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CHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY SCREENING OF THE LEAVES OF *PICRALIMA NITIDA* (*APOCYNACEAE*)

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ABSTRACT

The ethanolic extract of the leaves of *Picralima nitida* was analyzed by Gas chromatography-mass spectrometry (GC-MS). Ten different phytochemical compounds have been characterized, including : 2,6-bis(1,1-dimethylethyl)-4-methyl phenol(0.65%), N1-(4-fluorobenzylideno)-N2-(4-quinolinyl-1-oxide) hydrazine(2.19%), sulfurous acid butyl cyclohexylmethyl ester(1.15%), 1,2,3,5-cyclohexanetetrol(40.73%), alpha-methyl mannofuranoside(39.88%), hexadecanoic acid, methyl ester(2.65%), 7-octadecenoic acid, methyl ester(2.95%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(5.17%), N,N-dimethyldodecanamide(2.10%) and N,N-dimethyl decanamide (2.51%). The extract showed marked antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*. These results give credence to the use of the extract in herbal medicine for the treatment of diseases and infections.

Keywords: *Picralima nitida* leaves, Chemical constituents, GC-MS analysis, Antibacterial activity, Herbal medicine.

INTRODUCTION

The availability of several organic compounds from living things, especially plants, has been the focus of the Natural Products Chemists for decades. However, there is a tremendous pressure on these plants probing their bioactive chemical constituents for a possible drug development and delivery. Many of these plants are utilized in herbal medicine in Nigeria for the treatment of enormous number of diseases and infections. One of such plants is *Picralima nitida* which is being studied for the chemical constituents of its leaves with respect to the ecosystem where it is grown. *Picralima nitida* is a member of the family *Apocynaceae*. The plant is primarily found in West Africa. It is a shrub or tree that grows up to 35 metres tall with white latex in all parts. The bark is hard, brittle, pale to dark greyish black or brown, smooth to slightly rough or finely striped. The leaves are blade elliptical to oblong in shape, 10-26 cm by 2-13 cm. The fruits consist of 2 free obovoid to ellipsoid follicles 11-20 cm long, smooth, apex rounded, yellow to orange, 2-valved, several to many seeds. The seeds are obliquely ovate, obovate to oblong, flattened, 2.5-4.5 cm long, smooth, brown to orange, embedded in soft white to orange pulp. *P. nitida* can be found flowering and fruiting throughout the year. The plant is an under storey tree in rainforest, also in mature secondary forest and semi-deciduous forest along river banks [1, 2, 3].

P. nitida seeds, stem bark and roots throughout its distribution area have a reputation as a febrifuge and remedy for malaria. The extracts of *P. nitida* seeds, fruit rind and stem bark have been reported to possess in vitro anti-malarial activity [4]. These extracts showed highly significant inhibitory effects against *Plasmodium falciparum*, including chloroquine-resistant strains, even in low concentrations. The anti-malarial activity is also present in the seeds and leaves, but at a lower level [4]. They are also extensively used for pain relief and to treat chest and stomach problems, pneumonia and intestinal worms in which case the seeds or bark are crushed or chewed and eaten or a decoction from the roots, seeds or

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bark is drunk [1]. In Nigeria, a decoction of the leaves is taken by mouth or used as a lotion against measles. In Ghana, the dry leaves are boiled in water and taken to treat guinea worm while in Cameroon a fruit decoction is taken to cure cough or typhoid fever. The leaf sap is dripped into the ear to treat otitis. *P. nitida* leaves have been reported to possess both antidiabetic and antioxidant properties [5] while the seeds contain a mixture of alkaloids producing antipyretic and anti-inflammatory effects along with analgesia [6, 7]. Antidiarrhoeal activity of the fruit-rind of *P. nitida* has been reported and shown to possess significant antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion [8]. Also, the antiplasmodial activity of ethanolic seed extract of *P. nitida* has been reported [9]. The plant has been investigated experimentally to exhibit antimicrobial, antipyretic and anti-inflammatory activities [10, 11]. It has been demonstrated that *P. nitida* has a broad activity for treating parasitic diseases, which lends credibility for its use against diarrhoea, gonorrhoea and intestinal worms [8, 10].

Among the Igbo tribe of South-eastern Nigeria, *P. nitida* plant is used in traditional herbal medicine for the treatment of diabetes, fibroid, typhoid fever, gonorrhoea, syphilis, malaria, and skin infections. Some herbalists including individuals cultivate and own this plant in their compounds since they consider it as an asset to them. The above highlighted therapeutic dividends of *P. nitida* and the different uses of the plant parts in herbal medicine in South-eastern Nigeria led to research on the chemical constituents of the leaves of *P. nitida* (*Apocynaceae*) grown in this area of Nigeria.

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℃ held at 60℃ for 1 minute, and at 160℃ for 2 minutes (rate 10℃/min), at 220℃ for 3 minutes (rate 10℃/min) and finally at 280℃ for another 2 minutes (rate 10℃/min) , injection temperature 220℃. GC-MS (Gas chromatography mass spectrometry) analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with column oven temperature of 60℃, 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℃, interface temperature was 250℃, 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 1364-2918.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

Picralima nitida leaves were harvested from Avodim Ubakala, Umuahia South Local Government Area of Abia State, South Estern Nigeria on November 19, 2013. The plant material was 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 sample (500 g) was successfully extracted with 2 L of ethanol (8hrs/3 times/30℃). The extract was concentrated under reduced pressure and the supernatant leaf (7.35g) extract was decanted 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.

Components Identification

The components of the ethanolic extract of *P. nitida* leaves were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature [12, 13].

Antibacterial Activity

The *in vitro* antibacterial activity of the extract from the leaves of *Picralima nitida* 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 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^{o}C for 15 minutes. 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 [14, 15]. 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 h in an incubator at 37^{o}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.

RESULTS AND DISCUSSION

The ethanolic extract of *Picralima nitida* leaves showed ten peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of ten compounds (1-10) in the extract (Figs. 2-12). The molecular formulae, percentage composition and molecular masses of these compounds are shown in Table 1. The compounds comprise phenol (0.65%), alkaloid (6.80%), sulphurous acid ester (1.15%), cycloalcohol (40.73%), glycoside (39.88%), fatty acid ester (5.60%) and alcohol (5.17%). The major constituents in the leaf extract of *P. nitida* include 1,2,3,5-cyclohexanetetrol (40.73%) and alpha-methyl mannofuranoside (39.88%).

As shown in Table 2, six bacteria organisms were used for the antibacterial activity of the leaf extract of *P. nitida* 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]. It is pertinent to give a brief summary of the diseases these organisms can cause. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils, cellulitis, scalded skin syndrome and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia and sepsis [17]. *E. faecalis* can also cause endocarditis and bacteraemia, urinary tract infections, meningitis and other infections of humans [18] while *B. cereus* is responsible for a minority of food-borne illnesses, causing severe nausea, vomiting and diarrhoea [19]. *Bacillus* food borne illnesses occur due to survival of the bacterial endospores when food is improperly cooked [20] and compounded when not properly refrigerated [21]. Some *E. coli* can cause bloody diarrhoea, urinary tract infections, severe anaemia or kidney failure which can lead to death [22] while *S. typhi* causes typhoid fever. *P. mirabilis* causes kidney infection, wound infections, septicaemia and pneumonias mostly in hospitalized patients [23].

The extract of the leaves of *P. nitida* exhibited marked antibacterial activity against the six pathogens tested. The order of activity is shown thus:

Salmonella typhi > *Escherichia coli* > *Bacillus cereus* > *Proteus mirabilis* > *Staphylococcus aureus* > *Enterococcus faecalis*

The minimum inhibitory concentration (MIC) of *P. pellucida* leaf extract was 25-100%. From the result, greater antibacterial activity was shown against *S. tyhpi* and *E. coli* suggesting that the leaves of *P. nitida* could be used in the treatment of typhoid fever, bloody diarrhoea, urinary tract infections, severe anaemia and kidney failure. From the chemical analysis result of *P. nitida* leaf extract, the leaf contains high quantities of 1,2,3,5-cyclohexanetetrol (40.73%) and alpha-methyl mannofuranoside (39.88%) while alkaloids (6.80%) and phenol (0.65%) were in low quantities. Glycosides [24], alkaloids [25] and phenols [12] have been reported to exhibit antimicrobial activities. The ability of *P. nitida* leaf extract to show potent inhibition on these human pathogens gives corroborative evidence to its application in herbal medicine for the treatment of diarrhoea, typhoid fever, gonorrhoea, syphilis, fever, fatigue and skin infections.

Table 1. Phytochemicals identified from the GC-MS analysis of the leaf extract of *Picralima nitida*

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	$C_{15}H_{24}O$	220	14.359	0.65	Phenol
2	N1-(4-fluorobenzylideno)-N2-(4-quinolinyl-1-oxide) hydrazine	$C_{16}H_{12}FN_3O$	281	15.175	2.19	Alkaloid
3	Sulfurous acid, butyl cyclohexylmethyl ester	$C_{11}H_{22}O_3S$	234	17.081	1.15	Sulfurous acid ester
4	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	148	17.798	40.73	Cyclo alcohol

5	alpha-Methyl mannofuranoside	$C_7H_{14}O_6$	194	18.442	39.88	glycoside
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	19.857	2.65	Fatty acid ester
7	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	22.579	2.95	Fatty acid ester
8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{38}H_{68}O_8$	296	22.901	5.17	Alcohol
9	N,N-Dimethyldodecanamide	$C_{14}H_{29}NO$	227	25.000	2.10	Amide (Alkaloid)
10	N,N-Dimethyldecanamide	$C_{12}H_{25}NO$	199	27.312	2.51	Amide (Alkaloid)

Table 2. Antibacterial activity of the leaf extract of *Picralima nitida*

Test Microorganism	Concentration (%)				
	25	**50**	**75**	**100**	**MIC (%)**
Staphylococcus aureus	7.59	11.77	14.65	18.35	25
Enterococcus faecalis	7.33	10.99	14.25	18.23	25
Bacillus cereus	8.53	12.10	15.67	18.67	25
Escherichia coli	8.91	13.73	17.33	20.37	25
Salmonella typhi	9.77	14.67	18.55	22.32	25
Proteus mirabilis	8.33	11.87	14.99	18.37	25

Figures are in mm and include the diameter of the paper disc (5mm). Data are means of triplicate determinations.
MIC = Minimum Inhibitory Concentration

Fig 1. GC-MS chromatogram of ethanolic extract of *Picralima nitida* leaves

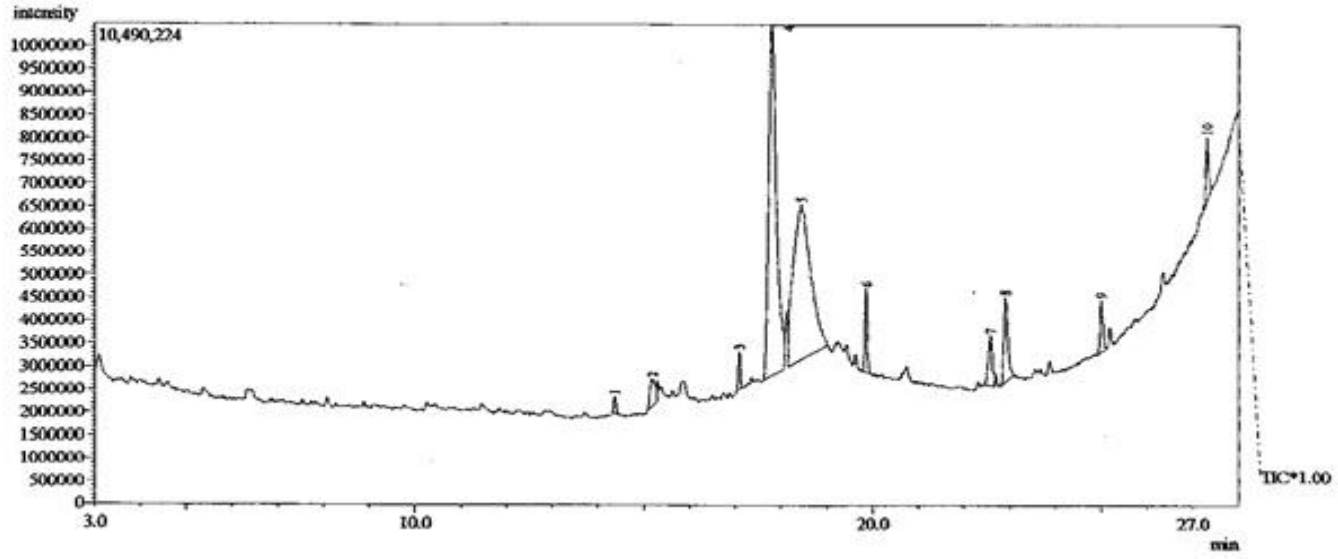


Fig 2. 2,6-bis(1,1-dimethylethyl)-4-methyl phenol

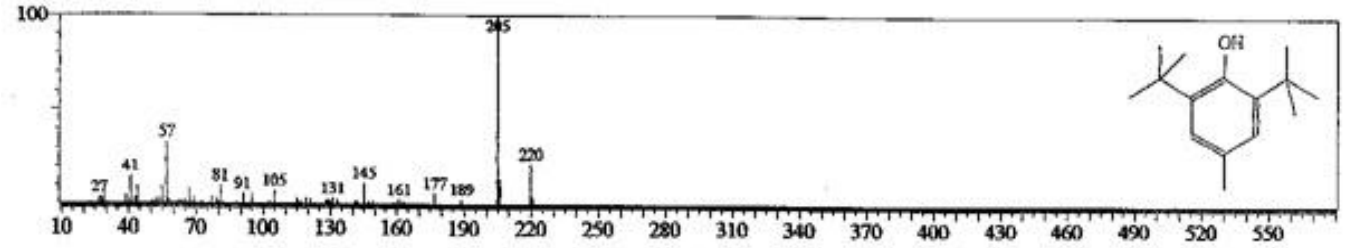


Fig 3. N1-(4-fluorobenzylideno)-N2-(4-quinolinyl-1-oxide) hydrazine

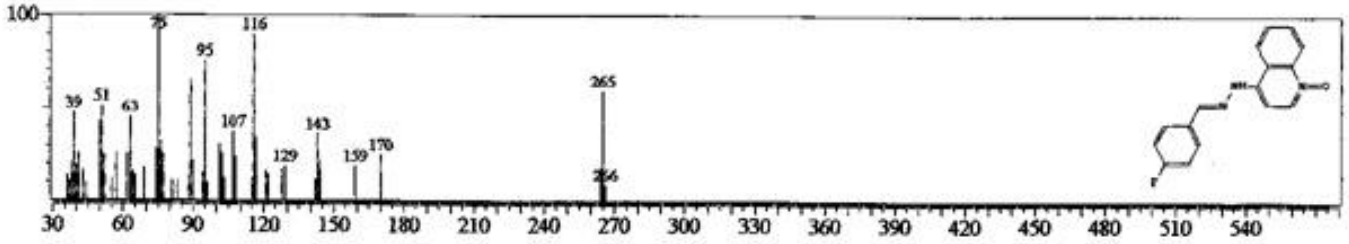


Fig 4. Sulfurous acid, butyl cyclohexylmethyl ester

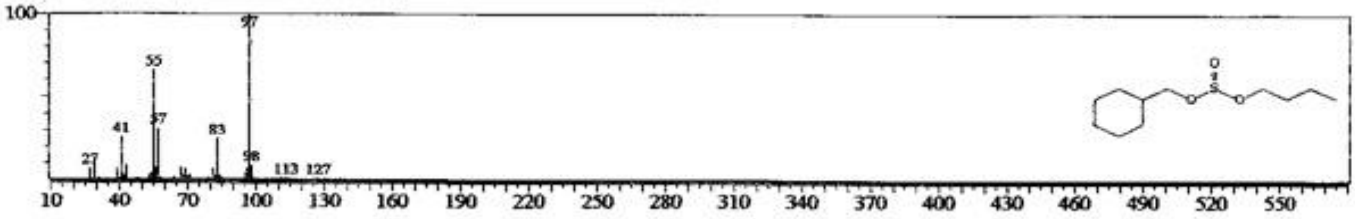


Fig 5. 1,2,3,5-Cyclohexanetetrol

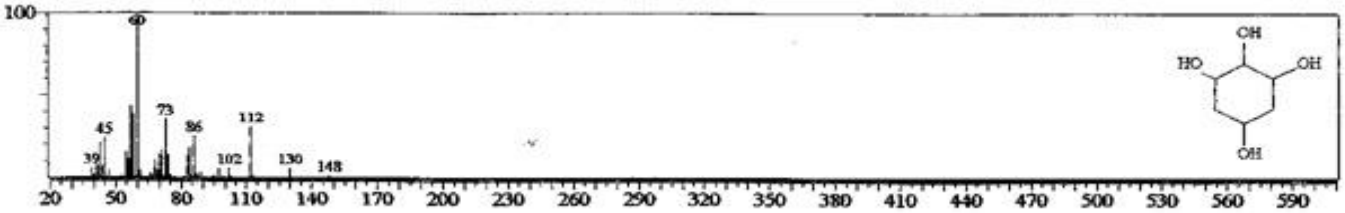


Fig. 6. alpha-Methyl mannofuranoside

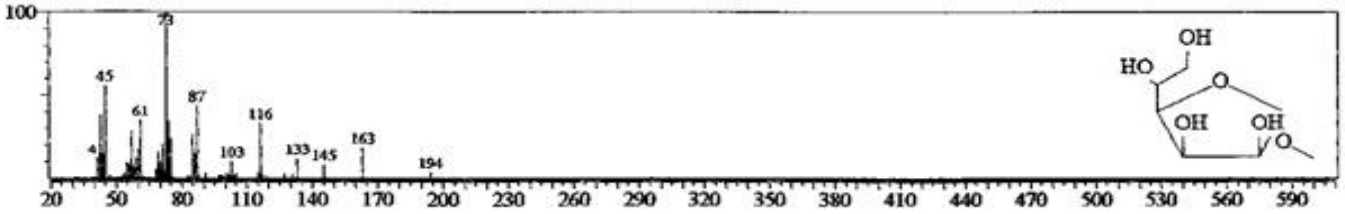


Fig 7. Hexadecanoic acid, methyl ester

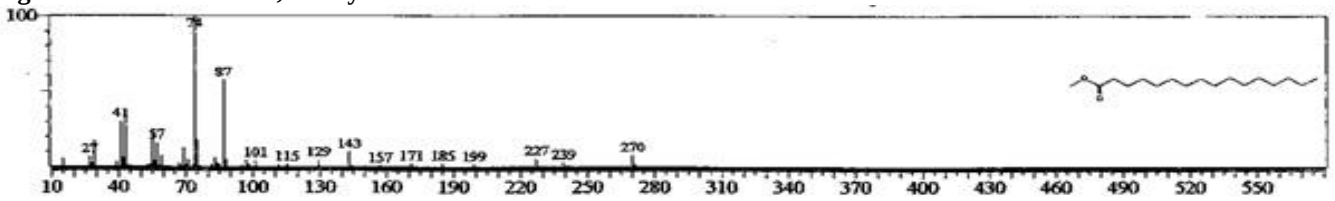


Fig 8. 7-Octadecenoic acid, methyl ester

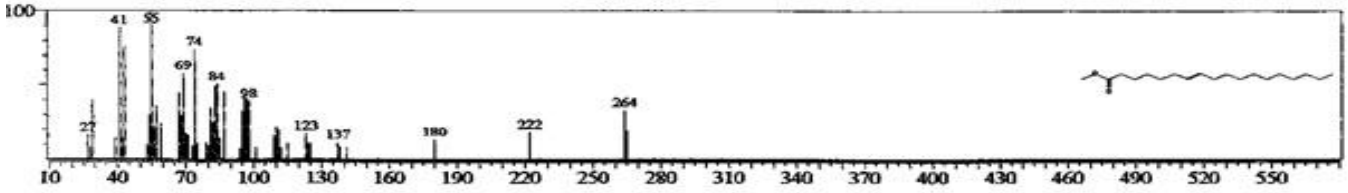


Fig 9. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

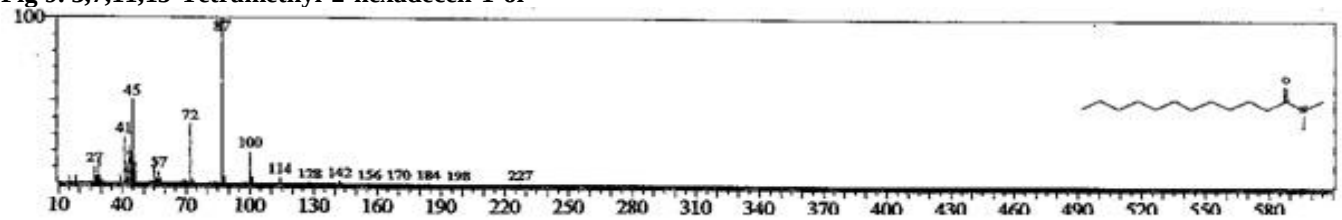


Fig 10. N,N-Dimethyldodecanamide

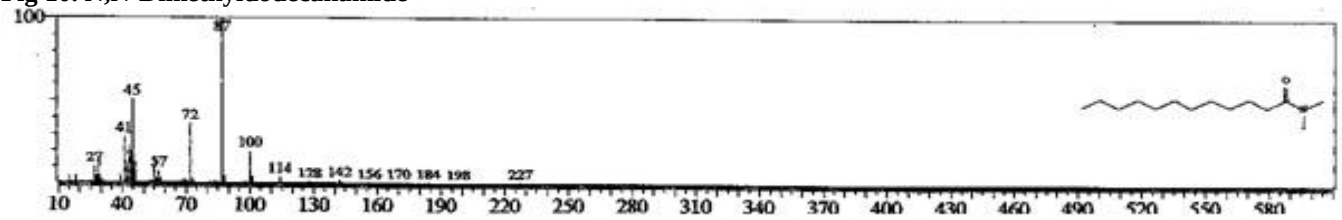
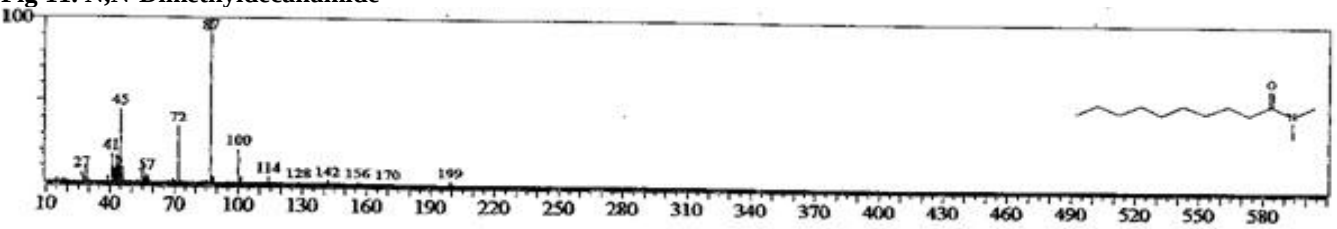


Fig 11. N,N-Dimethyldecanamide



CONCLUSION

The GC-MS results of the ethanolic leaf extract of *P. nitida* have revealed some of the chemical components of the leaves of the plant. The strong antibacterial activities exhibited by the extract are attributed to the presence of glycosides, alkaloid and phenols by way of their synergistic effects. These investigations provide supporting evidence to the use of the leaves of *P. nitida* in herbal medicine in Nigeria for the treatment of diseases and infections.

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