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Phytochemical and Anti – Microbial Screening of the Aerial Parts of *Eleusine indica*

Alaekwe, I.O[1]* Ajiwe, V.I.E[2], Ajiwe, A.C[2] and Aningo, G.N[2]

[1]Chemistry Education Department, Federal College Of Education (Technical), Umunze

[2]Department Of Pure And Industrial Chemistry, Nnamdi Azikiwe University, Awka

*Corresponding Author E-mail: iykemome.io@gmail.com

ABSTRACT

Phytochemical screening was carried out on the aerial parts of Eleusine indica with the aim of establishing the ethnomedicinal claims on the vegetative part. The results revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins and acidic compounds. The chloroform and methanol extracts had antibacterial and anti fungal activities against the test organisms – S.aureus, E. coli, Enterobacter aerogens, P. Vulgaris, Streptococcus specie, Bacillus specie, Pseudomonas aerogenes, Klebsiella aerogenes, Aspergillus flavus, Aspergillus niger and Candida albicans. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the two extracts confirmed that they were active at very low concentrations. Pure compounds from the extract gave yellow crystalline solids which were characterized using spectroscopic instruments such as GC-MS, UV, FTIR, H^1- NMR and C^{13} – NMR. Hexadecanoic acid, [[[(2-aminoethoxy)hydroxyphosphinyloxy]methyl]-,2 –ethanediylester was the pure chloroform extract while Hexadecanoic acid ($C_{16}H_{32}O_2$)is the pure methanol extract.

Keywords:*Phytochemical/Antimicrobial analysis, Chloroform/Methanol extracts, Structural elucidation.*

INTRODUCTION

Plants have remained a major source of medicinal drugs. The use of plants for medicinal purposes dates back to thousands of years ago as they are employed by early men in the treatment of ailments[4]. Hundreds of plants species are recognized as having medicinal values and four out of every five of those plants are collected from the wild forest while most of them are from the floras of developing countries including Nigeria[8,9].

The use of herbs in the treatment of diseases is almost universal. Medicinal plants are considered to play a beneficial role in health care as they have been used in treating and preventing specific ailments. Biological activities have been discovered in the roots, stems, leaves, barks, flowers, seeds or fruits of various plant species[9].

These medicinal plants are particularly important to rural dwellers that may not be well served by formal health care systems.

Eleusine indica is a tufted annual grass belonging to the family *poaceae* formally known as *Gramineae* family. The plant is a common weed of cultivation in all territories and the probable ancestor of *Eleusina Coracana*[5]. The common names include; wire grass (English), Dogon (Mali), and Fula – pulanar (Senegal). The grass grows to 60cm high, slender or moderately robust or sprawling prostrate to 1.2m long. It is a common wayside plant and of disturbed sites having two sub species of which Ssp. Africana is the most robust and tetraploid. The plant has been associated with the treatment of haemoptysis in Cameroon[5],treatment of menstrual cycle disorder and oedema[3].

MATERIALS AND METHOD

The grasses were harvested from the permanent site of Nnamdi Azikiwe University, Awka in Anambra State. It was identified by a taxonomist Prof. J.C. Okafor of Fame Consultancy Services Enugu, Enugu state Nigeria. The grass was washed under running water to remove soil particles after which it was air dried at room temperature. The dried sample was pulverized and stored in a plastic container and taken to the laboratory for analysis.

Extraction procedures

The cold method of extraction was used. 500g of the pulverized sample was soaked in 3500ml of chloroform. This was allowed to stand for 24hours after which it was filtered. The residue was spread out and the chloroform allowed to evaporate at room temperature after which the residue was re – soaked in methanol and allowed to stand for 24hours. This was filtered and both chloroform and methanol extracts were concentrated by boiling off the solvents.

Phytochemical Screening

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

Bioassay Analysis of the Chloroform and Methanol Extracts

The antimicrobial activities were determined using the agar diffusion method[2]. 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 result of the phytochemical screening showed the presence of alkaloid, tannin, flavonoid, cardiac glycosides and acidic compounds (Table 1).

The result of the antibacterial activity of the chloroform and methanol extracts showed that both extracts were active against all the test organisms with the highest activity shown with *Enterobacter aerogenes* (in both extracts) having 38mm and 33mm average diameter zones of inhibition (Table 2).

The result of antifungal activity of both chloroform and methanol extracts showed that they were effective in inhibiting the growth of all three fungi used. The growth of *candida albican* was most inhibited with an average diameter of zone of inhibition 20mm in the chloroform extract and 18mm in methanol extract (Table 3).

The result of the MIC and MBC of the chloroform extract showed activity on the subcultures at 0.125 dilutions denoted by (+) and visible growth on both subculture and control at 0.125, 0,0625, 0.03125, 0.015625, and 0.0078125 dilutions denoted by (++). These results confirmed that the chloroform extract was highly bactericidal at very low concentrations (Table 4). The result of the MIC and MFC of the chloroform extract showed that at minimum concentrations, the extract inhibits the growth of all the test organisms used (Table 5).

Table 6 gave the result of MIC and MBC of the methanol extract. At 0.5 dilutions, there was inhibition of growth on the subculture. The result of the MIC and MFC of the methanol extract showed that at dilutions between 0.5 – 0.125, there were no growth on both the subculture and the control except for *Aspergillus flavus* which showed little growth at 0.125 dilution (Table 7). This showed that the methanol extract was effective in treating ailments associated with *Aspergillus niger* and *Candida albican.*

The FTIR Spectrum of the chloroform extract (Table 8) gave six absorption peaks. The carbonyl region showed a band at 1731.17 cm^{-1}, an indication of the presence of a Ketone or an ester.

The FTIR Spectrum Showed C-H deformation bonds for methyl groups at 461.97cm^{-1} while C-H deformation bonds for aromatics and alkyl groups occurred at 734.9 cm^{-1}. C-O deformation bonds for esters and ethers appeared at 1264.38 cm^{-1} while C=C stretch for aromatics occurred at 1443.77 cm^{1}. C=O stretch of Ketones occurred at 1731.17 cm^{-1} whereas C-H stretch of alkanes and alkenes appeared at 2929.97 cm^{-1}.

UV-visible spectroscopic analysis for Chloroform extract gave absorption at λmax of 740.50 (nm) (Table 9). This appeared in the visible region. The UV-Visible spectrum showed that the compound was highly conjugated.

The result of C^{13} NMR for the chloroform extract (Table10) showed the presence of nine signals having a carbonyl group, methylene and methyl group. There were three carbon environment having nine distinct carbon atoms in the carbon spectra. A sharp singlet of 2.25ppm in the H^1NMR spectrum suggested that the methyl group was part of a methyl ketone. The triplet at 0.80ppm of the H^1NMR with a large coupling constant and a doublet at 1.60ppm indicated the type of coupling by the hydrogen atoms.

The FTIR Spectrum for the methanol extract (Table 11) gave four absorption peaks. The spectrum showed the presence of O-H stretch at a weak band of 3057.27 cm^{-1}. There was also the presence of C-H bonds for alkyl and methyl groups at strong bands of 469.68 cm^{-1} and 733.94 cm^{-1}. The carbonyl region showed a band at 1264.38 cm^{-1} indicating the presence of acid groups which appear in the yellow continuum of UV and the O-H stretch in the FTIR. These supported the structure in fig 2.

The UV-Visible Spectroscopic analysis for Methanol extract (Table 12) gave absorption at 664.00 (nm). This appeared in the visible region of the spectrum and showed the compound to be conjugated.

The C^{13} NMR for the methanol extract (Table 13) showed the presence of five signals. The signals conform to the presence of four carbon environment having five distinct carbon atoms in the carbon spectra. The triplet at 2.0 ppm of the H^1NMR showed the presence of two neighboring proton for an equivalent methyl proton. The high coupling constant of 38.12Hz confirmed the influence of other methyl protons. The duplets at 1.2 and 0.8 ppm are due to methylene protons close to a methyl group and a COH respectively.

The compounds isolated from the chloroform and methanol extracts were found to be derivatives of Hexadecanoic acid, phosphatidylethanolamine and ethanediyl ester. The compounds isolated are 1 – [[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]1-,2-ethandiylester and Hexadecanoic acid for the chloroform and methanol extracts respectively (fig 1 and 2).

Table 1: Result of the phytochemical screening on each solvent extract of the crude sample

	Chloroform Extract	**Methanol Extract**
Alkaloids	+	+
Tannins	-	+
Flavonoids	+	-
Cardiac Glycosides	-	+
Saponins	-	-
Acidic Compounds	+	+

Table 2: Result of antibacterial activities of chloroform and methanol extract

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**	**36**	**30**	**38**	**26**	**24**	**16**	**22**	**20**
2	**Methanol**	**0.05**	**30**	**26**	**33**	**16**	**18**	**14**	**19**	**18**
	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 antifungal activities of chloroform and Methanol extract

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	$CHcl_3$	0.05	10	14	20
2	MeOH	0.05	14	16	18
	Control 50%Acetone	0.05	NA	NA	NA

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

Table 4: Result of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chloroform pure extract

Extracts/Solvents	**Presences or Absence of Growth or Turbidity of Test Organisms**								
	Dilutions Neat	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**
	0.5	-	-	-	-	-	-	-	-
Chloroform	0.25	-	-	-	-	-	-	-	-
	0.125	-	-	-	-	-	+	-	-
	0.0625	-	-	-	-	-	++	+	+
	0.0313	-	-	-	+	+	++	++	++
	0.0156	+	+	+	++	++	++	++	++
	0.0078	++	++	++	++	++	++	++	++
CONTROL TUBE 8		++	++	++	++	++	++	++	++
TUBE 9		-	-	-	-	-	-	-	-
TUBE 10		-	-	-	-	-	-	-	-
M.I.C mg/ml		0.0155	0.0155	0.0155	0.0313	0.0313	0.125	0.125	0.125
M.B.C mg/ml		0.0313	0.0313	0.0313	0.125	0.125	0.25	0.125	0.125

KEY: A = *S.aureus* NCTC 6571, B = *E.Coli* NCTC 1048, C = *Enterobacter aerogenes,*
D = *Proteus vulgaris* E = *Streptococcus specie,* F = *Bacillus specie*
G = *Pseudomonas aerogenes,* H = *Klebsiella aerogenes*
- = No growth on subculture (MBC) + = Growth on subculture (MIC)
++ = Visible growth in media and control.

TABLE 5: Result of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of chloroform pure 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
	Neat	-	-	-
	0.5	-	-	-
Chloroform	0.25	+	-	-
	0.125	++	++	++
	0.0625	++	++	-
	0.0313	++	++	++
	0.0156	++	++	++
	0.0078	++	++	++
CONTROL TUBE 8		-	-	-
MIC mg/ml		0.25	0.125	0.0313
MFC mg/ml		0.50	0.25	0.0625

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 minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of pure methanol extract

Extracts/ Solvents		Presences or Absence of Growth or Turbidity of Test Organisms							
	Dilutions	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
	Neat	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
Methanol	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	0.0313 0.0625	0.0156 0.0313	0.1250 0.25	0.125 0.25	0.25 0.50	0.125 0.25	0.125 0.25
M.B.C mg/ml									

KEYS: A = *S.aureus* NCTC 6571, B = *E.Coli* NCTC 1048
C = *Enterobacter aerogenes*, D = *Proteus vulgaris*
E = *Streptococcus specie*, F = *Bacillus specie*
G = *Pseudomonas aerogenes* , H = *Klebsiella aerogenes*

Table 7: Result of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of pure methanol 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
	Neat	-	-	-
	0.5	-	-	-
	0.25	-	-	-
MeOH	0.125	+	-	-
	0.0625	++	+	+
	0.0313	++	++	++
	0.0156	++	++	++
	0.0078	++	++	++
CONTROL				
TUBE 8		-	-	-
MIC mg/ml		0.125	0.0625	0.0625
MFC mg/ml		0.25	0.125	0.125

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

Table 8: FTIR spectroscopic analysis for purified chloroform extract

Wave Band cm^{-1}	Description (Functional Group)
461.97	C-H deformation bonds for alkyl and methyl groups
734.9	C-H deformation bond for Aromatics and alkyl groups
1264.38	C-O deformation bond for esters and ethers
1443.77	C=C stretch for aromatics and alkyl groups
1731.17	C=O stretch for Ketones and esters
2929.97	C-H stretch vibration for alkanes and alkenes

Table 9: UV-visible spectroscopic analysis result for chloroform extract

Extract	λmax (nm)	Chromospheres/Description
Chloroform	740.50	C=O bonds, n→π* transition

Table 10: Summary of the H^1 and C^{13} NMR result for chloroform extract

H^1(ppm)& Multiplicity	Coupling Constant (J)	Types of Proton	C^{13} (ppm)	Types of Carbon
7.25 (s)		OCH_3	77.648	C-O
5.40 (s)		$R_2C=CHR$	77.208	C-O
4.20 (s)		$R_2C=CH_2$	77.018	C-O
2.25 (s)		CHO	76.374	C-O
1.60 (d)	8.60	$CHCH_2$	31.934	CH_2
1.20 (s)		CH_2	29.708	CH_2
0.80 (t)	25.30	CH_3	29.371	CH_2
			22.674	CH_2
			14.128	CH_3

Table 11: FTIR spectroscopic analysis for purified methanol extract

S/N	**Wave Band cm^{-1}**	**Description (Functional Group)**
1	469.68	C-H deformation bonds for alkyl and methyl groups
2	733.94	C-H deformation bonds for aromatics and alkyl groups
3	1264	C-O deformation bonds for esters and ethers
4	3057.27	O-H stretch of acids and alcohols

Table 12: UV- Visible spectroscopic analysis for purified methanol extract

Extract	λmax (nm)	Chromospheres/Description
Methanol	664.00	C=O bonds, n→π* transition

Table 13: Summary of the H^1 and C^{13} NMR result for pure methanol extract

H^1(ppm)& Multiplicity	Coupling Constant (J)	Types of Proton	C^{13} (ppm)	Types of Carbon
2.0 (t)	38.12	CHO	77.648	C=O
1.2 (d)	35.75	CH_2	77.208	C-O
0.8 (d)	15.50	CH_3	77.003	CH
			76.374	CH_2
			29.693	CH_3

Fig 1: Hexadecanoic acid, 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy]methyl]- 1,2- ethanediyl ester. ($C_{37}H_{74}NO_8P$)

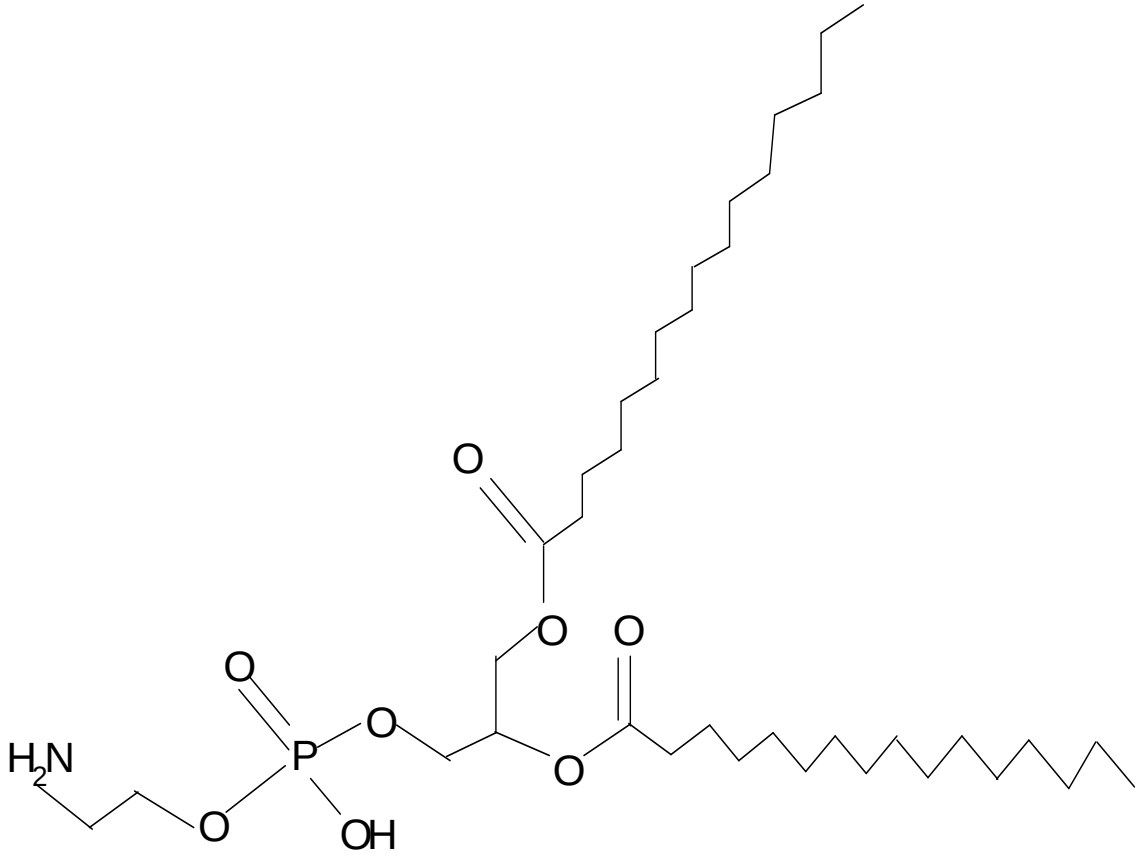
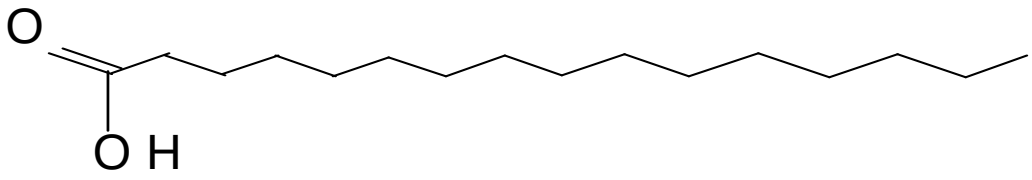


Fig 2: Hexadecanoic acid ($C_{16}H_{32}O_2$)



CONCLUSION

These extracted compounds are known to be anti cancerous, antifungal , and anti – convulsant and are used in treating various viral and fungal related diseases. This confirmed the traditional uses of *E. indica*.

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