



## Chemical Investigation and Antibacterial Activity of the Leaves of *Peperomia pellucida* L. HBK (*Piperaceae*)

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### Abstract

The chemical components of the ethanolic extract of the leaves of *Peperomia pellucida* was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) technique and fifteen compounds were identified which include bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene(2.27%), 10,12-octadecadiynoic acid(2.81%), 3,7,11,11-tetramethylbicyclo [8.1.0] undeca-2,6-diene (5.45%),2,6-bis (1,1-dimethylethyl)-4-methyl phenol (1.18%), 1,2-dimethoxy-4-(2-methoxyethenyl) benzene(8.09%), 1,3-benzodioxole, 4,7-dimethoxy-5-(2-propenyl)(14.96%), oxalic acid, cyclohexylmethyl tridecyl ester(1.48%), ethyl alpha-d-glucopyranoside (10.91%), hexadecanoic acid methyl ester(9.13%), hexadecanoic acid ethyl ester(3.30%), 10-octadecenoic acid methyl ester(15.32%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(16.61), (Z)6,(Z)9-pentadecadien-1-ol(3.87%), 9,12,15-octadecatrienoic acid ethyl ester(2.73%) and N,N-dimethyldodecanamide(1.88%).The extract exhibited marked antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*. The synergistic effect of these compounds might have caused the high antibacterial activity shown by the extract against the test organisms. These results therefore offer cogent evidence that strengthens the reason behind the wide applicability of *Peperomia pellucida* in herbal medicine in Nigeria.

**Keywords:** *Peperomia pellucida*, Chemical Investigation, GC-MS Analysis, Antibacterial Activity, Herbal Medicine.

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### 1. Introduction

There is no gainsaying that plants remain the greatest source of bioactive compounds which can be developed and used as drugs. A lot of them are still being used raw in herbal medicine for disease prevention and treatment. One of such plants is *Peperomia pellucida* which prompted research on its chemical constituents as well as its antibacterial efficacy. *Peperomia pellucida* L. HBK belongs to the family *Piperaceae* comprising about 5 genera and 1400 species. The plant is found in Nigeria, all over Asia and in many South American countries growing in clumps,

thriving in loose, humid soils under the shade of trees and a tropical to subtropical climate. *P. pellucida* is an annual, shallow rooted herbaceous plant that grows to a height of 15 to 45 cm. It is characterized by succulent stems, shiny light-green, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. The plant flowers year-round [1,2,3,4,5].

The leaves of *P. pellucida* are used topically for treatment of athlete foot and skin infections caused by bacteria and fungi in Igbo tribe of South Eastern Nigeria. The plant has been reported to be used ethno-medicinally for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders and rheumatic joint pain [6] and to treat breast cancer, impotence, measles, mental disorders and smallpox [7]. The roots are used to treat fevers and the aerial parts are used as dressing for wounds [8] while the whole plant has been reported to be used in stopping haemorrhage when crushed, mixed with water, heated and then orally administered [8]. The chloroform extracts of *P. pellucida* have been reported to exhibit antifungal activity against *Trichophyton mentagrophytes* in vitro [9] while the whole plant showed anti-malarial activity [8, 10]. In North Eastern Brazil, the plant has been used as a diuretic and to treat proteinuria; and in the Amazon region, it has been used as a cough suppressant, diuretic and emollient and to treat cardiac arrhythmia [2,3,4]. It has also been reported that the extracts from *P. pellucida* exhibited cytotoxicity against the cancer cell lines HL-60, MCF-7 and Hela[11]. The anti-inflammatory, chemotherapeutic and analgesic properties of the crude extracts of *P. pellucida* have been reported [2, 7]. The analgesic properties of the plant seemed to be related to its effect on prostaglandin synthesis [7].

*P. pellucida* has been used as a food item as well as a medicinal herb. Although mostly grown for its ornamental foliage, the entire plant is edible, both cooked and raw [7]. As aforementioned, the plant is abundantly used in herbal medicine in South Eastern Nigeria for the treatment of athlete foot, eczema, ringworm, boils and other skin infections caused by fungi and bacteria. This research is predicated on exploring the bioactive compounds present in the leaves of this plant and their bio-effect. Herein is therefore reported the chemical investigation and antibacterial activity of *Peperomia pellucida* leaves.

## 2. Materials and methods

### Experimental

GC analyses were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 60-280°C held at 60°C for 1 minute, and at 160°C for 2 minutes (rate 10°C/min), at 220°C for 3 minutes (rate 10°C/min) and finally at 280°C for another 2 minutes (rate 10°C/min), injection temperature 220°C. GC-MS (Gas chromatography mass spectrometry) analysis was conducted using GCMS-QP 2010 Plus Shimadzu Japan with column oven temperature of 60°C, injection mode was split, flow control mode was linear velocity, carrier gas pressure was 100.2 Kpa, total flow was 6.2 mL/min, column flow was 1.61 mL/min, linear velocity was 46.3 cm/sec, purge flow was 3.0 mL/min and split ratio was 1.0. Also, ion source temperature was 200°C, interface temperature was 250°C, solvent cut time was 2.5 min., detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spec., start time was 3.0 min., end time was 28.0 min, event time was 0.5 sec, scan speed was 1250, and start m/z was 50 while end m/z was 600. The scan range was 1171-2640. The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge Germany was used. All solvents used were all of analytical grade and were procured from Merck, Germany.

### Plant Materials

*Peperomia pellucida* leaves were harvested from Avodim Ubakala, Umuahia South Local Government Area of Abia State, South Eastern Nigeria on November 19, 2013. The plant materials were identified and authenticated on November 21, 2013 by Mr. I. K. Ndukwe, a specialist in Plant Taxonomy of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were then dried for 30 days and thereafter milled into a uniform and fine powder by a mechanically driven attrition mill.

### Extraction of Plant Materials

The powdered plant samples (400 g) were successfully extracted with 2 L of ethanol (8hrs/3 times/30°C). The extract was concentrated under reduced pressure and the supernatant extract was decanted (6.81g) after complete removal of the solvent. The extract was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant extract was subjected to systematic GC-MS analysis [12,13].

### Components Identification

The components of the extracts were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [14, 15].

### Antibacterial Activity

The *in vitro* antibacterial activity of the leaf extract of *Peperomia pellucida* was carried out for 24h culture of six selected bacteria i.e. three gram-positive and three gram-negative bacteria. The bacteria organisms used were *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the

Central Laboratory Services Unit of National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 15 minutes. They were however used within 48 hours of production. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique [16, 17]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculums was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs was carefully place on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 hours in an incubator at 37°C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimetre rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the extract having different zones and selecting the lowest concentration.

### 3. Results and Discussion

The ethanolic extract of the leaves of *Peperomia pellucida* showed fifteen peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of fifteen compounds (1-15) in the extract (Figs. 2-17). The molecular formulae, percentage composition and molecular masses of these compounds are shown in Table 1. The compounds comprise sesquiterpenes (7.72%), fatty acid (2.81%), phenol (1.18%), methoxy benzene (8.09%), benzodioxole (14.96%), fatty acid ester (31.96%), glycoside (10.91%), alcohol (20.48%) and alkaloid (1.88%).

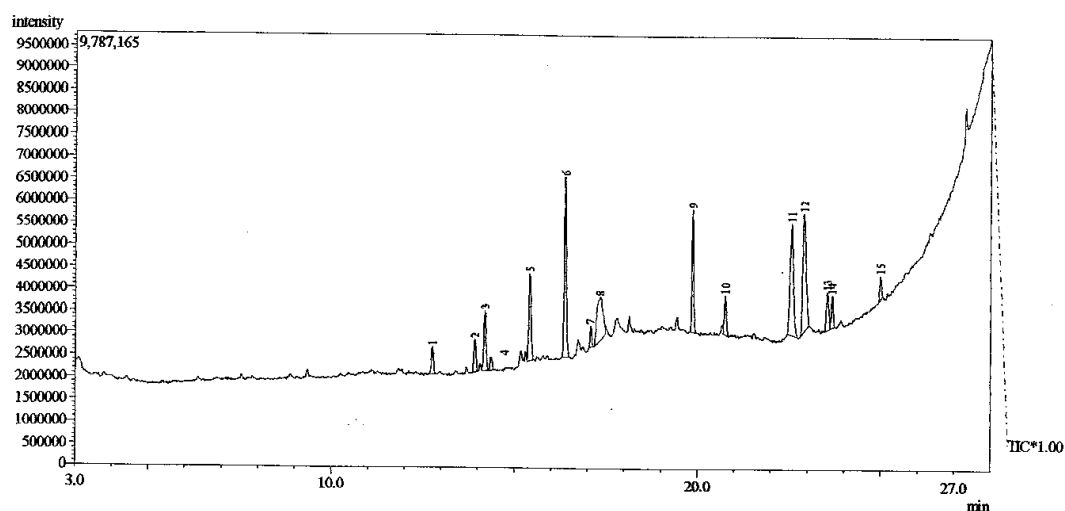


Fig. 1: GC-MS chromatogram of ethanol extract of *Peperomia pellucida*

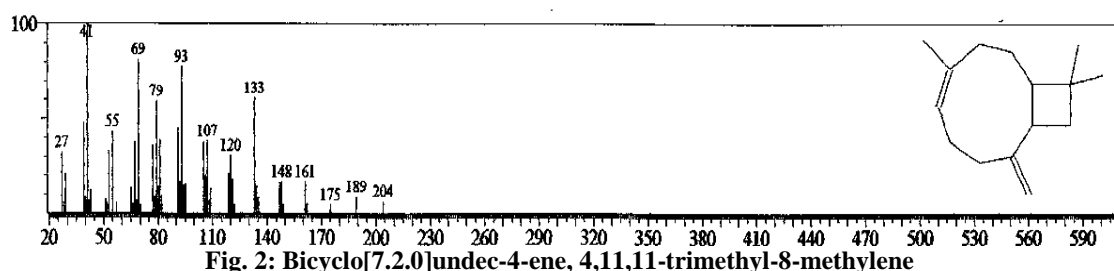


Fig. 2: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene

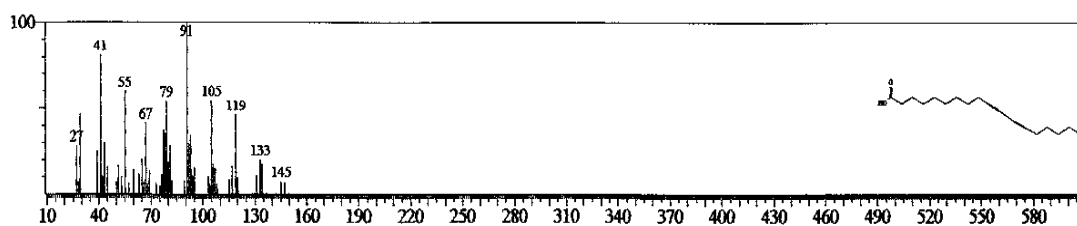


Fig. 3: 10,12-Octadecadienoic acid

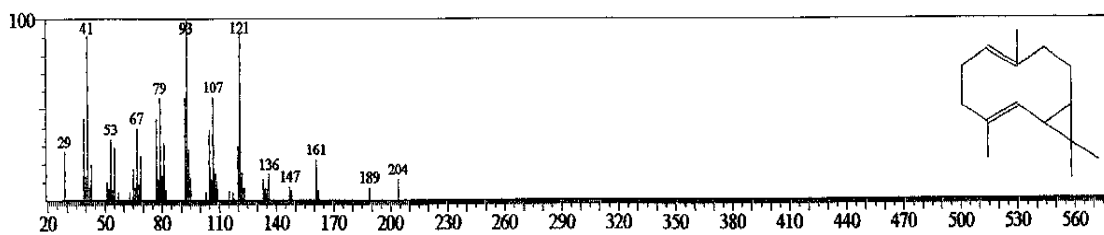


Fig. 4: 3, 7, 11, 11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene

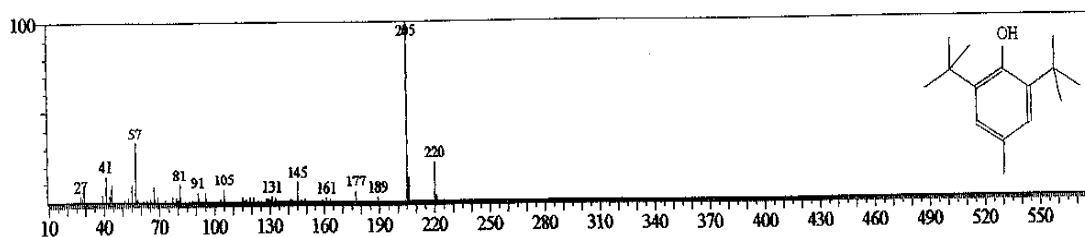


Fig. 5: 2,6-bis(1,1-dimethylethyl)-4-methyl phenol

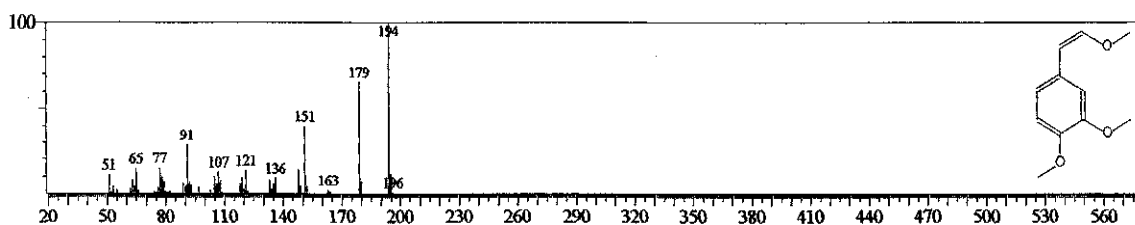


Fig. 6: 1,2-Dimethoxy-4-(2-methoxyethenyl) benzene

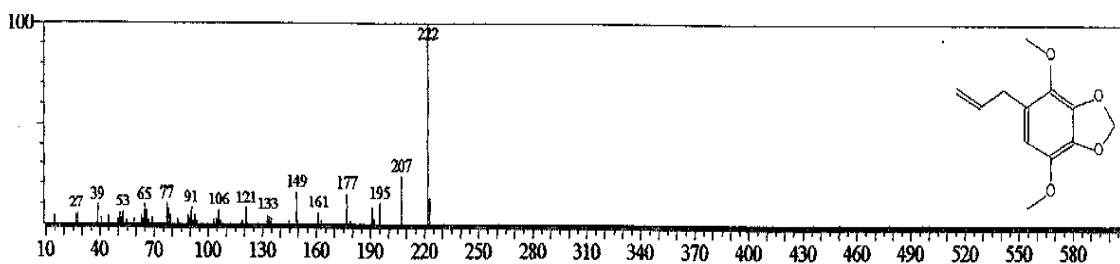


Fig. 7: 1,3-Benzodioxole, 4,7-dimethoxy-5-(2-propenyl)

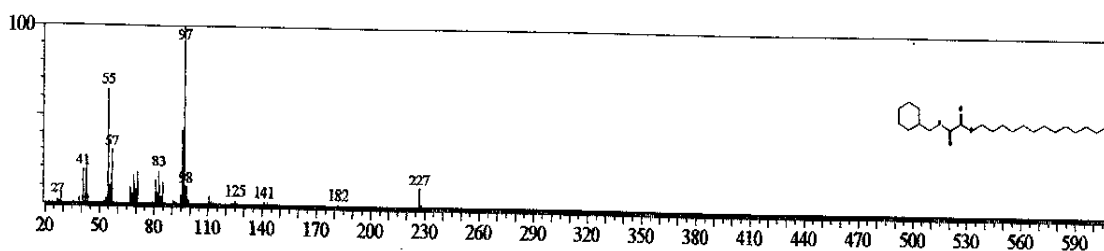


Fig. 8 : Oxalic acid, cyclohexylmethyl tridecyl ester

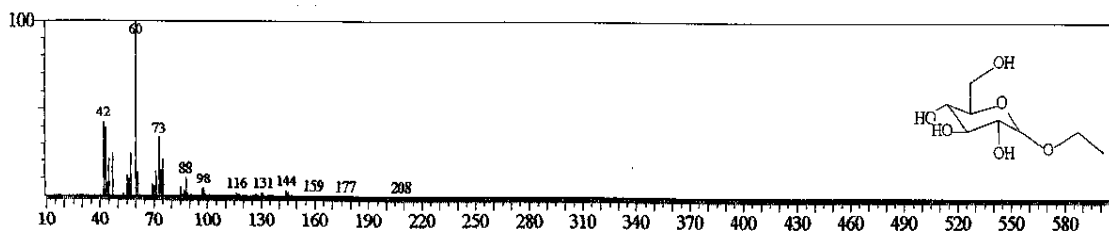


Fig. 9: Ethyl alpha-d-glucopyranoside

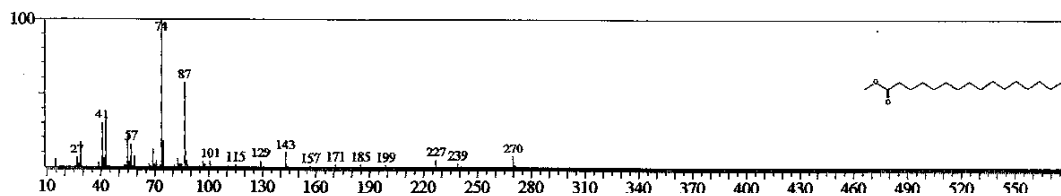


Fig. 10: Hexadecanoic acid methyl ester

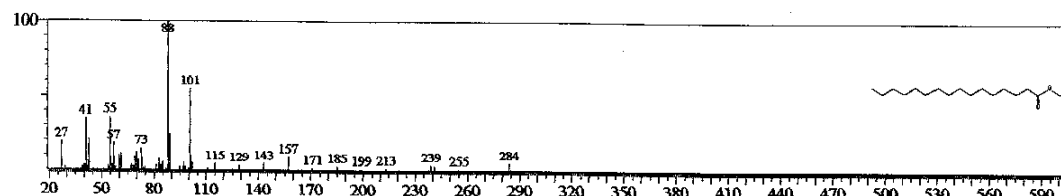


Fig. 11: Hexadecanoic acid ethyl ester

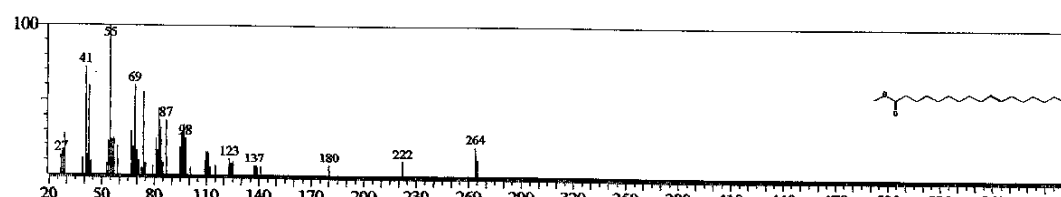


Fig. 12: 10-Octadecenoic acid methyl ester

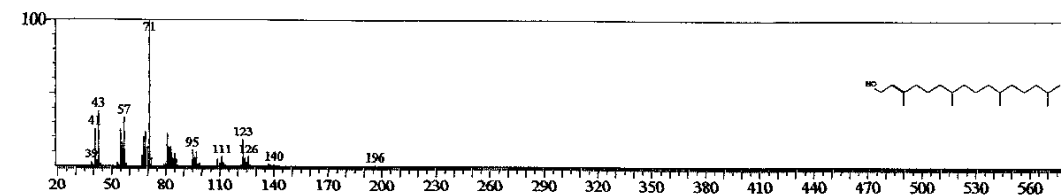


Fig. 13: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

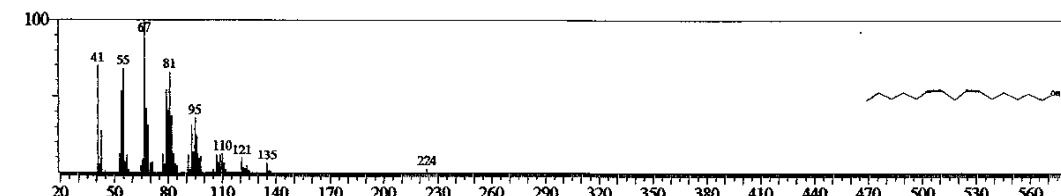


Fig. 14: (Z)6,(Z)9-Pentadecadien-1-ol

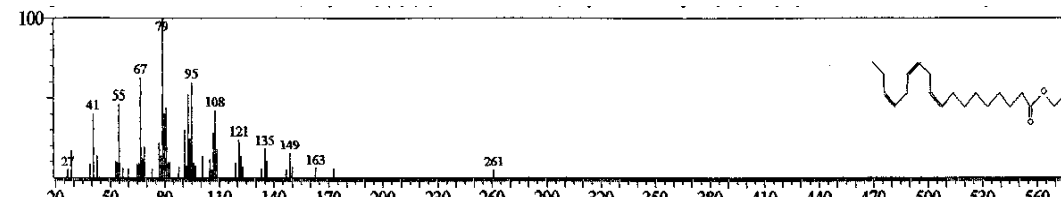


Fig. 15: 9,12,15-Octadecatrienoic acid ethyl ester

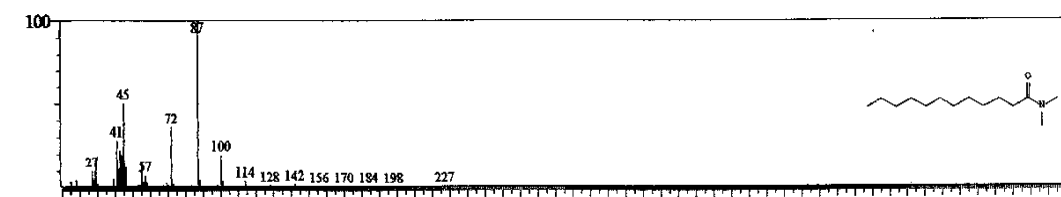
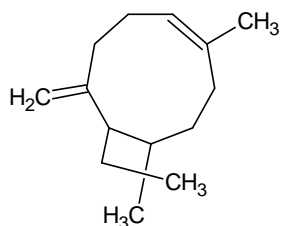
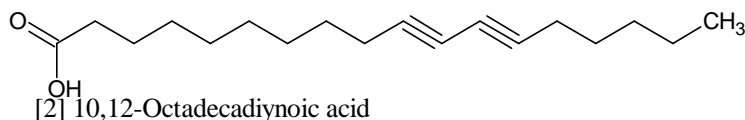


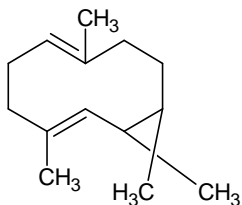
Fig. 16: N,N-Dimethyldodecanamide



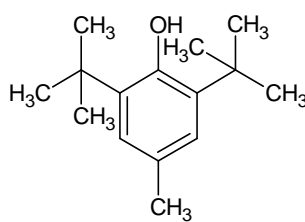
[1] Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene



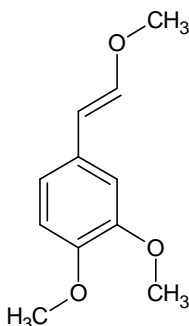
[2] 10,12-Octadecadiynoic acid



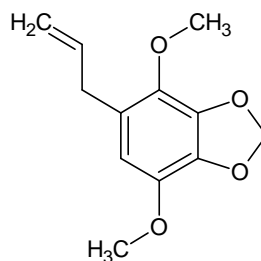
[3] 3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene



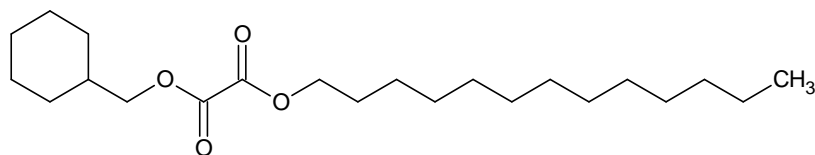
[4] 2,6-bis(1,1-dimethylethyl)-4-methyl phenol



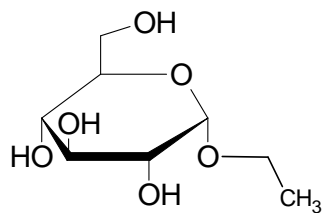
[5] 1,2-Dimethoxy-4-(2-methoxyethenyl)benzene



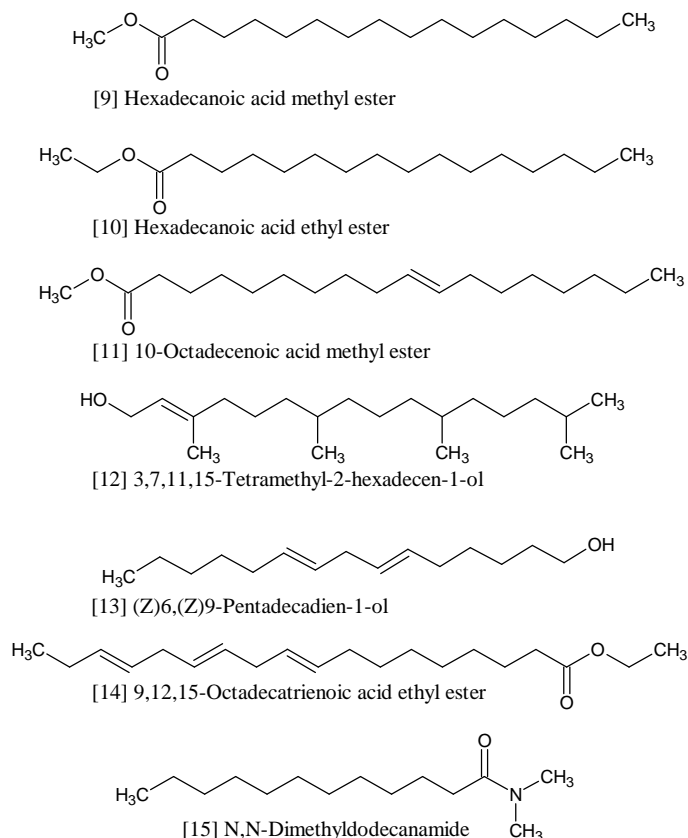
[6] 1,3-Benzodioxole, 4,7-dimethoxy-5-(2-propenyl)



[7] Oxalic acid cyclohexylmethyl tridecyl ester



[8] Ethyl alpha-D-glucopyranoside

Fig. 17: Structures of phytochemicals from the ethanolic extract of *Peperomia pellucida* leaves**Table 1: Phytochemicals identified from the GC-MS analysis of the leaf extract of *Peperomia pellucida*.**

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak area(%)	Nature of compound
1	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	C <sub>15</sub> H <sub>24</sub>	204	12.751	2.27	Sesquiterpene
2	10,12-Octadecadiynoic acid	C <sub>18</sub> H <sub>28</sub> O <sub>2</sub>	276	13.912	2.81	Fatty acid
3	3,7,11,11-Tetramethylbicyclo[8.1.0]undec-2,6-diene	C <sub>15</sub> H <sub>24</sub>	204	14.180	5.45	Sesquiterpene
4	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	C <sub>15</sub> H <sub>24</sub> O	220	14.353	1.18	Phenol
5	1,2-Dimethoxy-4-(2-methoxyethenyl) benzene	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	15.408	8.09	Methoxy benzene
6	1,3-Benzodioxole, 4,7-dimethoxy-5-(2-propenyl)	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222	16.371	14.96	Benzodioxole
7	Oxalic acid, cyclohexylmethyl tridecyl ester	C <sub>22</sub> H <sub>40</sub> O <sub>4</sub>	368	17.078	1.48	Fatty acid ester
8	Ethyl alpha-d-glucopyranoside	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	208	17.332	10.91	glycoside
9	Hexadecanoic acid methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	19.853	9.13	Fatty acid ester
10	Hexadecanoic acid ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	20.748	3.30	Fatty acid ester
11	10-Octadecenoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	22.574	15.32	Fatty acid ester
12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O	296	22.899	16.61	Alcohol
13	(Z)6,(Z)9-Pentadecadien-1-ol	C <sub>15</sub> H <sub>28</sub> O	224	23.535	3.87	Alcohol
14	9,12,15-Octadecatrienoic acid ethyl ester	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306	23.668	2.73	Fatty acid ester
15	N,N-Dimethyldodecanamide	C <sub>14</sub> H <sub>29</sub> NO	227	24.992	1.88	Amide (Alkaloid)

As shown in Table 2, six bacteria organisms were used for the antibacterial activity of the leaf extract of *Peperomia pellucida* i.e. three gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus*) and three gram-negative (*Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*) bacteria. These microorganisms are human pathogens that have been involved in causing diseases and infections in man [16]. Some of the diseases they can cause include skin infections, pneumonia, meningitis, bacteraemia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, diarrhoea, anaemia, typhoid fever, kidney infections, osteomyelitis, endocarditis, septicaemia and wound infections [19,20,21,22,23,24,25].

The minimum inhibitory concentration (MIC) of *P. pellucida* leaf extract was 50-100%. From the result, greater antibacterial activity was shown against *E. faecalis* and *P. mirabilis* suggesting that the leaves of *P. pellucida* could be used in the treatment of endocarditis and bacteraemia, urinary tract infections, meningitis, kidney infections, wound infections, septicaemia and pneumonias since these diseases are caused by them. From the GC-MS chemical analysis result of *P. pellucida* leaf extract, the leaf contains sesquiterpenes, glycosides, alkaloids and phenols. These compounds are bioactive and have been reported to exhibit antibacterial activities [26,27,28]. The ability of *P. pellucida* leaf extract to show potent inhibition on these human pathogens gives credence to its application in herbal medicine for the treatment of abdominal pain, abscesses, acne, boils, colic, fatigue, fever, gout, headache, renal disorders and rheumatic joint pain.

**Table 2: Antibacterial activities of the leaf extract of *Peperomia pellucida***

Test Microorganism	Concentration (%)				
	25	50	75	100	MIC (%)
<i>Staphylococcus aureus</i>	6.33	8.67	13.89	17.33	25
<i>Enterococcus faecalis</i>	8.67	10.46	15.68	19.77	25
<i>Bacillus cereus</i>	7.38	10.12	15.33	17.67	25
<i>Escherichia coli</i>	-	6.67	10.33	15.33	50
<i>Salmonella typhi</i>	6.33	7.57	11.67	17.12	25
<i>Proteus mirabilis</i>	7.67	10.33	15.51	18.67	25

Figures are in mm and include the diameter of the paper disc (5mm). Data are means of triplicate determinations.

MIC = Minimum Inhibitory Concentration

- = Zone of no inhibition

#### 4. Conclusion

Fifteen phytochemical compounds were observed from the GC-MS analysis of the leaves of *Peperomia pellucida* L. HBK. These constituents comprise primarily sesquiterpenes, glycosides, alkaloids and phenols. The extract proved to be potent antibacterial agent against the six organisms tested. The synergistic effect of these compounds could be the reason behind its antibacterial activity. This investigation further reveals the rationale behind the wide applicability of the leaves of *P. pellucida* in herbal medicine.

#### 5. Acknowledgement

We are grateful to Mr I. K. Ndukwe of Taxonomy Section, Forestry Department of Michael Okpara University of Agriculture, Umudike, Nigeria, for his kindness in identifying and authenticating our plant samples. We are also grateful to Mr Y. O. Usman of Instrumentation Unit, National Research Institute for Chemical Technology, Zaria, Nigeria, for his assistance in operating the GC-MS machine.

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