Chromatography-Flame Ionization Detector (GC-FID) analytical techniques. The FTIR result showed ten (10) functional groups that include halo-compound, alkene, alkane, sulphate, aldehyde, thiol, isothiocyanate, carboxylic acid, alcohol, and aromatic compound. Furthermore, the GC-FID analyses indicated the occurrence of nineteen (19) different components in the lemongrass extract which include α-pinene (12.9%), 2-nonanone (10.3%), acetonitrile (7.5%), β-cedrene (6.6%), camphor (6.4%), citronellal (6.0%), 4-nonanone (5.9%), terpinolene (5.4%), decanal (5.3%), α-cedrol (5.3%), citral A (5.0%), β-pinene (4.4%), atlantone (3.6%), pentane (3.3%), p-cymene (3.1%), γ-terpinene (2.8%), α-cedrene (2.6%), thujopsene (2.0%) and genanol (1.7%). In this research work, the highest constituent obtained was α-pinene making up of 12.9% of the oil sample and the citral content was 5.0%. It is concluded that lemongrass oil is made up of different bioactive chemicals which sometimes vary according to geographical origin, biodiversity and culture.

Keywords: Essential Oil, Ethanol, Lemongrass, Modelling, Physicochemical, Soxhlet Extraction

I. INTRODUCTION

Essential oils of plant origin are natural occurring volatile and odoriferous aromatic extracts of plants. These plant essences are mostly located in cells, ducts and glands of leaves, barks, roots, buds, flowers and fruits of most plant matrix. Lemongrass (Cymbopogon citratus) is a rich source of lemon-scented essential oil. Lemongrass last all season of the year (perennial plant). The plant has long, thin leaves, and is largely cultivated medicinal plants in parts of tropical and subtropical areas of Asia, Africa and America (Suryawanshi et al., 2016; Chantalet al., 2012). The chemical compositions of lemongrass (Cymbopogon citratus) essential oil may vary widely due to geographical locations and agronomic treatment of the culture. The leaves of lemongrass (Cymbopogon citratus) possess lemon like odour characteristic aromatic flavours due to its citral contents and this made it of great importance to the industry. Lemongrass essential oil found broad application in food, pharmaceutical, perfumery and cosmetics industries (Ganjewala and Luthra, 2010). Lemongrass oil has a pleasant, refreshing aroma, antifungal and antibacterial properties (Ukponget al., 2016; Anarumae et al., 2010). Many researchers have reported that plants contain valuable chemicals (Morrison and Boyd, 1987). These natural chemicals and their synthetic components have continued to serve as feedstock or intermediate feedstock in most industries. While some are used in pharmaceutical and chemical allied industries, others are applied as food flavours, fragrances, sweeteners, pesticides etc. Many researches carried out on lemongrass extraction and characterizations are wholly centered on steam distillation technique. Moreover, there are no reports on characterization of lemongrass oil extracted with Soxhlet extraction apparatus using ethanol as solvent especially from lemongrass sample from Ozoro and its environ- Delta State Nigeria. Therefore, the present study is aimed to characterize bioactive chemical constituentlemongrass essential oil with the aid of FTIR and GC-FID analytical methods.

II. MATERIALS AND METHODS

Materials collection and preparation
Fresh lemongrass leaves used for this research work were harvested from a private garden in Ozoro located at 5° 32’ 18” N, 6° 12’ 58” E, Delta State, Nigeria. Sample were washed and dried for eight (8) hours in an oven to reduce the moisture content. The dried lemongrass leaves were kept in sealed bag to avoid direct
sunlight. Thereafter, the dried lemongrass leaves were cut with a knife into various sizes of 0.5cm, 1.0cm, 1.5cm, 2.0cm and 2.5cm so as to increased contact area of the plant matrix.

**Reagents**
Ethanol used as solvent for the extraction process. Reagent is of analytical grade purchased by the technologist attached to Chemical Engineering Department, University of Port Harcourt, River state.

**Equipment**
The equipment used for the study include; 250ml Shuniu GG-17 Soxhlet extractor, Setra analytical weighing balance BL-410s, Buck scientific M530 USA Fourier Transform-Infrared Spectroscopy (FT-IR) machine and Buck scientific M910 USA Gas Chromatography-Flame Ionization Detector (GC-FID) machine.

**III. METHOD:**

100g of 0.5cm particle size lemongrass samples was measured using weighing balance. The weighed sample was put into extractor thimble and 300ml of ethanol were added into the flask. The heating mantle was set at a pre-determine temperature according to the boiling point of the solvent use for the experiment and the extraction was conducted for the set time (say 1 hour). Thereafter, experimental set up was dismantled and mixture of solvent and extracted oil obtained was placed on rotary evaporator set at initial speed 110rpm. This is done to enable ethanol recovery from extracted oil by evaporation under reduced pressure. Thereafter, the oil was kept in desiccator for about 25 minutes and the mass in grams of oil extract was determined with a digital electronic weighing balance. The results were recorded accordingly.

The percentage oil yield was estimated accordingly using Equation 1;

\[
\text{Oil yield} = \frac{\text{Weight of extracted oil}}{\text{Weight of lemongrass sample}} \times \frac{100\%}{1}
\]

**Determination of lemongrass oil composition using GC-FID analysis**

GC-FID analysis was performed using a Buck M910 scientific gas chromatography instrument equipped with a capillary column. Helium was applied as carrier gas at constant (steady) flow rate of 1.2ml/min and split ratio 1:30. Oven temperature was initially held at 50°C for 1 minute, and later programmed to be 280°C at a rate of 5°C/min. Helium flux is 30ml/min and air flux is 300ml/min. The injector temperature was 280°C and that of detector (FID) temperature was 300°C. Injection volume is 1μl. The percent (%) compositions of various compounds were obtained by GC-FID analysis. Identification of lemongrass oil composition was based on retention index (RI) in co-injection with standards (Sigma Aldrich and standard isolates), MS Library search (NIST 98 and WILEY), the same time comparing with the MS literature data (Adams, 2007; Adams, 2001; Shibamoto, 1987; Jennings and Shibamoto, 1980). And finally, the percentage (%) composition of individual constituent of the sample lemongrass oil was determined from GC-FID peak areas without using any correction factors.

**Determination of functional groups using FT-IR analysis**

The determination of functional groups in the lemongrass oil was done with Buck scientific M530 USA FTIR Spectrometer at SpringLab Awka Anambra state Nigeria. The instrument was fitted with deuterated triglycerine sulphate detector and potassium bromide beam splitter. Gram A1 software was used to obtain spectra. About 1.0g of sample was properly placed on the salt pellet. Thereafter, the FT-IR spectrogram were obtained at frequency range between 4,000 – 600cm\(^{-1}\) and co-added at 32 scans and at 4cm\(^{-1}\) resolution. The functional groups were numerated with the assistance of IR correlation charts.

**IV. RESULTS AND DISCUSSION**

**FT-IR analysis**

FT-IR’s absorption spectrum of lemongrass oil extract using ethanol is given in Figure 1. The FT-IR’s absorption spectra result is presented in Table 1. FT-IR spectra confirmed seven (7) functional groups. The FT-IR spectra established peak values were 803, 1387, 1860, 2059, 2554, 3291, and 3430 as presented in the chromatogram in Figure 1.
The FTIR spectra analysis of lemongrass oil is shown in Table 1. The corresponding absorptions were compared with standard IR spectrum table and chart using frequency range, the lemongrass oil spectra affirmed the presence halo compound, alkene, alkane, sulphate, aldehyde, aromatic compound, isothiocyanate, thiol, carboxylic acid, alcohols as functional groups. These identified functional groups were in agreement with compounds reported in the work of Olayemi et al. (2018). In addition, the functional groups identified confirmed GC-FID analysis. For instance, camphor, allantone, 2- nonanone, 4-nonanone are ketone, beta-pineane, pentane are alkanes. Beta-cedrenes, terpinolene, alpha-pinene, gamma terpinene are alkene. Citrals A, citronellal, decanal are aldehydes while p-cymene is aromatic compound, alpha- cedrol, gananiol are alcohol and thujopsene belong to amines.

**GC-FID analysis**

The GC-FID’s analysis is presented in Table 2 and the chromatogram of the lemongrass oil extracts is presented in Figure 2. The peaks in chromatogram were compared and integrated with database spectrum of known components stored in GC-FID library. The GC-FID analysis confirmed that lemongrass oil contains nineteen (19) compounds.
Figure 2: Chromatogram for lemongrass oil using ethanol
The observed chemical composition (constituent) of lemongrass oil is as presented in Table 2 in their descending order. The sample oil is dominated with \( \alpha \)-pinene, 2-nonanone, acetonitrile, \( \beta \)-cedrenes, camphor, citronellal, 4-nonanone, terpinolene, decanal, \( \alpha \)-cedrol, citral A for both extract. The minor constituents were \( \beta \)-pinene, atlantone, pentane, p-cymene, \( \gamma \)-terpinene, \( \alpha \)-cedrenes, thujopsene and genaniol. It has been proved that the composition of lemongrass oil varies according to geographical origin, biodiversity and culture (Ganjewela, et al., 2008; Khanuja et al., 2005). In this research work, the highest constituent obtained was \( \alpha \)-pinene making up of 12.9% of oil. This result is at variance with literature reports where citral has always been reported to be the major constituent of lemongrass oil. Joy et al., (2001) have reported 74-76% citral. In this work the citral content was 5.0%. Bleasale et al., (2002) reported that dry lemongrass oil yields of 0.4% contain 72.3% citral. Citral has immense commercial significance due to its characteristic lemon like odour in flavor, cosmetics, perfumery and pharmaceutical industries (Ganjewela et al., 2008). Citral is used in the formulations of perfumes, coloured soaps and synthesis of vitamin-A (Deepak et al., 2012). Ganjewala (2009) also report antimicrobial, antiparasitic, antispasmodic, analgesic, anti-inflammatory activities of citral. On the other hand, camphor is used to grow hair loss and treat cold, sores, poor blood recirculation, anxiety, heart disease symptoms, ear aches, acne, flatulence, depression, muscle spasms, low libido, hemorrhoids etc. \( \alpha \)-pinene is used as ingredient in food flavoring and it has anti-depressant, anticonvulsant, antioxidant effects. \( \alpha \)-pinene has a physiological effect on human. Finally, comparing chemical compositions of lemongrass oil from Nigeria with results of other researchers shows that there are variations in chemical composition of lemongrass oil gotten from different location. These variation in composition could be traced to diverse climatic, geographical differences, varied harvesting time and extraction technique adopted (Ashgariet al., 2010).

V. CONCLUSION AND RECOMMENDATIONS

The FT-IR and GC-FID analytical techniques are important methods of characterizing plant extracts. The FTIR analysis confirmed ten(10) functional groups that include halo-compound, alkene, alkane, sulphate, aldehyde, thiol, isothiocyanate, carboxylic acid, alcohol, and aromatic compound. GC-FID analysis shows the sample lemongrass oil is made up of nineteen(19) bioactive chemicals which include \( \alpha \)-pinene, 2-nonanone, acetonitrile, \( \beta \)-cedrenes, camphor, citronellal, 4-nonanone, terpinolene, decanal, \( \alpha \)-cedrol, citral A, \( \beta \)-pineane, atlantone, pentane, p-cymene, \( \gamma \)-terpinene, \( \alpha \)-cedrenes, thujopsene and genaniol. The major constituent was \( \alpha \)-pinene making up of 12.9% of oil for ethanol extract. From this study, it can be affirmed that composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>External ppm</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-pinene</td>
<td>10.136</td>
<td>24648.2274</td>
<td>477.515</td>
<td>0.3117</td>
<td>12.9</td>
</tr>
<tr>
<td>2- nonanone</td>
<td>10.366</td>
<td>19567.5278</td>
<td>378.811</td>
<td>18.7428</td>
<td>10.3</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>32.996</td>
<td>14322.7033</td>
<td>277.023</td>
<td>2.1934</td>
<td>7.5</td>
</tr>
<tr>
<td>( \beta )-cedrenes</td>
<td>20.313</td>
<td>12615.9522</td>
<td>244.427</td>
<td>8.5812</td>
<td>6.6</td>
</tr>
<tr>
<td>Camphor</td>
<td>2.39</td>
<td>12226.532</td>
<td>238.796</td>
<td>19.9044</td>
<td>6.4</td>
</tr>
<tr>
<td>Citronellal</td>
<td>27.536</td>
<td>11449.1788</td>
<td>222.627</td>
<td>2.4687</td>
<td>6.0</td>
</tr>
<tr>
<td>4- nonanone</td>
<td>17.966</td>
<td>11229.8418</td>
<td>218.863</td>
<td>10.7566</td>
<td>5.9</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>44.17</td>
<td>10204.4334</td>
<td>201.491</td>
<td>2.5817</td>
<td>5.4</td>
</tr>
<tr>
<td>Decanal</td>
<td>39.2</td>
<td>10162.0437</td>
<td>197.604</td>
<td>0.6984</td>
<td>5.3</td>
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<tr>
<td>( \alpha )-cedrol</td>
<td>25.65</td>
<td>10042.2102</td>
<td>194.954</td>
<td>1.0521</td>
<td>5.3</td>
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<tr>
<td>Citral A</td>
<td>22.73</td>
<td>9509.6778</td>
<td>184.883</td>
<td>9.197</td>
<td>5.0</td>
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<tr>
<td>( \beta )-pineane</td>
<td>7.47</td>
<td>8378.2564</td>
<td>163.997</td>
<td>11.7914</td>
<td>4.4</td>
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<tr>
<td>Atlantone</td>
<td>36.876</td>
<td>6866.2699</td>
<td>134.457</td>
<td>2.8276</td>
<td>3.6</td>
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<tr>
<td>Pentane</td>
<td>12.97</td>
<td>6200.4714</td>
<td>120.729</td>
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<td>3.3</td>
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<tr>
<td>p-cymene</td>
<td>34.6</td>
<td>5959.5226</td>
<td>116.801</td>
<td>4.11</td>
<td>3.1</td>
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<tr>
<td>( \gamma )-terpinene</td>
<td>29.86</td>
<td>5413.8648</td>
<td>105.734</td>
<td>0</td>
<td>2.8</td>
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<tr>
<td>( \alpha )-cedrenes</td>
<td>15.46</td>
<td>4927.4465</td>
<td>96.039</td>
<td>0.8865</td>
<td>2.6</td>
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<tr>
<td>Thujopsene</td>
<td>0.495</td>
<td>3773.8654</td>
<td>404.399</td>
<td>1.4897</td>
<td>2.0</td>
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<tr>
<td>Genaniol</td>
<td>42.276</td>
<td>3192.6428</td>
<td>65.133</td>
<td>0.2183</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>190690.6682</td>
<td>99.3804</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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of lemongrass oil varies according to diverse climatic conditions, geographical differences, varied harvesting time and may be extraction technique adopted. It is therefore recommended that further study be carried out using Gas Chromatography Mass Spectrometry (GC-MS) technique for the characterization. In addition, further research should be carried out on lemongrass obtained from different locations of the world in order to compare chemical composition of their essential oils.

REFERENCES


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