

## Full Length Research Paper

# Volatile compound analysis of the leaves and seeds of *Piper guineense* using gas chromatography-mass spectrometry (GC-MS)

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The volatile compounds in the leaves and seeds of *Piper guineense* plant from South East Nigeria, were investigated using gas chromatography-mass spectrometry (GC-MS). The analysis was carried out to identify the compounds responsible for the characteristic flavor of the flavouring spices. A total of thirty-three volatile constituents were identified in the leaves and seeds of *Piper guineense* using GC-MS. The major compounds identified include acids, esters, alcohols, hydrocarbons and others. Acids were found to be the predominant constituent group followed by esters. The leaves and seeds of *Piper guineense* had acids in the values of 65.56 and 53.72%, respectively. The esters were also found to be more in the leaves (25.63%) than in the seeds (5.22%) of *P. guineense*. The hydrocarbons identified appeared more in the seeds (11.47%) of *P. guineense*. The monoterpene (beta-myrcene) and sesquiterpenes (aromadendrene, trans-alpha-bergamotene, copaene) hydrocarbons identified were also present in the seeds than in the leaves of the *P. guineense*. Gamma-elemene, a sesquiterpene were identified in both the leaves and seeds of *P. guineense* at the same retention time of 10.242 min but at different concentrations of 0.41 and 0.72%, respectively.

**Key words:** *Piper guineense*, volatile compounds, leaves, seeds.

## INTRODUCTION

*P. guineense* is a spice plant from the family, *Piperaceae* and from the genus *piper*. The genus *Piper* is made up of about 1050 species of tropical shrubs, lianas, and small trees, many of which are important as spices and flavoring agents and medicines (Owolabi et al., 2013; Maberley, 2008). *P. guineense* is a climbing plant that can grow up to 20 m in length. The seeds are smooth

and are prolate-elliptically shaped. The seeds of the plant are commonly known in English-speaking countries as "West African black pepper", "uziza" in Igbo "iyeree" in Yoruba and "poivrie" in French. West African black pepper (*Piper guineense*) is an important plant used in traditional medicine and as spice. The fruits (the part of the plant traditionally used) are rich in a wide range of

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natural products including volatiles oils, lignans, amides, alkaloids, flavonoids and polyphenols (Rodolfo et al., 2013). The leaves and seeds are usually sold in Nigerian markets as an edible medicinal plant or additive in foods to offer aroma and flavor.

The seeds, leaves and sometimes the stems are used in preparing soup. It imparts "heat" and a spicy pungent aroma to food. The medicinal properties of *P. guineense* exert bacteriostatic and bacteriocidal effects on some bacteria. The leaves are considered aperitive, carminative and eupeptic. They are also used for the treatment of cough, bronchitis, intestinal disease and rheumatism (Okoye and Ebeledike, 2013; Sumathykutty et al., 1999). The leaves are also used for female infertility while, the fruits are used as an aphrodisiac (Echo et al., 2012). *P. guineense* has culinary, medicinal, cosmetic and insecticidal uses (Nwinyi et al., 2013; Okwute, 1992). The powder obtained from the ground seeds is used for its stimulating properties (Tchoumboungang et al., 2009; Sofowora, 1982). *P. guineense* is added to food meant for pregnant and nursing mothers as a medicinal spice and among the post partum women, it is claimed that it assists in the contraction of the uterus (Achinewhu et al., 1995). Okoye and Ebeledike (2013) reported that the leaf extracts of *P. guineense* have antimicrobial effect on some test organisms. The leaf extracts exhibited antimicrobial effect activity due to the presence of tannins, saponins, flavonoids and alkaloids (Rabe and Vonstanden, 1987). The essential oils of *P. guineense* from Cameroon (Tchoumougoungang et al., 2009) and from Nigeria (Oyededeji et al., 2005) have also been studied.

Monoterpenes, sesquiterpenes and benzenoids (e.g. dillapiole and myristicin) have been identified as the main compounds in *P. guineense*. In addition to their importance as a spice and for the flavoring of food products, this *Piper* species is also used in traditional African medicine, because of its various pharmacological effects (e.g. antimicrobial, insecticidal, anticonvulsive, antihypertensive, sedative and tranquillizing activities (Jirovetz et al., 2002). An investigation of the volatile compounds that contribute to the flavor characteristics of *P. guineense* from South East Nigeria by means of GC-MS has not been performed previously. Therefore, the objective of this study was to identify the entire volatile compounds of the leaves and seeds of *P. guineense* responsible for the characteristic aroma of these natural flavourings and spices and to compare the volatile constituents in the leaves and seeds of the *P. guineense* plant.

## MATERIALS AND METHODS

The seeds and leaves of *P. guineense* were purchased from Umuahia modern market, Urbani Ibeku Abia State, Nigeria. The leaves and seeds of *P. guineense* were separated from the stem, sorted to remove debris. The leaves and seeds were washed separately and oven dried at 65°C for 4 h and milled into powder before further analysis.

**Table 1.** Percent composition of the different volatile compounds identified in the leaves and seeds of *Piper guineense* (%).

| S/N | Compounds    | Samples (%)  |             |
|-----|--------------|--------------|-------------|
|     |              | Uziza leaves | Uziza seeds |
| 1   | Acids        | 65.56        | 53.72       |
| 2   | Esters       | 25.63        | 5.22        |
| 3   | Alcohol      | -            | 1.90        |
| 4   | Hydrocarbons | 6.96         | 11.47       |
| 5   | Others       | 1.88         | 27.66       |

## Determination of volatile compounds using gas chromatographic-mass spectral analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising of a AOC-20i auto-sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µm df, composed of 100% dimethylpoly diloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 0.5 µL was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature of 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5 C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and Fragments from 40 to 450 Da. Total GC running time was 36 min. N/B: Before GC-MS analysis, the plant is subjected to cold extraction process using ethanol as the solvent.

## Identification of components

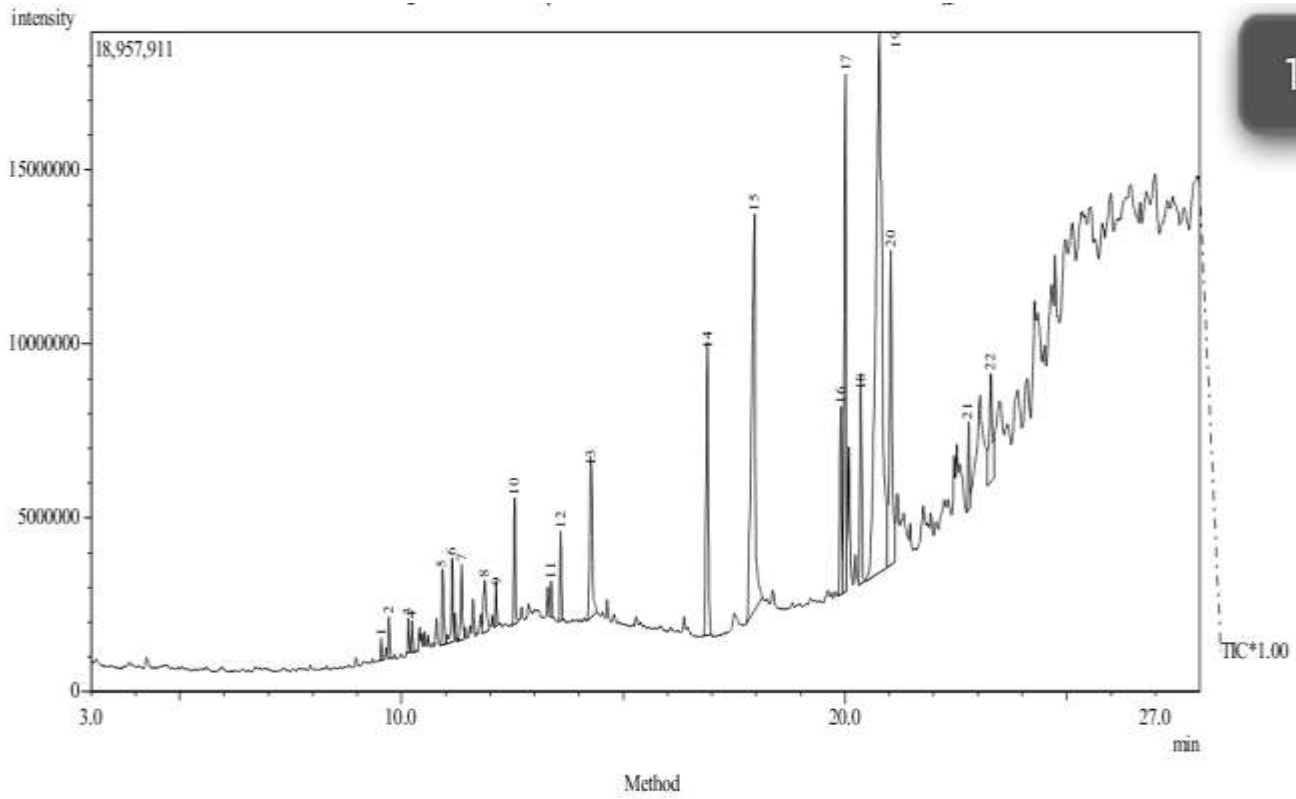
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

## RESULTS AND DISCUSSION

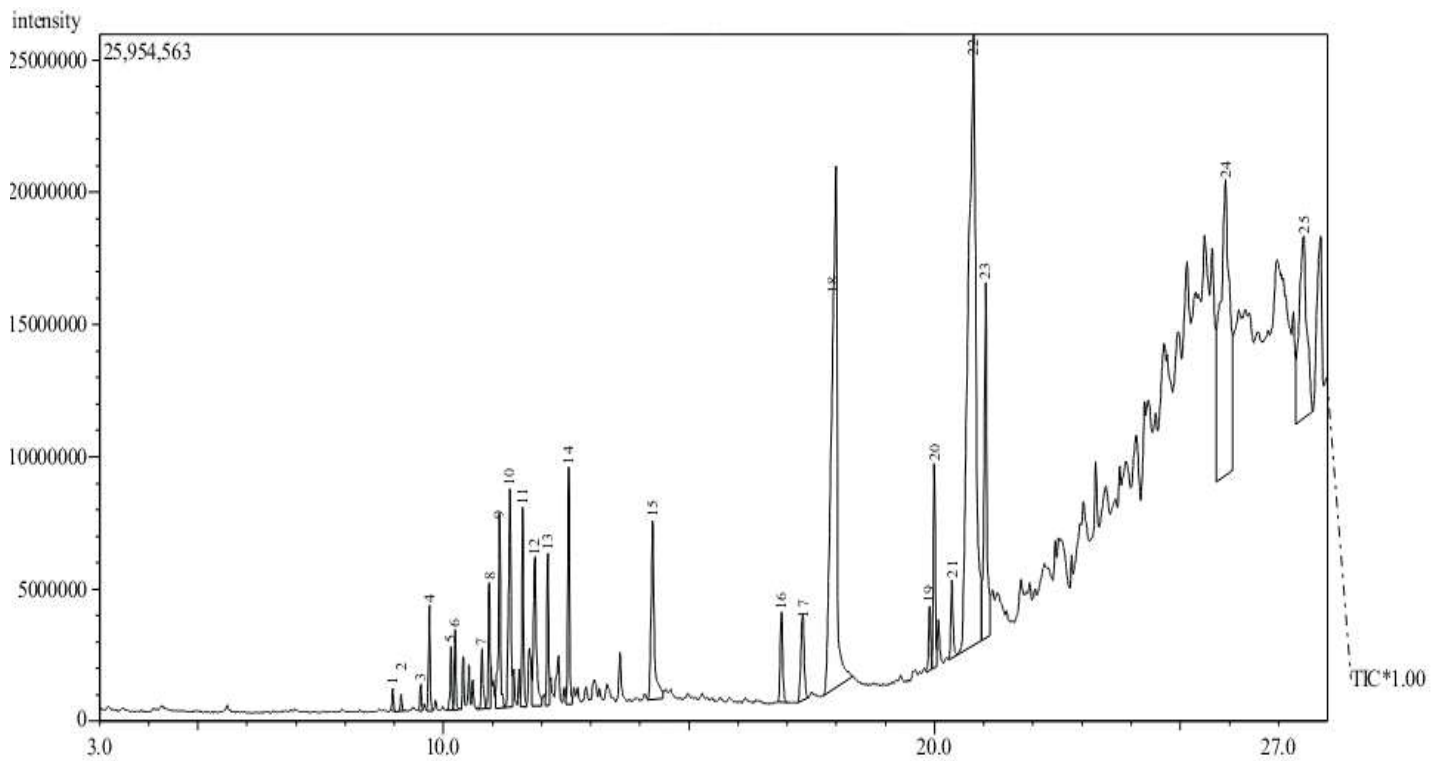
The volatile compounds of the leaves and seeds of *P. guineense* analyses using GC-MS are shown in Tables 1 and 2. The gas chromatogram showed the relative concentrations of various compounds getting eluted as a function of retention time (Figures 1 and 2). The heights of the peak indicate the relative concentrations of the compounds of the components present in the plant extract (Negbenebor et al., 1999). A total of thirty-three volatile constituents were identified in the leaves and seeds of *P. guineense*. The compounds which were identified by GC-MS analysis were twenty-two for the leaves and twenty-five for the seeds of *P. guineense*.

**Table 2.** Concentrations of volatile compounds in the leaves and seeds of *Piper guineense*.

| S/N                 | Retention time (minute) | Compounds  | Area  |              | Normalized (%) |
|---------------------|-------------------------|--|---|--------------|----------------|
|                     |                         |  | Formula   | Uziza leaves | Uziza seeds    |
| <b>Acids</b>        |                         |  |   |              |                |
| 1.                  | 11.858                  | Dodecanoic acid  | C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>                | -            | 2.48           |
| 2.                  | 11.875                  | Dodecanoic acid  | C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>                | 1.47         | -              |
| 3.                  | 14.258                  | Tetradecanoic acid   | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>                | -            | 3.26           |
| 4.                  | 14.267                  | Tetradecanoic acid   | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>                | 4.03         | -              |
| 5.                  | 17.942                  | n-Hexadecanoic acid  | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>                | -            | 16.28          |
| 6.                  | 17.958                  | n-Hexadecanoic acid  | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>                | 15.14        | -              |
| 7.                  | 20.783                  | Oleic Acid   | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                | 33.37        | -              |
| 8.                  | 20.800                  | Oleic Acid   | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                | -            | 26.09          |
| 9.                  | 21.033                  | Octadecanoic acid  | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                | 8.22         | -              |
| 10.                 | 21.042                  | Octadecanoic acid  | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                | -            | 5.61           |
| 11.                 | 23.292                  | Eicosanoic acid  | C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>                | 3.33         | -              |
| <b>Esters</b>       |                         |  |   |              |                |
| 12.                 | 13.592                  | Methyl tetradecanoate  | C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>                | 1.19         | -              |
| 13.                 | 16.883                  | Pentadecanoic acid,14-methyl-,methyl ester                           | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>                | -            | 1.28           |
| 14.                 | 16.892                  | Hexadecanoic acid,methyl ester                                       | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>                | 6.53         | -              |
| 15.                 | 19.900                  | 9,12-Octadecadienoic acid, methyl ester (E,E)                        | C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>                | -            | 0.74           |
| 16.                 | 19.908                  | 9,12-Octadecadienoic acid,methyl ester (E,E)-                        | C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>                | 3.42         | -              |
| 17.                 | 19.992                  | 11-Octadecenoic acid, methyl ester                                   | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                | -            | 2.26           |
| 18.                 | 20.008                  | 11-Octadecenoic acid, methyl ester                                   | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                | 9.64         | -              |
| 19.                 | 20.350                  | Octadecanoic acid methyl ester                                       | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>                | 3.49         | 0.94           |
| 20.                 | 22.792                  | Eicosanoic acid, methyl ester  | C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>                | 1.36         | -              |
| <b>Alcohol</b>      |                         |  |   |              |                |
| 21.                 | 11.617                  | 3,5,7-Cycloheptatriene-1,3-dimethanol                                | C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>                 | -            | 1.90           |
| 22.                 |                         |  |   |              |                |
| <b>Hydrocarbons</b> |                         |  |   |              |                |
| 23.                 | 8.967                   | 1, 3, 6-Heptatriene, 2, 5, 5-trimethyl                               | C <sub>10</sub> H <sub>16</sub>                               | -            | 0.17           |
| 24.                 | 9.142                   | Copaene  | C <sub>15</sub> H <sub>24</sub>                               | -            | 0.14           |
| 25.                 | 9.542                   | Cedrene  | C <sub>15</sub> H <sub>24</sub>                               | 0.26         | -              |
| 26.                 | 9.542                   | trans-alpha-Bergamotene  | C <sub>15</sub> H <sub>24</sub>                               | -            | 0.22           |
| 27.                 | 9.717                   | Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)              | C <sub>15</sub> H <sub>34</sub>                               | 0.60         | 1.00           |
| 28.                 | 10.158                  | 1, 3, 6, 10-Dodecatetraene,3, 7, 11-trimethyl-,(Z,E)                 | C <sub>15</sub> H <sub>24</sub>                               | 0.44         | 0.62           |
| 29.                 | 10.242                  | gamma-Elemene  | C <sub>15</sub> H <sub>24</sub>                               | 0.41         | 0.72           |
| 30.                 | 10.783                  | Aromadendrene  | C <sub>15</sub> H <sub>24</sub>                               | -            | 0.66           |
| 31.                 | 10.925                  | Tridecane  | C <sub>13</sub> H <sub>28</sub>                               | 1.27         | -              |
| 32.                 | 10.933                  | Cyclopropane, 1-(2-methylene-3-butenyl)-1-(1-methylenepropyl         | C <sub>12</sub> H <sub>18</sub>                               | -            | 1.14           |
| 33.                 | 11.150                  | beta-Myrcene   | C <sub>10</sub> H <sub>16</sub>                               | -            | 2.26           |
| 34.                 | 11.150                  | Cyclohexene,1-methyl-4-(5-methyl-1-methyl-4-hexenyl)                 | C <sub>15</sub> H <sub>24</sub>                               | 1.49         | -              |
| 35.                 | 11.358                  | Cyclohexe,3-(1,5-dimethyl-4-hexenyl)-6-methylene,[S-(R*,S*)]-        | C <sub>15</sub> H <sub>24</sub>                               | 1.43         | 2.95           |
| 36.                 | 12.142                  | Undecane   | C <sub>11</sub> H <sub>24</sub>                               | 0.56         | -              |
| 37.                 | 13.375                  | Heptadecane,2, 6-dimethyl-   | C <sub>19</sub> H <sub>40</sub>                               | 0.50         | -              |
| 38.                 | 17.325                  | Cyclohexene,1-nonyl-   | C <sub>15</sub> H <sub>28</sub>                               | -            | 1.59           |
| <b>Others</b>       |                         |  |   |              |                |
| 39.                 | 12.125                  | 1-Hydroxyl-1, 7-dimethyl-4-isopropyl-2, 7-cyclodecadiene             | C <sub>15</sub> H <sub>26</sub> O                             | -            | 1.48           |
| 40.                 | 12.550                  | Pyridine,3-(5-phenyl-4H-1, 2, 4-triazol-3-yl                         | C <sub>13</sub> H <sub>10</sub> N <sub>4</sub>                | 1.88         | 2.36           |
| 41.                 | 25.917                  | 3-[(4-methoxyl-benzoyl)-hydrazono]-N-(1-phenyl-ethyl)-butyramide     | C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>3</sub> | -            | 15.94          |
| 42.                 | 27.508                  | 3-[2-(3, 4-Dimethoxy-phenyl)-2-oxo-ethyl-3H-[1, 3, 4]oxadiazol-2-one | C <sub>18</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub> | -            | 7.88           |



**Figure 1.** Chromatograms of the volatile compounds in the leaves of *Piper guineense*.



**Figure 2.** Chromatograms of the volatile compounds in the seeds of *Piper guineense*.

The thirty-three volatiles are of various types; acids, esters, alcohol, hydrocarbons (mainly terpenes) and others (Table 1). Acids are the dominant constituent group from the result shown in Table 1 and constitute over 50% of total volatiles in the leaves and seeds of *P. guineense*. Ali and Ibiam (2014), also identified acids in their work on the phytochemical studies and GC-MS analysis of *Gongronema latifolium* and *P. guineense*. Acids identified in the leaves and seeds of *P. guineense* include dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, oleic acid, octadecanoic acid and eicosanoic acid. They however occurred at different retention times. The highest concentration of the acids were found in oleic acids - 33.37% in *P. guineense* leaves and 26.09% in *P. guineense* seeds at retention time of 20.783 mins and 20.800 mins, respectively. However, oleic acid and hexadecanoic acid have been reported in the leaves of *G. latifolium* (Ali and Ibiam, 2014). Jirovetz et al. (2002) in their study of the aroma compounds in black *Piper nigrum*, white *P. guineense* and black *P. guineense* also identified some acids (acetic acid, propanoic acid, butanoic acid, nonanoic acid). Some of these acids are used in various industries as flavouring agents. Some organic acids have been determined as major aroma compounds in Korean soy sauces and barley bran sauces (Steinhaus and Schieberle, 2007).

Table 1 also shows that esters were also identified in the volatile compound analysis. The leaves of *P. guineense* had higher ester concentration than the seeds. Octadecanoic acid methyl ester occurred at the same retention time of 20.350 min in the leaves and seeds of *P. guineense*. But their concentrations were different; *P. guineense* leaves had a higher concentration of 3.49%, while the *P. guineense* seeds had 0.94%. Other ester compounds shown in Table 2 occurred at different time and with different constituents. Jirovetz et al. (2002) also identified esters (benzyl benzoate, phenylethyl benzoate) in *P. nigrum* and *P. guineense*. Esters, mainly formed by esterification of carboxylic acids and alcohols were reported to determine the characteristic pleasant aromatic notes (Klesk and Qian, 2003). The importance of ester contributions toward food aroma is undisputed with the fact that esters with low carbon atoms are highly volatile at ambient temperatures and the perception thresholds are ten times lower than their alcohol precursors (Nogueira et al., 2005). In addition to imparting a fruity floral character, esters can diminish or mask the sharpness of unpleasant FFA-derived notes. These esters are formed by esterification between the short-chain FFAs and the alcohols (Qin and Ding, 2007). 3,5,7-Cycloheptatriene-1,3-dimethanol was the only alcohol identified at 1.90% in the seeds of *P. guineense* at 11.617 min retention time. Alcohols are known to act as antifungal and prevent food spoilage (Onyenekwe et al., 2012).

Hydrocarbons (mainly terpenes) were also identified in

the leaves and seeds of *P. guineense* plant. The seeds of *P. guineense* had higher concentration of the hydrocarbons which were mainly terpenes. Beta-myrcene (monoterpene) was identified only in the seeds of *P. guineense* at 2.26% concentration at a retention time of 11.150 min. Gamma-elemene (sesquiterpenes) was identified in both the leaves and the seeds of *P. guineense* at the same retention time of 10.242 min at concentrations of 0.41 and 0.72%, respectively. Other hydrocarbon sesquiterpenes identified only in the seeds of *P. guineense* include copaene, trans-alpha-bergamotene and aromadendrene. Owolabi et al. (2013) in their study of the aroma chemical composition of *P. guineense* from Lagos identified myrcene, alpha-copaene and beta-elemene in fruit (berries) of the plant. They also reported linalool as the major oil responsible for their characteristic flavor. Cedrene is a sesquiterpene that was identified only in the leaves of *P. guineense* at concentration of 0.26%. Jirovetz et al. (2002) reported that monoterpenes, sesquiterpenes and benzenoids have been identified as the main compounds in *P. guineense* responsible for their characteristic flavor. They also identified similar aroma compounds in their analysis of the seeds of black *P. nigrum*, white *P. guineense* and black *P. guineense*. Some of the similar terpenes identified include myrcene, gamma-elemene, copaene and trans-alpha-mergamotene.

They reported that monoterpenes and sesquiterpenes particularly were found to be of essential importance for the fine and pleasant pepper aroma of the black pepper (*P. nigrum*) and "Ashanti pepper" (*P. guineense*). Sruthi et al. (2013) in their study on the correlation between chemical profiles of black pepper (*P. nigrum*) collected from different locations in India reported the presence of monoterpenes like thujene, alpha-pinene, sabinene, limonene, alpha-phellandrene and linalool in samples collected at relatively higher altitudes as compared to plains. Other volatile compounds that were identified in the leaves and seeds of *P. guineense* were 1-hydroxyl-1, 7-dimethyl-4-isopropyl-2,7-cyclodecadiene, pyridine,3-(5-phenyl-4H-1, 2, 4-triazol-3-yl), 3-[(4-methoxyl-benzoyl)-hydrazono]-N-(1-phenyl-ethyl)-butyramide and 3-[2-(3, 4-dimethoxy-phenyl)-2-oxo-ethyl-3H-[1, 3, 4] oxadiazol-2-one. Pyridine,3-(5-phenyl-4H-1, 2, 4-triazol-3-yl) occurred in both the leaves and seeds of *P. guineense* at the same retention time of 12.550 min with concentrations 1.88 and 2.36%, respectively.

## Conclusion

A total of thirty-three volatile compounds were identified in the leaves and seeds of *P. guineense* comprising acids, esters, alcohols, hydrocarbons (mainly monoterpenes and sesquiterpenes) and others. The different volatile constituents isolated using the gas chromatography-mass spectrometry contributes to the

aroma in the leaves and seeds of *P. guineense* and the final characteristic aroma of *P. guineense* leaves and seeds depend on the balance and interaction between the different components identified.

### Conflict of interests

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. The authors have not declared any conflict of interests.

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