



## Studies of the Chemical Constituents of the Fruit-rind of *Picalima nitida* (*Apocynaceae*) and Screening of its Antimicrobial Potency

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### Abstract

The ethanolic extract of the fruit-rind of *Picalima nitida* was analyzed by Gas chromatography-mass spectrometry (GC-MS). Seven different phytochemical compounds have been characterized, including : 1,2,3,5-cyclohexanetetrol(23.04%), alpha-methyl mannofuranoside(70.61%), hexadecanoic acid, methyl ester(1.46%), tetradecanoic acid ethyl ester(0.70%), 12-octadecenoic acid methyl ester(1.28%), Z,E-3,13-octadecadien-1-ol (1.65%) and N,N-dimethyldodecanamide (1.26%). The antimicrobial screening of the fruit-rind extract of *Picalima nitida* showed marked antimicrobial potency against certain strains of bacteria which include *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:** *Picalima nitida* fruit-rind, Chemical constituents, GC-MS analysis, Antimicrobial screening, Herbal plant.

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### 1. Introduction

South Eastern Nigeria is endowed with a lot of vegetative natural resources used as food and medicine. The treatment of diseases in this area is not limited to synthetic drugs as most people still depend on botanical preparations as medicine. These are always available in form of infusions, decoctions, macerations and concoctions. Nevertheless, exhaustive knowledge of the bioactive compounds in these herbal plants and their bioactivity are necessary to fully understand the effects and medicinal characteristics of these plants. Also, the pharmacological profiles of some of these herbal plants are yet to be fully documented. Most people believe that herbal preparations are more efficacious than conventional synthetic drugs but are sceptical about its use since there is no clear-cut dosage hence, making it a major constraint.

It is in view of the foregoing that interest on the chemical studies and antimicrobial screening potency of *Picralima nitida* fruit-rind stemmed up. *Picralima nitida* belongs to the family, *Apocynaceae*. It is a shrub or tree that grows up to 35 metres tall with white latex in all parts and is usually found in West Africa. 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. The plant 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].

The 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 [4]. The extracts of *P. nitida* seeds, fruit rind and stem bark have been reported to possess in vitro anti-malarial activity [5]. 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 [5]. 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 bark is drunk [1]. 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.

*P. nitida* seeds, stem bark and roots throughout its distribution area have a reputation as a febrifuge and remedy for malaria. 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 [6] while the seeds contain a mixture of alkaloids producing antipyretic and anti-inflammatory effects along with analgesia [7, 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 [4, 10]. It does appear that the fruit-rind of *P. nitida* has not been thoroughly researched upon and this has engendered a probe trying to beam a search light on the chemical constituents of the fruit-rind of the plant. The various aforementioned curative benefits and potentials of *P. nitida* as well as the different uses of the plant parts in herbal medicine in South-eastern Nigeria led to research on the chemical constituents of the fruit-rind of *P. nitida* (*Apocynaceae*).

## 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 1775-2641. 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* fruits were harvested from Avodim Ubakala, Umuahia South Local Government Area of Abia State, South Eastern Nigeria on November 19, 2013. Fresh green matured fruits were used for the analysis. The fruit has an average length of 14.0 cm and width of 11.8 cm. 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 fruits were chopped into pieces after removing the seeds. The fruits 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 (300 g) was successfully extracted with 2 L of ethanol (8hrs/3 times/30°C). The extract was concentrated under reduced pressure and the supernatant fruit (5.94g) 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<sup>[12,13]</sup>.

### Components Identification

The components of the ethanolic extract of the fruit-rind of *P. nitida* was identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature<sup>[14,15]</sup>.

### Antimicrobial Screening

The *in vitro* antimicrobial activity of the fruit-rind extract of *Picalima 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°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 [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 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 *Picalima nitida* fruit-rind showed seven peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of seven compounds (1-7) in the extract (Figs. 2-9). The names, molecular formulae, percentage compositions and molecular masses of these compounds are shown in Table 1 while the mass spectra of each of the compounds are shown from Figures 2 to 8. The compounds comprise cycloalcohol (23.04%), glycoside (70.61%), fatty acid ester (3.44%), alcohol (1.65%) and alkaloid (1.26%).

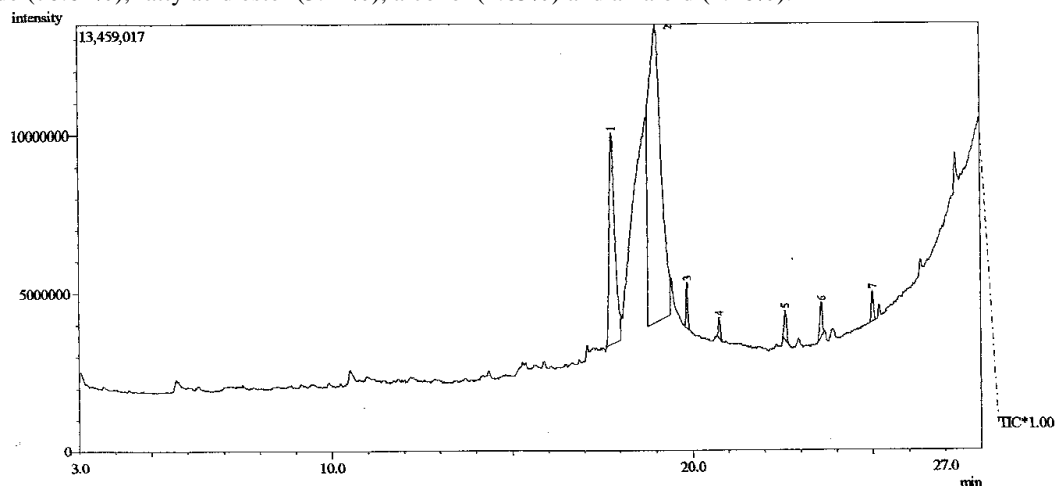


Fig. 1: GC-MS chromatogram of ethanolic extract of *Picalima nitida* fruit-rind

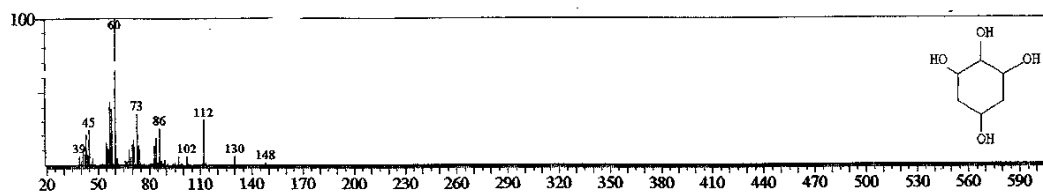


Fig. 2: 1,2,3,5-Cyclohexanetetrol

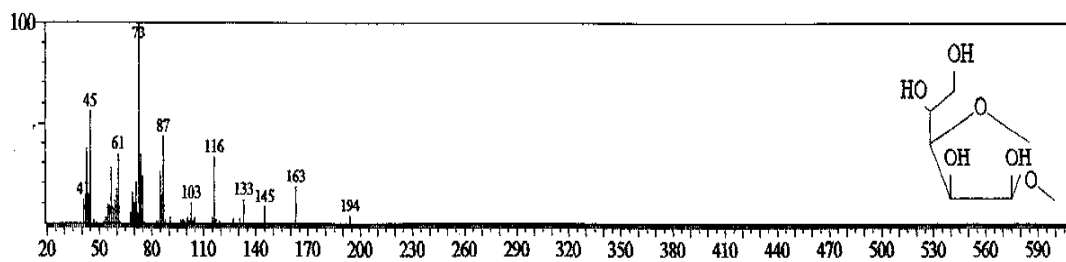


Fig. 3: alpha-Methyl mannofuranoside

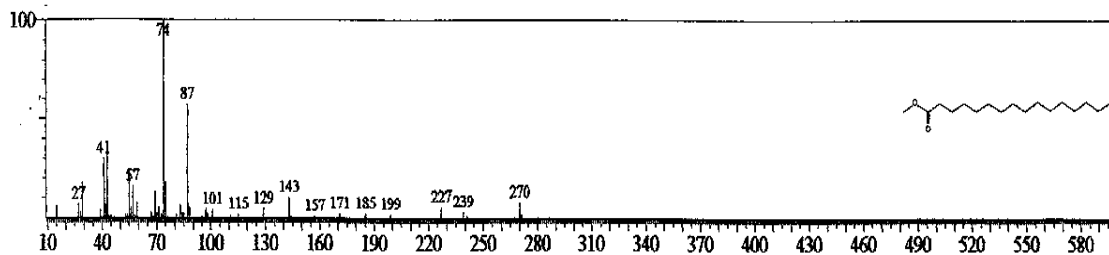


Fig. 4: Hexadecanoic acid, methyl ester

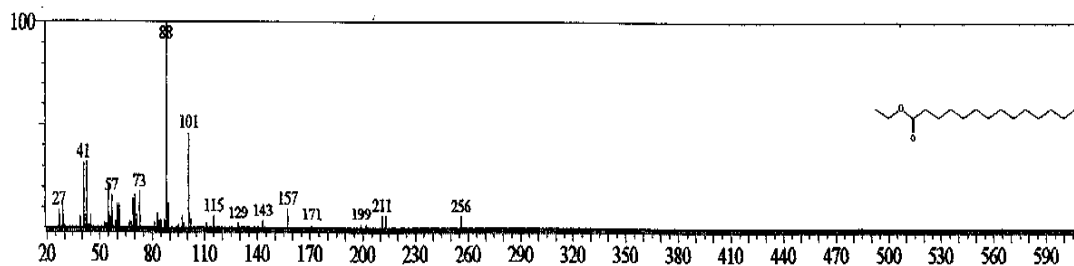


Fig. 5: Tetradecanoic acid ethyl ester

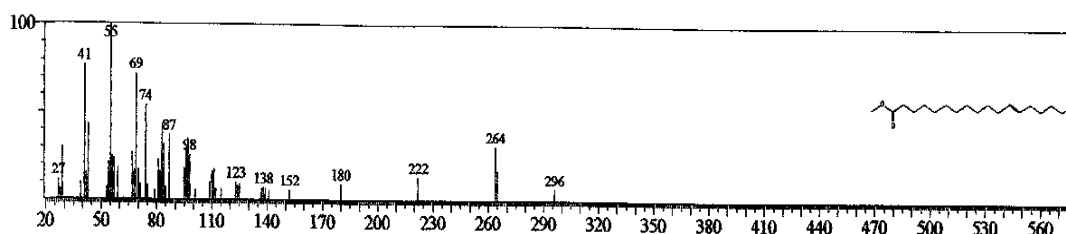


Fig. 6: 12-Octadecenoic acid methyl ester

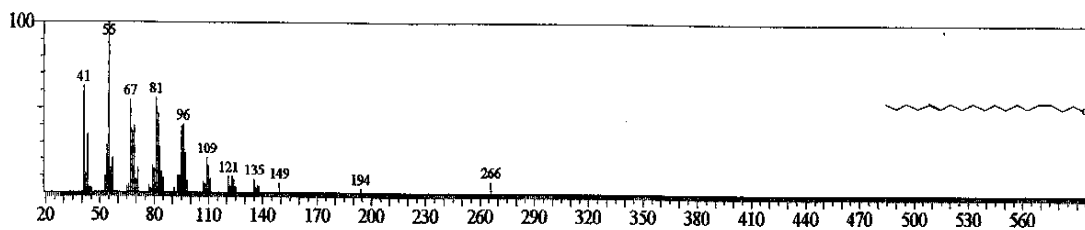


Fig. 7: Z,E-3,13-Octadecadien-1-ol

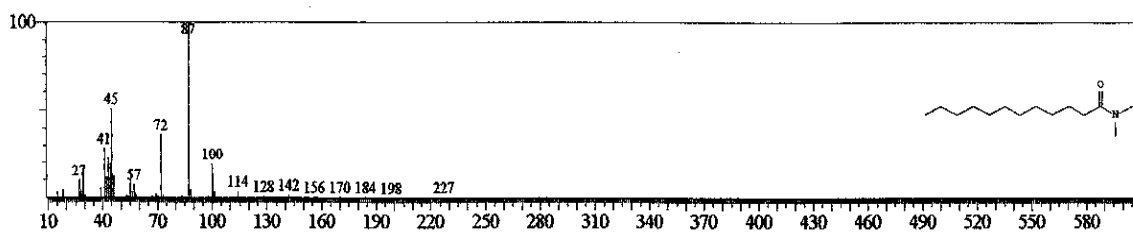
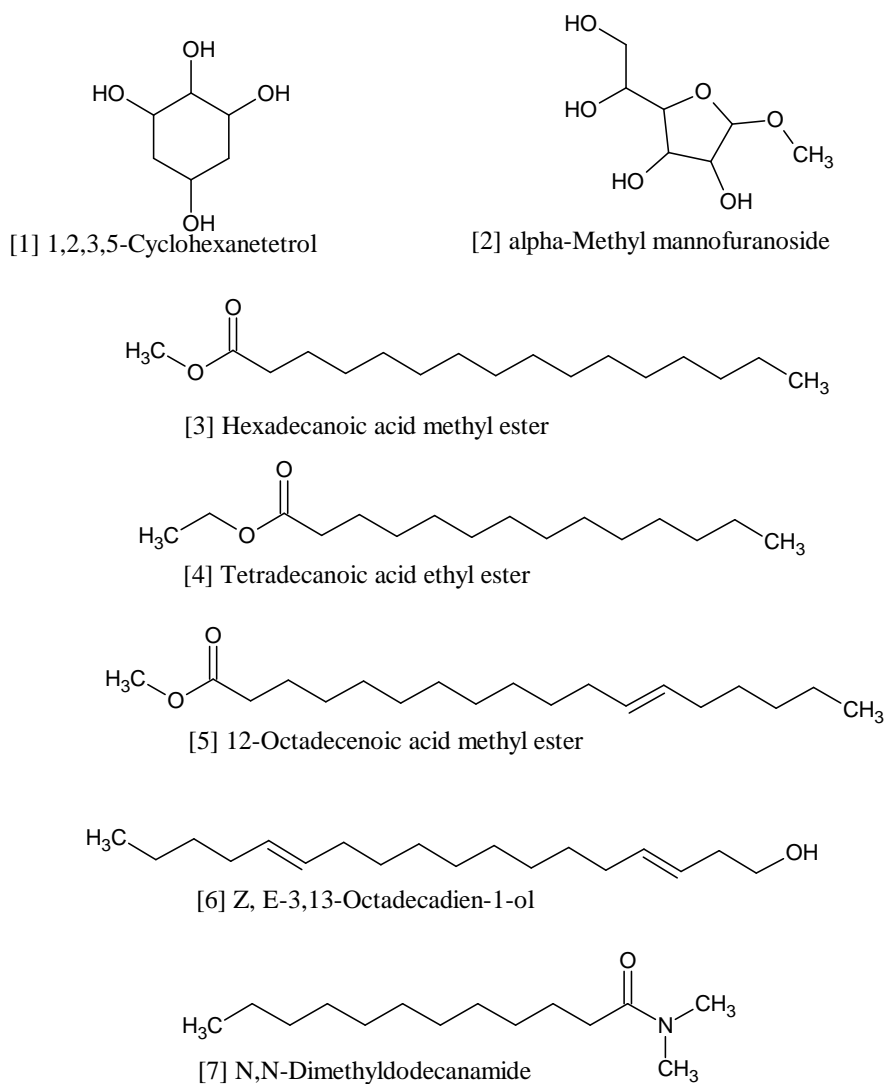


Fig. 8: N,N-Dimethyldodecanamide

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

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak area(%)	Nature of compound
1	1,2,3,5-Cyclohexanetetrol	C <sub>6</sub> H <sub>12</sub> O <sub>4</sub>	148	17.780	23.04	Cyclo alcohol
2	alpha-Methyl mannofuranoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	19.016	70.61	glycoside
3	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	19.857	1.46	Fatty acid ester
4	Tetradecanoic acid ethyl ester	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	20.751	0.70	Fatty acid ester
5	12-Octadecenoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	22.578	1.28	Fatty acid ester
6	Z,E-3,13-Octadecadien-1-ol	C <sub>14</sub> H <sub>29</sub> NO	227	23.582	1.65	Alcohol
7	N,N-Dimethyldodecanamide	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	24.999	1.26	Amide (Alkaloid)

Fig. 9: Structures of phytochemicals from the ethanolic extract of *Picralima nitida* fruit-rind

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

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

The minimum inhibitory concentration (MIC) of *P. nitida* fruit-rind extract was 25-100%. From the GC-MS photochemical screening in Table 1, the sensitivity shown to the extract by the pathogens might be due to the presence of tremendously high amount of alpha-methyl mannofuranoside (70.61%) which is a glycoside. Glycosides have broad spectrum pharmacological and therapeutic profiles as they have been reported to possess antimicrobial [18,19] and antitumor properties [19]. Other uses include as cardiac drugs, laxatives, counter irritants, analgesics, antirheumatic, anti-inflammatory and anticancer agents [20]. The microorganisms tested are human commensals and have been incriminated in the infection of wounds [21]. These findings give credence to the use of *P. nitida* fruit-rind in South Eastern Nigeria in traditional herbal medicine for the treatment of typhoid fever, diarrhoea, gonorrhoea, syphilis, skin infections and sexually transmitted diseases. This investigation has also revealed that the extract of the fruit-rind of *P. nitida* could be used in treating diseases and infections caused by these organisms.

**Table 2. Antimicrobial screening potency of the fruit-rind extract of *Picralima nitida***

Test Microorganism	Concentration (%)				
	25	50	75	100	MIC (%)
<i>Staphylococcus aureus</i>	7.67	10.67	13.33	17.83	25
<i>Enterococcus faecalis</i>	7.60	9.33	13.05	17.67	25
<i>Bacillus cereus</i>	9.33	12.53	16.33	21.67	25
<i>Escherichia coli</i>	8.33	11.33	15.33	18.37	25
<i>Salmonella typhi</i>	8.67	11.50	15.50	21.33	25
<i>Proteus mirabilis</i>	8.59	11.33	15.67	19.67	25

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

The mechanism of inhibitory action of these phytochemicals on the microorganisms may be due to impairment of variety of enzyme systems, including those involved in energy production, interference with the integrity of the cell membrane and structural component synthesis [19]. 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 [21].

#### 4. Conclusion

This research has provided information on the major constituents of the fruit-rind of *P. nitida*. It consist mainly glycosides which have considerable therapeutic values. Other components like alkaloids and phenol are in smaller quantities. This study has also shown potent antibacterial activities of the fruit-rind extract of *P. nitida* against the test organisms. The use of the fruit-rind of the plant in herbal medicine could be due to the presence alpha-methyl mannofuranoside which constitutes 70.61% of the extract.

#### 5. Acknowledgement

The authors grateful to Mr I. K. Ndukwe of Taxonomy Section, Forestry Department of Michael Okpara University of Agriculture, Umudike, for his kindness in identifying and authenticating our plant samples

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