

## Chemical Characterization and Investigation of the Bio-effects of the Leaves of *Acanthus montanus* (*Acanthaceae*) on Some Selected Microorganisms

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**Abstract :** The leaves of *Acanthus montanus* are used in traditional herbal practices in South Eastern Nigeria and in some other parts of West Africa for the treatment of gonorrhoea, syphilis, wounds and boils. Other uses of *A. montanus* in herbal medicine include the treatment of hypertension, cardiac dysfunctions, hepatitis and heart diseases. The chemical constituents of the ethanolic extract of the leaves of *A. montanus* were characterized using Gas Chromatography-Mass Spectrometry (GC/MS) technique and nine compounds were identified which include 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (13.68 %), allyl(2-tetrahydrofuryl methoxy)dimethylsilane (3.86 %), sulfurous acid cyclohexylmethyl hexyl ester (5.67 %), alpha-methyl 4-methylmannoside (8.41 %), hexadecanoic acid methyl ester (16.12 %), 11-octadecenoic acid methyl ester (19.03 %), docosane (5.85 %), N,N-dimethylvaleramide (18.62 %) and 2,6,10,15-tetramethyl heptadecane (8.76 %). The extract exhibited marked antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. The presence of these bioactive compounds in the leaves of *A. montanus* could be the reason behind its bacterial extermination effects as well as its concomitant use in the treatment of diseases and infections in herbal medicine in Nigeria.

**Keywords:** *Acanthus montanus*, Chemical characterization, GC/MS analysis, Antibacterial activity

### 1. Introduction

The flora and fauna that exist and evolve within the rainforest vegetation of South Eastern Nigeria no doubt provide a lot of benefits to the people. Prominent among these benefits is the provision of plant materials used in herbal medicine. These herbal plants possess bioactive compounds that impart desirable therapeutic properties in humans. Most of the compounds in these plants are yet to be fully explored. It is against this backdrop that the chemical composition and antimicrobial effects of the leaves of *A. montanus* are being investigated with a view to substantiate claims of its use as antibiotics in herbal medicine in South-east Nigeria. *A. montanus* (Nees) T. Anders belongs to the family *Acanthaceae* and is native to West Africa. The plant grows luxuriantly in Nigeria. It is an erect, prickly perennial plant that can grow up to 2 m tall. It has clusters of oblong to lance-shaped glossy, dark green leaves reaching up to 30 cm<sup>1,2</sup>. Leaves are opposite, glossy and papery in texture, deeply pinnately-lobed and the lobes have spines. The stem is stout woody and sparsely branching. The plant prefers shady situations and occasional deep watering, but tolerates sunny, dry situations too. It can perfectly survive steep areas because of its aggressive roots. The plant has been introduced to the rest of the world as an ornamental plant<sup>1,2</sup>.

A decoction of the leaves of *A. montanus* is used in the treatment of hypertension, cardiac dysfunctions

and heart diseases in Nigeria and in some other countries of West Africa <sup>3</sup>. The leaves are used by Africans in the treatment of hepatitis and hepatosplenomegaly and these liver protective properties have been reported experimentally <sup>4</sup>. The anticonvulsant, sedative <sup>5</sup>, antispasmodic <sup>6</sup> and anti-abortifacient <sup>7</sup> activities of the leaves of *A. montanus* have also been experimentally reported. The leaves of *A. montanus* are used in traditional herbal medicine for the treatment of inflammatory conditions, pains and fever while the root is popularly used effectively in the treatment of boils in Nigeria <sup>8</sup>. In Cameroon, the leaf extracts had been used in the treatment of threatened abortion, menstrual irregularities, dysmenorrhoea, gonorrhoea and syphilis <sup>9, 10, 11</sup> while the traditional women of South-eastern Nigeria use the roots instead to treat dysmenorrhoea <sup>9</sup>. The macerated leaves of *A. montanus* are used to induce vomiting in children amongst the Geviya tribe of Gabon. Women with stomach-ache and nausea are given the young shoots cooked with peanut butter to relieve them of the complaints <sup>2</sup>. In South Eastern Nigeria, the leaves of *A. montanus* are used in traditional herbal practices for the treatment of gonorrhoea, syphilis, deep and dangerous wounds and boils among other uses. Farmers in Ubakala, Umuahia South Area of South Eastern Nigeria, use the fresh leaves of *A. montanus* to ward off rodents from their produce probably because of the thorns associated with the leaves. Given the above invaluable applications of the plant in herbal medicine, this research was aimed at chemically characterising and investigating the bio-effects of the leaves of *A. montanus* on some selected microorganisms.

## 2. Materials and Methods

### 2.1 Plant Materials

*A. montanus* leaves were harvested from Oyimo shady farm, Ubakala, Umuahia South Local Government Area of Abia State, South Eastern Nigeria on November 18, 2013. Identification and authentication were done on November, 21, 2013 at the Taxonomy Section of Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, where a herbarium specimen is on file with voucher number FHI 23965. The leaves were then dried under a shade and thereafter milled into a uniform and fine powder by a mechanically driven attrition mill.

### 2.2 Extraction of Plant Materials

The powdered plant samples (300 g) were successfully extracted with 2 L of ethanol (8 h/3 times/30 °C). The extract was concentrated under reduced pressure and the supernatant extract was decanted (7.23 g) after complete removal of the solvent. The extract was centrifuged at 10,000 rpm for 20 min and the clear supernatant extract was subjected to systematic GC/MS analysis.

### 2.3 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC analysis was 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 min, and at 160 °C for 2 min (rate 10 °C/min), at 220 °C for 3 min (rate 10 °C/min) and finally at 280 °C for another 2 min (rate 10 °C/min). The injection temperature was 220 °C. GC/MS analysis was conducted using GCMS-QP 2010 Plus Shimadzu Japan with column oven temperature of 60 °C. The carrier gas was Helium with a pressure of 100.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 6.2 mL/min, column flow was 1.61 mL/min, injection mode was split, flow control mode was linear velocity, 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 s, scan speed was 1250, and start m/z was 50 while end m/z was 600. 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 of analytical grade and were procured from Merck, Germany.

### 2.4 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 <sup>12,13</sup>.

### 2.5 Bioassay

The *in vitro* antimicrobial activity of the leaf extract of *A. montanus* was carried out for 24 h 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 Federal Medical Centre, Umuahia, 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 1 h to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminum foil and sterilized in an autoclave at 121 °C for 15 min. They were however, used within 48 h of production. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique<sup>14, 15</sup>. 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 inoculum 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 placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h 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 *A. montanus* showed nine peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of nine compounds (1-9) in the extract (Figs. 2-11). The molecular formulae, percentage composition and molecular masses of these compounds are shown in Table 1. The compounds comprise phenol (13.68 %), furysilane (3.86 %), sulphurous acid ester (5.67 %), glycoside (8.41 %), fatty acid ester (35.15 %), alkane (14.61 %) and alkaloid (18.62 %). The prevailing components observed in the extract include 11-octadecenoic acid methyl ester (19.03 %), N,N-dimethylvaleramide (18.62 %), hexadecanoic acid methyl ester (16.12 %), and 2,6-bis(1,1-dimethylethyl)-4-methyl phenol (13.68 %).

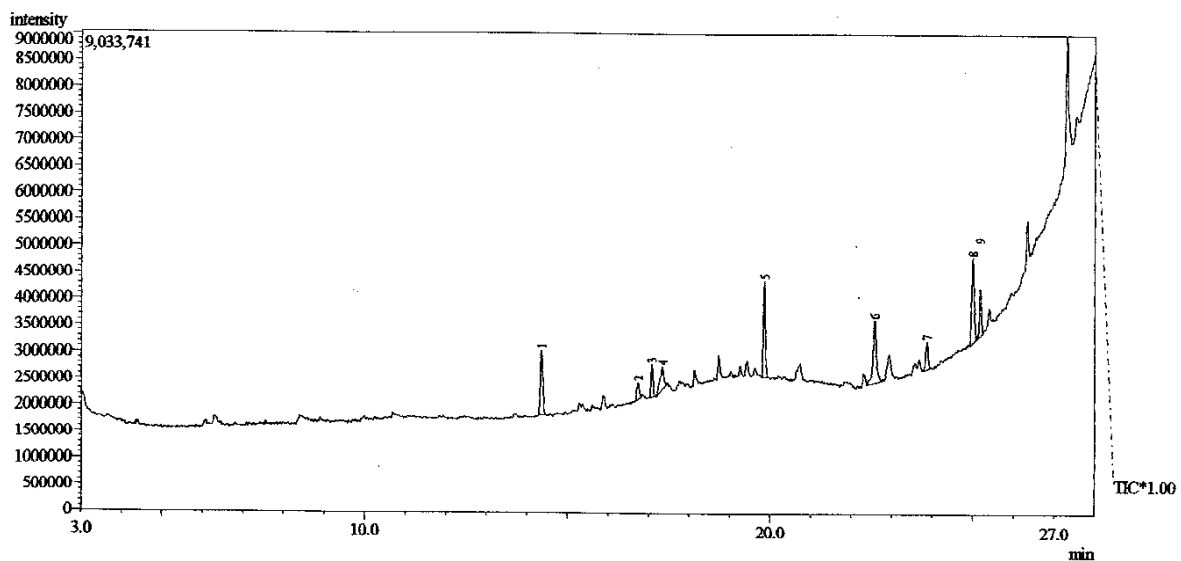


Fig. 1: GC/MS chromatogram of ethanolic extract of *A. montanus*

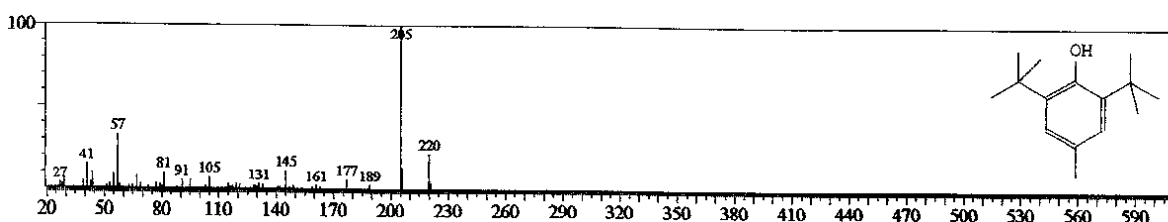


Fig. 2: 2,6-bis(1,1-dimethylethyl)-4-methyl phenol

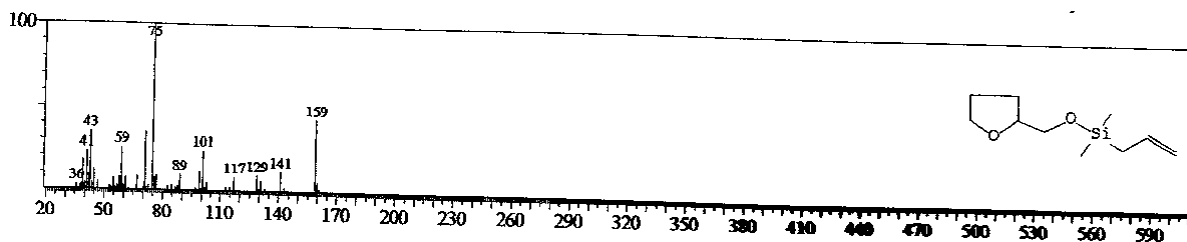


Fig. 3: Allyl(2-tetrahydrofurylmethoxy)dimethylsilane

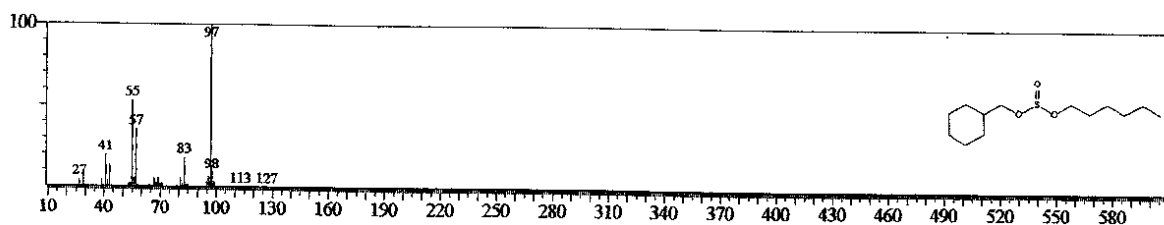


Fig. 4: Sulfurous acid, cyclohexylmethyl hexyl ester

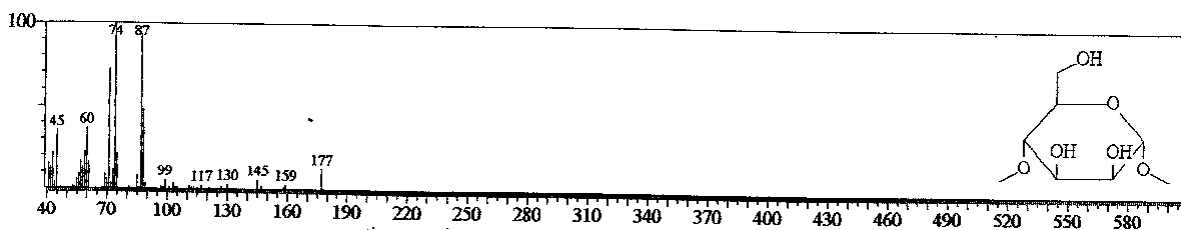


Fig. 5: alpha-Methyl 4-methylmannoside

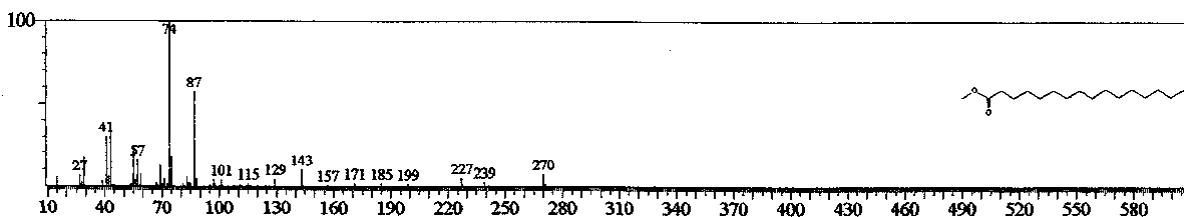


Fig. 6: Hexadecanoic acid methyl ester

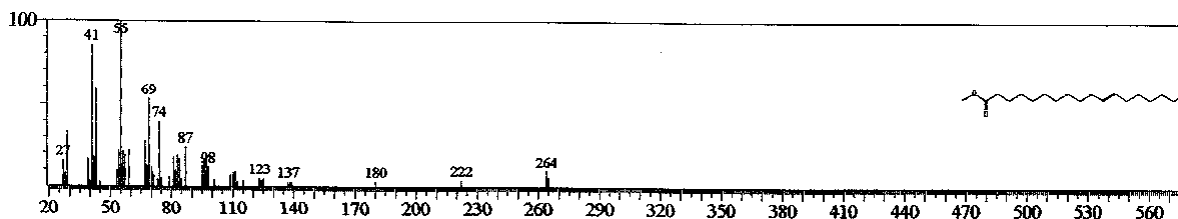


Fig. 7: 11-Octadecenoic acid methyl ester

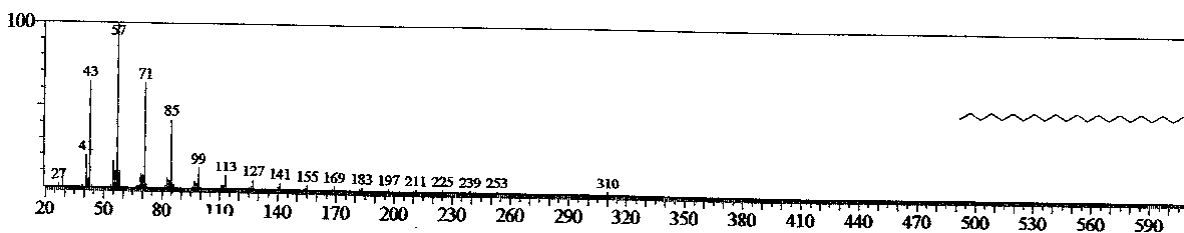


Fig. 8: Docosane

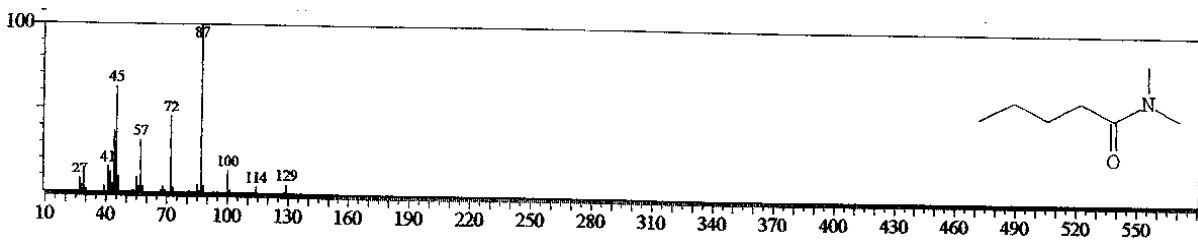


Fig. 9: N,N-Dimethylvaleramide

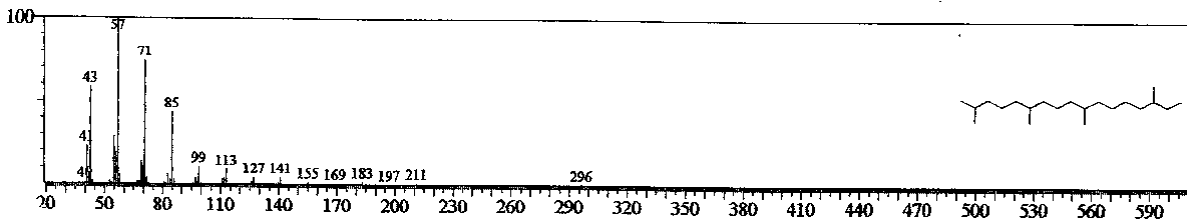
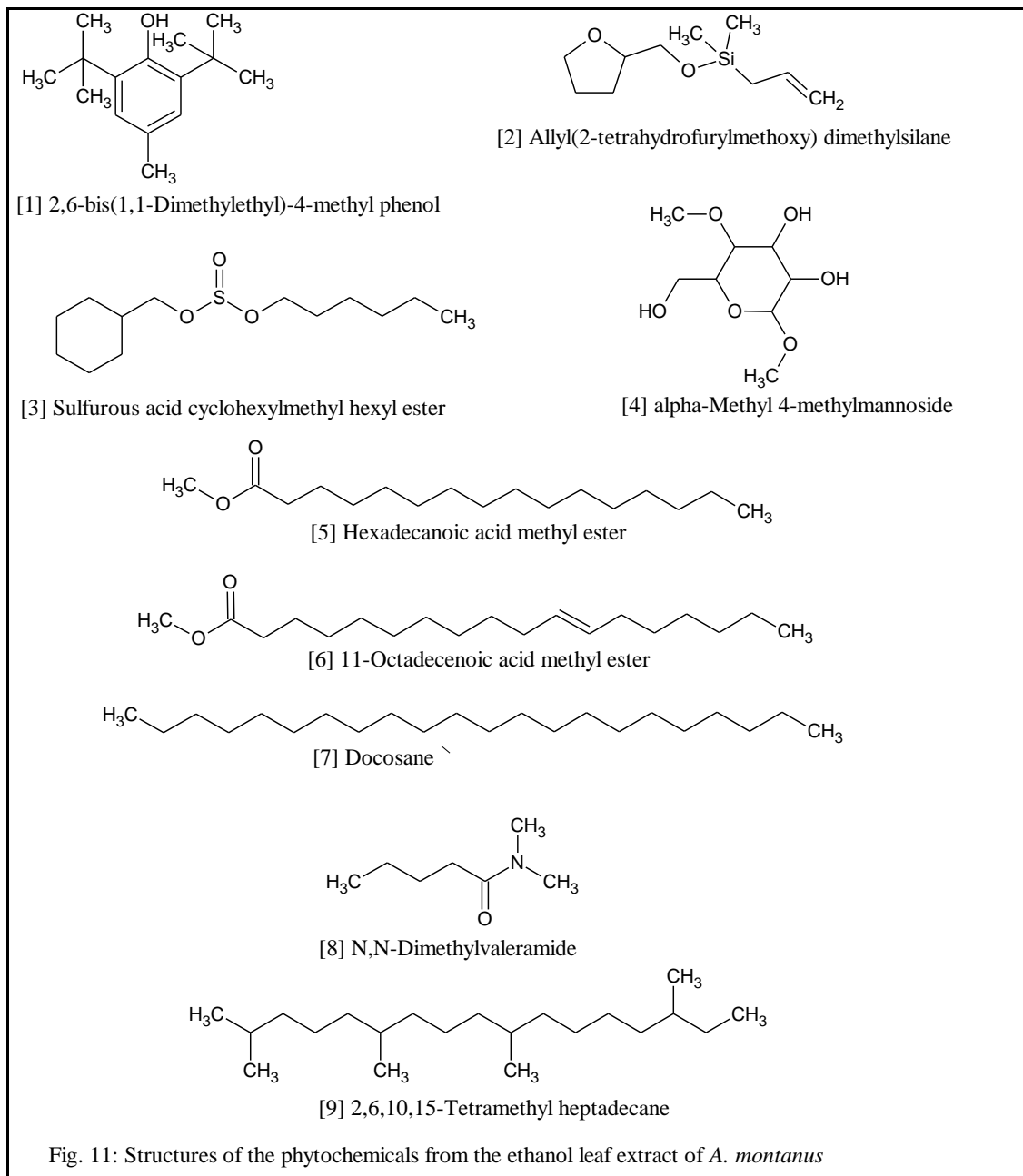


Fig. 10: 2,6,10,15-tetramethyl heptadecane



**Table 1: Phytochemicals identified from the GC/MS analysis of the leaf extract of *Acanthus montanus*.**

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)	Nature of compound
1	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	C <sub>15</sub> H <sub>24</sub> O	220	14.353	13.68	Phenol
2	Allyl(2-tetrahydrofurylmethoxy)dimethylsilane	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> Si	200	16.735	3.86	Furyl silane
3	Sulfurous acid, cyclohexylmethyl hexyl ester	C <sub>13</sub> H <sub>26</sub> O <sub>3</sub> S	262	17.078	5.67	Sulfurous acid ester
4	alpha-Methyl 4-methylmannoside	C <sub>8</sub> H <sub>16</sub> O <sub>6</sub>	208	17.338	8.41	glycoside
5	Hexadecanoic acid methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	19.852	16.12	Fatty acid ester
6	11-Octadecenoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	22.580	19.03	Fatty acid ester
7	Docosane	C <sub>22</sub> H <sub>46</sub>	310	23.865	5.85	Alkane
8	N,N-Dimethylvaleramide	C <sub>7</sub> H <sub>15</sub> NO	129	24.995	18.62	Amide (Alkaloid)
9	2,6,10,15-Tetramethyl heptadecane	C <sub>21</sub> H <sub>44</sub>	296	25.178	8.76	Alkane

**Table 2: Antimicrobial activities of the leaf extract of *Acanthus montanus***

Test Microorganism	Concentration (%)				
	25	50	75	100	MIC (%)
<i>Staphylococcus aureus</i>	7.33	10.62	12.88	14.33	25
<i>Enterococcus faecalis</i>	7.67	11.67	13.33	14.67	25
<i>Bacillus cereus</i>	-	8.68	11.33	13.50	50
<i>Escherichia coli</i>	7.78	11.66	12.92	14.67	25
<i>Salmonella typhi</i>	8.13	12.38	13.33	15.67	25
<i>Proteus mirabilis</i>	8.12	11.46	13.21	15.33	25

Values are in mm and include the diameter of the paper disc (5 mm).

Data are means of triplicate determinations.

The antimicrobial activities of the ethanolic extract of the leaves of *A. montanus* are shown in Table 2. The extract showed potent inhibition on six microorganisms which include three gram-negative bacteria (*E. coli*, *S. typhi* and *P. mirabilis*) and three gram-positive bacteria (*S. aureus*, *E. faecalis* and *B. cereus*). As shown in Table 2, the extract successfully inhibited these organisms at different concentrations and highest antimicrobial activity was exhibited against *S. typhi* and *P. mirabilis*. In general, the order of activity of *A. montanus* leaf extract against the test organisms was:

*S. typhi* > *P. mirabilis* > *E. coli* > *E. faecalis* > *S. aureus* > *B. cereus*

The minimum inhibitory concentration (MIC) of *A. montanus* leaf extract was 50-100 %. The sensitivity shown to the extract by the pathogens might be due to the presence of high amounts of phenols (13.68 %) and alkaloids (18.62 %) as shown by the GC/MS phytochemical screening in Table 1. The compounds detected from the leaf extract of *A. montanus* might have synergistically played antimicrobial role since they are mostly bioactive compounds. Alkaloids are plant bases which exhibit certain physiological properties when used in herbal medicine. A lot of them have antimalaria and antimicrobial activities<sup>16</sup>. Also, phenolic compounds have been reported to have high antibacterial and antifungal activities in humans<sup>12</sup>. The microorganisms tested are human commensals and have been incriminated in the infection of wounds<sup>17</sup>. These findings give credence to the use of *A. montanus* leaves in South Eastern Nigeria in traditional herbal practices for the treatment of gonorrhoea, syphilis, deep and dangerous wounds and boils among other uses. The

inhibitive activity of the extracts against *S. typhi* and *S. aureus* suggests the use of the extracts in the treatment of typhoid fever and sexually transmitted diseases (STDs).

The mechanism of inhibitory action of these phytochemicals on the microorganisms may be due to impairment of a variety of enzyme systems, including those involved in energy production, interference with the integrity of the cell membrane and structural component synthesis<sup>17</sup>. The use of plants or plant extracts in controlling diseases have several advantages, including their being pathogen specific, biodegradable, inexpensive, readily available and more environmentally friendly than synthetic chemicals<sup>13,17</sup>.

MIC = Minimum Inhibitory Concentration

= Zone of no inhibition

#### 4. Conclusion

The GC/MS results of the leaf extract of *A. montanus* have given insight on the chemical constituents of the leaves of the plant. The strong antibacterial activities exhibited by the extract give credence to the use of the plant in herbal medicine for the treatment of diseases and infections especially in South Eastern Nigeria.

#### 5. Acknowledgements

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