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Phytochemical, Anti-microbial analysis and structural elucidation of *Urena Lobata* root extracts.

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ABSTRACT

The Harbone method of extraction was used in the extraction of Urena Lobata roots. The crude extracts were separated using TLC. Result of the phytochemical screening showed the presence of saponins, cardiac glycosides, and tannins. The pure chloroform extract of the roots of *Urena lobata* and that of ethyl acetate were analyzed using spectroscopic instruments; Fourier Transform Infra-red(FTIR), UV-Visible, Nuclear Magnetic Resonance (NMR), and GC-MS. Combination of the results from these analysis suggested the compounds Phathalic acid, dibutylglycolate ($C_{18}H_{24}O_6$) for the chloroform extract and 1,2-Benzenedicarboxylic acid, 2-methylpropyl, 3- methylbutyl ester ($C_{17}H_{24}O_4$) for ethyl acetate extract as the active compounds. These compounds are phthalates which are known to have antimicrobial properties. Antimicrobial analysis carried out on the extracts and the chloroform extract showed very high sensitivity to the test organisms: *Aspergillus flavus, Aspergillus niger, Candida albican; S. aureus, E. coli, Enterobacter aerogenes, Proteus vulgaris, Streptococcus species, Bacillus species, Pseudomonas pyocyania and Klebsiella aerogenes.*

Keywords: Phytochemical, Anti microbial, *urena lobata*, phathalates

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INTRODUCTION

Urena lobata, also called aramina, bun ochra, caesar weed or Congo jute, is a genus of plants which grows in various tropical and subtropical areas throughout the world. The name *Urena* derives from the name given to the plant on India's Malabar Coast (Adeloye *et al.*, 2007)[1]. The plant is an herbaceous, perennial one which grows to about 1 to 4.5 metres high with many branches. It is a specie of the *Malvaceae* family. The flowers of *Malvaceae* are always bisexual and actinomorphic. They are dicots (Carr, 2006)[2].

The leaves vary in size and shape and are usually round with three to five lobes and serrated edges. The flowers grow singly in the axial of the leaf, with five petals that are usually pink. The plant's seeds are small with hook-like appendages. *Urena lobata* grows best in hot humid climate, with direct sunlight and rich, well drained soil. It is found as wild plant in places like North and South America, Asia, Philippine, Indonesia and Africa but as cultivated plant in Central Africa and Congo Basin, Brazil, Madagascar and India. The plant is seen as weed by some people while to some other people, it is an economic plant because of its fibres which are used for various purposes. The fibre is one of the bast fibre groups and resembles jute in appearance and strength. It is often blended with jute or other fibres for sacking fabrics or carpeting materials. In Brazil, *Urena lobata* has long been used as fibre. Commercial cultivation of the plant started in the Belgian Congo in the 1920s and in Central Africa in the 1930s (Adeloye *et al.*, 2007)[1].The leaves and the flowers of *Urena lobata* are eaten as famine food in some parts of Africa. The extracts from its leaves and roots have broad spectrum anti-bacterial and antifungal qualities. *Urena lobata* is a medicinal plant used in the treatment of some ailments. It is effective in the treatment of many diseases which include pile, diabetes, diarrhea, intestinal inflammation, fever, gonorrhea, bruises, sprains, hydrophobia, malaria, toothache, snake bite, etc. (John, 2009)[3].

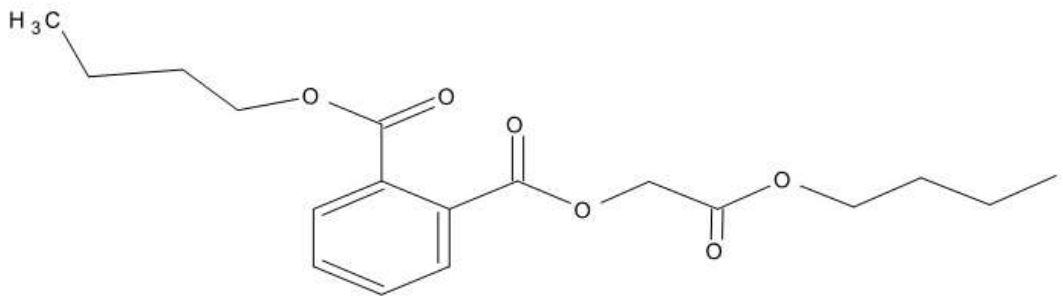

Figure 1: Phthalic acid, dibutylglycolate. Formula: $C_{18}H_{24}O_6$. Mol. weight: 278

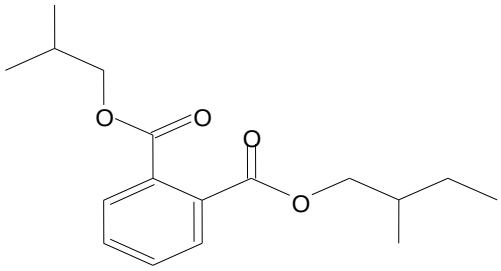

Figure 2: 1, 2-Benzenedicarboxylic acid, 2-methylpropyl, 3- methylbutyl ester. Formula: $C_{17}H_{24}O_4$.Mol. Weight: 292

MATERIALS AND METHOD

Urena lobata roots used for this research work were obtained from Oghe in Ezeagu Local Government Area of Enugu state Nigeria. It was air dried at room temperature. The dried sample was pulverized and stored in a plastic container prior to laboratory analysis.

Phytochemical Screening

The plant crude extracts were screened qualitatively for plant metabolites using the Harbone, (1998) method for the phytochemicals.

Bioassay Analysis of the Chloroform and Ethylacetate Extracts.

The antimicrobial activities were determined using the agar diffusion method (Bryant, 1972)[4]. The minimum inhibitory concentration of the extract against the microorganisms was carried out using glucose indicator broth. Punched agar diffusion method was used to determine the minimum inhibition concentration and minimum fungicidal concentrations of the extracts.

RESULTS AND DISCUSSION

The results of the phytochemical screening showed the presence of saponins, tannins and cardiac glycosides (Table 1).

The result of the antibacterial activity of the chloroform extract showed that the extract was active against the test organisms and having the highest activity with *Enterobacter aerogenes* at 36mm average diameter zones of inhibition. Ethylacetate extract had no activity on any of the test organisms (Table 2).

The result of the antifungal activity of the chloroform and ethylacetate extracts showed that only the chloroform extract inhibits the growth of all the three test fungi with *Aspergillus niger* and *Candida albican* being mostly inhibited at 6mm (Table 3).

The result of the MIC and MBC of the chloroform extract showed no visible activity on the subculture at 0.5 and 0.25 dilutions. At subculture 0.125 dilution, there is an activity and visible

growth on both subculture and control at 0.125, 0.0625, 0.0313, 0.0156 and 0.0078 dilutions denoted by (++). These results confirmed that the chloroform extract was bactericidal at veru low concentrations (Table 4).

The result of the MIC and MFC of the chloroform extract showed that at minimum concentrations, the extracts inhibited the growth of all the test organisms used (Table 5).

The FTIR spectrum of the Chloroform extract gave five absorption peaks (Table 6). The FTIR spectrum showed C – H stretch for alkyl groups at 2931cm^{-1}. The carbonyl region showed a band at 1715cm^{-1} indicating the presence of a six – membered ring ketone or an aldehyde. The FTIR spectrum showed C – O stretch for esters at

1263cm^{-1} while C – H deformation for aromatics and alkyl groups occurred at

735cm^{-1}. C – H deformation for methyl group also occurred at 449cm^{-1}.

The FTIR spectrum for the Ethyl acetate extract gave five absorption peaks (Table 7). The spectrum showed the presence of C-H stretch bands for alkyl groups at 2930cm^{-1}. There is the presence of strong C – H deformation bands for aromatics and methyl groups at 766cm^{-1}. The carbonyl region showed a band at 1265cm^{-1} indicating the presence of esters and a stronger band at 1720cm^{-1} indicating the presence of ketones. The FTIR spectrum showed a C = C stretch for aromatics at 1446cm^{-1}. These results supported the suggested structure for the extract (figure 2).

UV-Visible spectroscopic analysis of the chloroform extract gave absorptions in both the UV and Visible regions showing that the compound had high conjugation of pie bonds attached to alkyl chain or aromatic ring (Table 8).

The C^{13} NMR of the chloroform extract (Table 10) showed 18 signals corresponding to 18 distinct carbon atoms. Here, the aromatic carbon occurred at 127.974 and 125.836ppm. Four carbonyl signals were recorded at 77.677, 77.236, 77.033 and 76.403ppm. The quaternary carbon signals occurred at 34.115 and 33.596ppm confirming the conjugation in the benzene ring. The two methyl carbons of the attached alkane chain appeared at 22.69 and 14.143ppm.

The H^1 NMR (Table 10) showed an aromatic singlet proton at 7.3ppm and duplet at 7.0ppm. A duplet CH proton appeared at 6.5ppm which corresponded to the attached aromatic ring protons. A duplet CH_2 proton occurred at 1.7 and 1.3ppm corresponding to the methylene group attached to alkyl chains. A duplet CH_3 at 0.9ppm represent the two terminal methyl groups attached to the aromatic ring.

The GC-MS result gave two major fragments that corresponded to the H^1 NMR and C^{13} NMR signals when the fragments are coupled. There was a Phthalic ester moiety and a butylglycolate moiety which gave Phthalic acid, dibutylglycolate which was the suggested structure.

The suggested structure for the chloroform extract, from the combination of the results of the FTIR, NMR, UV-VISIBLE and GC-MS is shown in figure 1 while the suggested structure for the ethyl acetate root extract, from the combination of the results of the FTIR, NMR, UV-VISIBLE and GC-MS is shown in figure 2.

UV-visible spectroscopic analysis of the ethyl acetate root extract (Table 9) gave absorptions at visible regions (725.40, 666.60, 616.00, and 602.20nm) showing conjugation of pie bonds attached to alkyl chain or aromatic ring.

The C^{13} NMR result of ethyl acetate extract (Table 11) showed 17 signals corresponding to 17 distinct carbon atoms. The carbonyl signals were recorded at 77.662, 77.033 and 76.403ppm while carboxy signals appeared at 34.027 and 31.919ppm. Two quaternary carbon signals occurred at 29.693 and 29.444ppm confirming the conjugation in benzene ring. Five methine carbons appeared at 29.371, 29.254, 29.210, 29.093, and 28.610 ppm. Two methylene carbon atoms appeared at 28.288 and 22.700ppm. Three methyl signals were recorded at 22.694, 19.151 and 14.128ppm.

The H^1 NMR result (Table 11) showed an aromatic singlet proton at 7.3ppm and a singlet carboxyl proton at 4.2ppm. A triplet CH proton appeared at 2.4ppm, a duplet CH proton appeared at 2.1ppm and a singlet appeared at 1.6ppm. A duplet methylene proton signal occurred at 1.3ppm which corresponded to the long chain in the compound and a duplet methylene proton appeared at 0.9ppm which corresponded to the four terminal methyl groups in the compound.

The GC-MS result gave one major fragment that corresponded to the combined results of NMR, UV-Visible and FTIR with molecular formula $C_{17}H_{24}O_4$.

Table 1: Results of the Phytochemical Screening of the Root

Phyto compound	Inference
Alkaloids	- -
Saponin	+ +
Tannins	+ +
Flavonoids	- -
Cardiac glycosides	+ +
Terpenoids	- -
Acidic compounds	- -
Reducing sugar	- -

KEY: - - = absent + = slightly present**,** ++ = highly present

Table 2: Results of Antibacterial Activities of Chloroform and Ethyl Acetate Root Extracts

S/N		Volume Used (cm^3)	Average Diameter (mm) of zones of Inhibition on Test Organisms							
			A	B	C l.c.i	D l.c.i	E l.c.i	F l.c.i	G l.c.i	H l.c.i
1	Chloroform	0.05	28	24	36	28	16	18	22	26
2	Ethylacetate	0.05	NA	NA	NA	NA	NA	NA	NA	NA
	Control 50%Acetone	0.05	NA	NA	NA	NA	NA	NA	NA	NA

KEY: l.c.i = local clinical isolates, NA= No activity

A = *S.aureus* NCTC 6571, B = *E.Coli* NCTC 10418, C = *Enterobacter aerogenes*, D = *Protens Vulgaris*, E = *Streptococcus specie*, F = *Bacillus specie* G = Pseudomonas *aerogenes*, H = *Klebsiella aerogenes*

Table 3: Result of the Antifungal Activities of the Pure Root Extracts.

S/N	Extracts/ Solvents	Volume used (CM^3)	Average Diameter (mm) of zones of Inhibition on Test Organisms		
			***Aspergillus Flavus* l.c.i**	***Aspergillus Niger* l.c.i**	***Candida albican* l.c.i**
1	Chloroform	0.05	2	6	6
2	Ethylacetate	0.05	NA	NA	NA
	Control 5%Acetone	0.05	NA	NA	NA

KEY: NA= No activity. l.c.i =Local clinical isolates

Table 4: Result of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Of The Pure Chloroform Root Extract

Extracts/Solvents	Presences or Absence of Growth or Turbidity of Test Organisms								
	Dilutions Neat	1	2	3 l.c.i	4 l.c.i	5 l.c.i	6 l.c.i	7 l.c.i	8 l.c.i
Chloroform	0.5	-	-	-	-	-	-	-	-
	0.25	-	-	-	-	-	-	-	-
	0.125	-	-	-	-	+	+	-	-
	0.0625	-	-	-	-	++	++	+	-
	0.0313	-	+	-	+	++	++	++	+
	0.0156	+	++	+	+	++	++	++	++
	0.0078	++	++	++	++	++	++	++	++
CONTROL TUBE 8		++	++	++	++	++	++	++	++
TUBE 9		-	-	-	-	-	-	-	-
TUBE 10		-	-	-	-	-	-	-	-
M.I.C mg/ml		0.0156	0.0313	0.0156	0.0156	0.125	0.125	0.0625	0.0313
M.B.C mg/ml		0.0313	0.0625	0.0313	0.0313	0.25	0.25	0.125	0.0625

KEY: - = no growth on sub culture (MBC),+ = growth on sub culture (MIC), ++ = visible growth in media and control

1= *S. aureus* (NCTC 6571), 2 = *E. coli*, 3 = *Enterobacter aerogenes*, 4 = *Protoeus vulgaris*, 5 = *Streptococcus specie*, 6 = *Bacillus specie*, 7 = *Pseudomonas pyocyania*, 8 = *Klebsiella aerogenes*

Table 5: Result of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) Of Chloroform Pure Root Extract

Extract/ Solvent	Dilutions	Presence or Absence of Growth or Turbidity of Test Organisms		
		***Aspergillus flavus* l.c.i**	***Aspergillus niger* l.c.i**	***Candida albican* l.c.i**
Chloroform	Neat	-	-	-
	0.5	-	-	-
	0.25	+	-	-
	0.125	++	++	++
	0.0625	++	++	++
	0.0313	++	++	++
	0.0156	++	++	++
	0.0078	++	++	++
CONTROL TUBE 8		-	-	-
MI mg/ml		0.25	0.125	0.125
MFCmg/ml		0.50	0.25	0.25

KEY: l.c.i. = local clinical isolates, = No growth on subculture (MFC), + = No growth on subculture (MIC), ++ = Visible growth in media and control.

Table 6: Result of the FTIR spectroscopy of chloroform pure root extract.

Wave band (cm^{-1})	Functional group	Description
2931	C-H stretch	For alkanes and alkyl groups.
1715	C=O stretch	For aldehydes and 6 – membered ring ketones
1263	C – O Stretch	For esters and acids
735	C – H def	For aromatics and alkyl groups
449	C – H def	For methyl groups

Table 7: Result of the FTIR spectroscopy of ethyl acetate root extract.

Wave band (cm^{-1})	Functional group	Description
2930	C – H	For alkanes and alkyl groups.
1720	C = O	For aldehydes, 6-membered ring ketones
1446	C = C Stretch	For aromatics.
1265	C = O Stretch	For esters and ethers.
766	C – H def	For aromatics and methyl groups

Table 8: Result of UV-Visible Spectroscopy of Pure Chloroform Root Extract.

λmax (nm)	Chromophore	Description
725.40	O – C = O	$n \rightarrow \pi^*$ Bonds attached to aromatic ring
66.60	C = O	$n \rightarrow \pi^*$ Bonds attached to alkanes
616.00	C = O	$n \rightarrow \pi^*$ Bonds attached to alkanes
602.20	C = C	$n \rightarrow \pi^*$ Bonds attached to aromatic ring

Table 9: Result of The UV-Visible Spectroscopy of Pure Ethyl Acetate Root Extract

λmax(nm)	Chromorphore	Description
725.40	O = C - C= C	$n \rightarrow \pi^{*}$ Bonds attached to aromatic ring
666.60	O = C - C= C	$n \rightarrow \pi^{*}$ Bonds attached to aromatic ring..
616.00	O = C - C= C	$n \rightarrow \pi^{*}$ Bonds attached to aromatic ring.
602.20	O = C - C= C	$n \rightarrow \pi^{*}$ Bonds attached to aromatic ring.

Table 10: summary of the H^1 and c^{13} NMR results of pure chloroform root extract.

H^1(ppm) & multiplicity	Coupling Constant J(Hertz)	Type of Proton	C^{13} (ppm)	Type of carbon	position of carbon
7.3(s)	-	Ar- H	127.973	C – Ar	1
7.0(d)	8.16	Ar - H	125.836	C – Ar	2
6.5(d)	5.58	CH	77.677	C = O	3
4.1(d)	7.73	CH	77.236	C = O	4
2.3(q)	5.75	OCH2	77.033	C – O	5
1.7 (d)	9.21	CH2	76.403	C – O	6
1.3 (d)	45.31	CH2	34.115	C	7
0.9 (t)	18.25	CH3	33.896	C	8
			31.934	CH	9
			29.693	CH	10
			29.459	CH	11
			29.371	CH	12
			29.269	CH_2	13
			29.137	CH_2	14
			24.890	CH_2	15
			24.700	CH_2	16
			22.694	CH_3	17
			14.143	CH_3	18

Table 11: Summary of ^{1}H and ^{13}C NMR result of pure ethylacetate root extracts

H^1 (ppm)& Multiplicity	Coupling Constant (J)	Type of Proton	C^{13} (ppm)	Type of Carbon	Position of Carbon
7.3(s)	-	ArH	77.66	COH	1
4.2	24	OH	77.033	C=O	2
2.4	3.80	CH	76.403	C=O	3
2.1(d)	3.67	CH	34.027	C-O	4
1.6(s)	3.48	CH	31.919	C-O	5
1.3(d)	49.92	CH_2	29.693	C	6
0.9(d)	29.11	CH_3	29.444	C	7
			29.371	CH	8
			29.254	CH	9
			29.210	CH	10
			29.093	CH	11
			28.610	CH	12
			28.288	CH_2	13
			22.700	CH_2	14
			22.694	CH_3	15
			19.151	CH_3	16
			14.128	CH_3	17

CONCLUSION

From the results, root of *urena lobata* showed very strong antibacterial and antifungal properties and so could be used to formulate drugs for the treatment of ailments.

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