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EVALUATION OF THE MEDICINAL PROPERTIES OF IMPERATA CYLINDRICA (ATA) LEAF

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ABSTRACT

The leaves of Imperata Cylindrica were analysed to ascertain the active constituents responsible for the use of the plant in treatment of cough, toothache and as an astringent. The phytochemical analysis showed the presence of secondary metabolite such as flavonoids, tannins, alkaloids, acidic compounds, reducing sugars and proteins. The antibacterial and antifungal screening of the extracts showed considerable level of activity against the test organisms staphylococcus aureus, E. coli, Enterobacter aerogens, proteus vulgaris, streptoccus specie, bacillus specie, pseudomonas aerogenes, klebsiella aerogens, s. aureus, salmomonella species, pseudomonas pyocyanin, aspergillus flavus, aspergillus niger and candida albicans. Preparative thin layer chromatography and thin layer chromatography techniques were used to separate pure compounds from the extracts. The pure compounds were subjected to spectroscopic analysis such as GC – MS, UV – Visible, FTIR, H^1 – NMR and C^{13} NMR. The spectral analyses suggested the presence of Hexadecanoic acid, ethyl ester and cis – 9- Hexadecenal.

KEYWORDS: imperata Cylindrica, secondary metabolites, hexadecanoic acid.

INTRODUCTION

Traditional use of medicine is recognized as a way to learn about potential future medicines. Plants have remained a major source of medicinal drugs (Alaekwe, et al 2015). Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insect, fungi and herbivorous mammals. By chance some of these compounds whilst being toxic to plant predators turn out to have beneficial effects when used to treat human diseases (Roy, 2004).

Imperata Cylindrica is a perennial, rhizomatous grass belonging to the family Poaceae formally known as Gramineae family. The common name includes, Blady grass (English), Paille dedys (French), Cogon grass (USA). This invasive plant can be found throughout tropical and subtropical regions on every continent except Antarctica. It grows up to 120 cm high with narrow, rigid leaf-blades. Lower leaf-sheaths bearded at the mouth, upper usually glabrous; blades glabrous or hairy on the lower part, up to 100 cm long, often less, usually 3-10 mm wide, expanded; panicle 5-10 cm long; spikelets surrounded by hairs 10 - 15 mm long(www.fao.org).

The leaves are antibacterial, diuretic, and febrifuge (Yeung, 1985). The leaves and flowers are used in the treatment of hemorrhages and wounds (Duke and Ayensu, 1985). They are decocted and used to treat fevers, thirst etc (Duke and Ayensu, 1985).

MATERIAL AND METHODS

Phytochemical Screening

The plant crude extracts were screened using standard methods as described by Sofowora, 1983 and Harbone, 1998 to identify plant metabolites.

Analysis of the Chloroform and Chloroform – Methanol Extracts.

The antimicrobial activities were determined using the agar diffusion method (Bryant, 1972). 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 analysis showed the presence of major phytocompounds such as flavonoids, tannins, alkaloids, acidic compounds, Reducing sugar, and proteins. The presence of flavonoids may be responsible for the diuretics and antibacterial properties (Table 1).

The Chromatographic analysis for chloroform leaves extract and chloroform – methanol leaves extract gave R_f values of 0.616 and 0.561 respectively (Table 2).

Antibacterial activity of purified extracts showed activity against staphylococcus aureus, Eschericha coli, enterobacter aerogenes, proteus vulgaris and bacillus specie. Salmonella species was resistant to all extracts while klebsiella aerogenes, streptococcus species and pseudomonas pyocyania were resistant in chloroform – methanol leaf. Proteus vulgris had the highest average diameter zone of inhibition (26mm) (Table 3).

The result of the antifungal activity showed that candida albican has the highest diameter zone of inhibition of 8mm in the chloroform leaves (Table 4).

The FTIR spectrum result (Table 5) showed the major chromophores, C – H deformation bonds for alkyl groups at 741.65 cm^{-1}, C = C stretch for alkenes and aromatics at 895.96 cm^{-1}, C – O deformation at 1265.35cm^{-1}, and C – H bond for alkyl group at 1419.66 cm^{-1}. O – H stretch of alcohols and acids appeared at 3054.38 cm^{-1}.

The FTIR spectrum result for the chloroform – methanol extract (Table 6) showed major chromophores with frequencies ranging from 446.54cm^{-1} – 3055.35cm^{-1}. These results supported the suggested structures for the two extracts (Figure 1 and 2)

The UV – visible spectrum of the chloroform leaf extract gave absorption peaks at both the UV and visible region. The absorption in the visible region λmax 883.00 to 667.50nm showed that the compound had high conjugation of pie bonds (Table 7).

The UV – visible spectrum of the chloroform - methanol leaf extract gave absorption peaks at 883.50, 874.50, 742.50 and 659.50 nm respectively. The wave bands in the UV – Visible spectrum showed that the compound was conjugated (Table 8).

H^1 NMR spectrum for chloroform leaf showed a methyl hydrogen 0.8ppm with a coupling constant of 25.48J.

A methylene hydrogen at 1.2ppm, a $CHCH_2$ proton at 1.6ppm, two CHO protons at 2.0 and 2.3 ppm respectively and two $R_2C = CH_2$ duplets protons at 4.2 and 5.4 ppm with coupling constant of 1.73(MHZ) and 1.37(MHZ) respectively. C^{13} NMR spectrum (Table 9) recorded 9 signals equivalent to 9 distinct carbon atoms. One carbonyl carbon atom occurred at 77.648ppm while two C – O carbon atoms appeared at 77.018 and 76.374 respectively. Five methylene carbon atoms signal appeared at 31.919, 29.693, 29.356, 29.122 and 22.694ppm respectively with a methyl carbon atom signal occurring at 14.128 ppm.

H^1 NMR spectrum for chloroform – methanol leaf extract showed a triplet methylene proton at 1.3ppm with a coupling constant of 17.36MH_Z. A singlet methoxy proton at 3.3ppm and a duplet $R_2C = CH_2$ proton at 4.9ppm with a coupling constant of 70.00MH_z. The C^{13} NMR spectrum recorded 9 absorption signals equivalent to 9 distinct carbon atoms. A carbonyl carbon atom (C = O) appeared at 105.000ppm while seven methylene carbon atoms (carbon 2 to 8 position) occurred at 48.861, 48.436, 47.997, 47.572, 47.147,46.723 and 46.298ppm respectively. A methyl carbon atom (Carbon position 9) was recorded at 29.357ppm. (Table10).

The GC – MS result for the Chloroform extract gave Hexadecanoic acid, ethyl ester as one main molecular ion (Figure 1). This was supported by the C^{13} NMR result while the GC – MS results for the chloroform – methanol leaf extract gave Cis – 9 – Hexadecenal which corresponded to the C^{13} NMR 9 distinct carbon atoms (Figure 2) and supported by the combination of UV – Vis, NMR and GC – MS results.

The result of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the chloroform leaf extract (Table 11) showed that there were activity on both subculture with the exception of salmonella specie, klebsiella aerogenes, streptococcus specie and pseudomonas pyocyania which the chloroform extract had no activity on. These bacterial species that chloroform extract showed sensitivity on confirmed that the extract had high activity at very low concentrations. The high activity on microbes suggests the use of this extract for cure of diseases related to this micro – organisms. It is however, worthy to note that this compound had no fungi activity

The result of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the chloroform – methanol leaf extract (Table 12) showed that there were activities on the test organisms except for Klebsiella aerogenes, Streptococcus specie, Pseudomonas pyocyanin and Salmonella species and Bacillus specie . The purified extracts were very active at low concentrations of 0.5mg/ml for E. Coli, Enterobacter aerogenes, P roteus vulgaris, S. aureus and Streptococcus albus.

Table 1: Results of the Phytochemical Composition of the Leaf Crude Extract.

Phytocompound	Inference
Saponins	- -
Flavonoids	++
Proteins	++
Steroids	- -
Tannins	+ +
Alkaloids	+ +
Reducing Sugar	+ +
Carbohydrate	- -
Acidic Compounds	+ +
Cardiac glycosides	- -

Key: ++ = Positive - - = Negative

Table 2: Result of R_f Values For The Extracts.

Chloroform leaves extract	0.616
Chloroform – methanol leaves extract	0.561

Table 3: Results of Antibacterial Activities of Chloroform and Chloroform – Methanol Leaf Extracts.

Average Diameter of Zones of Inhibition												
S/N	**Volume**	**Used (cm^3)**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1.	Chloroform	0.05	12	14	10	16	18	NA	NA	NA	NA	12
2.	Chloroform –methanol	0.05	10	14	14	14	12	NA	NA	NA	NA	10
3.	Control 50% acetone	0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

KEY: NA = No activity
L. C. I = Local Clinical Isolate

A	=	E.Coli (NCTC 10418)	B	=	staphylococcus aureus (NCTC 6571)
C	=	Bacillus specie	D	=	Protens Vulgaris
E	=	Enterobacter aerogenes	F	=	Klebsiella aerogenes
G	=	Streptococcus specie	H	=	Pseudomonas pyocyania
I	=	Salmonella specie	J	=	streptococcus albus

Table 4: Antifungal Activity of Purified Leaf Extracts of Imperata Cylindrica.

S/N	Extracts	Volume used	Aspergillus niger (cm^3)	Aspergillus flavus L.c.i	Candida albican L.C.i
1.	Chloroform leaves	0.05	NA	NA	8
2.	Chloroform – Methanol	0.05	NA	NA	NA
3.	Control 50% Acetone	0.05	NA	NA	NA

KEY: NA = No Activity
L.C.i = Local Clinical Isolate.

Table 5: Ftir Spectra of the Chloroform Leaf Extract.

Frequency cm^{-1}	Description
741.65	C – H deformation bonds for alkyl groups
895.96	C = C stretch for alkenes and aromatics
1265.35	C – O deformation bonds for esters and acids
1419.66	C – H bond for alkyl groups
1736.96	C = O stretch for ketones and esters
2305.01	O – H stretch of alcohols and phenolics
2855.71	C – H stretch of alkanes
2927.08	C – H stretch for alkanes
3054.38	O – H stretch of acids and alcohols

Table 6: Ftir Spectra of the Chloroform - Methanol Leaf Extract.

Frequency cm^{-1}	Description
446.54	C – H deformation bonds for methyl groups
740.69	C – H deformation bonds for aromatics and alkyl groups
896.93	C – H deformation bonds for alkenes and aromatics
1266.31	C – O deformation bonds for esters and acids
1425.44	C = O bond stretch for alkenes
1589.40	C = O bond stretch for aldehydes and ketones
2304.05	C – H stretch of alkanes
2986.87	C – H stretch of alkanes
3055.35	O – H stretch of acids and alcohols

Table 7: UV – Visible Spectroscopic Analysis Result of Chloroform Leaf Extract.

Λ max (nm)	Chromophores	Description
883.00	C – C = O	n $\longrightarrow$ π* transition
801.00	C – C = O	n $\longrightarrow$ π* transition
739.50	C = O	n $\longrightarrow$ π* transition
667.50	C = O	n $\longrightarrow$ π* transition
320.00	C = C	n $\longrightarrow$ π* transition
304.00	C = C	n $\longrightarrow$ π* transition

Table: 8 UV – Visible Spectroscopic Analysis Result of Chloroform-Methanol Leaf Extract.

Λ max (nm)	Chromophores	Description
883.50	C – C = O	n $\longrightarrow$ π* transition
874.50	C – C = O	n $\longrightarrow$ π* transition
742.50	C = 0	n $\longrightarrow$ π* transition
659.50	C = 0	n $\longrightarrow$ π* transition

Table 9: Summary of the H^1 And C^{13} Nmr Result For Chloroform Leaf Extract.

$H^1\delta$ (ppm)	Coupling Constant (mh_z)	Types of Proton	$c^{13}\delta$ (ppm)	Types of Carbon	Position of Carbon
0.8 (d)	24.48	CH_3	77.648	C = O	1
1.2 (s)	-	CH_2	77. 018	C – O	2
1.6 (s)	-	$CHCH_2$	76.374	C – O	3
2.0 (s)	-	CHO	31.919	$CH = CH_2$	4
2.3(d)	4.92	CHO	29.693	CH_2	5
4.2(d)	1.73	$R_2C = CH_2$	29.356	CH_2	6
5.4(d)	1.37	$R_2C = CH_2$	29.122	CH_2	7
			22.694	CH_2	8
			14.128	CH_3	9

Table 10: Summary of the H^1 And C^{13} Nmr Result For Chloroform - Methanol Leaf Extract.

$H^1\delta$ (ppm)	Coupling Constant (MH_Z)	Types of Proton	$c^{13}\delta$ (ppm) Position of	Types of Carbon	Position of Carbon
1.3 (t)	17.36	CH_2	105.0001	C = O	1
3.3(s)	-	$ROCH_3$	48. 861	$CH_2=CH_2$	2
4.9(d)	70.00	$R^2C = CH_2$	48.4363	CH_2	3
			47.9974	CH_2	4
			47.5725	CH_2	5
			47.1476	CH_2	6
			46.7237	CH_2	7
			46.2988	CH_2	8
			29.3579	CH_3	9

Table 11: Result of Mic and Mbc of Chloroform Leaf Extracts.

Presence or absence of growth or turbidity of test organisms										
Dilutions	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Neat	-	-	-	-	-	NA	NA	NA	NA	-
0.5			-	-	-					-
0.25			+	-	-					+
0.125			++	-	-					++
0.0625			++	+	+					++
0.63125			++	++	++					++
0.015625			++	++	++					++
0.007813			++	++	++					++
Control										
Tube 8			++	++	++					++
Tube 9			-	-	-					++
Tube 10			-	-	-					++
MIC mg/ml			0.25	0.25	0.25	0.0625	0.0625			0.25
MBC mg/ml			0.5	0.5	0.5	0.125	0.125			0.5

KEY: NA = No activity

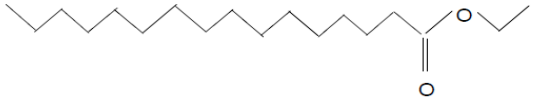
A = E.Coli (NCTC 1048). B = staphylococcus aureus (NCTC 6571).
C = Bacillus specie. D = Protens Vulgaris.
E = Enterobacter aerogenes. F = Klebsiella aerogenes.
G = Streptococcus specie. H = Pseudomonas pyocyania.
I = Salmonella specie. J = Streptococcus specie.

Table 12: Result of Mic and Mbc of Chloroform – Methanol Leaf Extracts.

Presence or absence of growth or turbidity of test organisms										
Dilutions	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Neat	-	-	NA	-	-	NA	NA	NA	NA	-
0.5	-	-		-	-				-	
0.25	+	+		+	+				+	
0.125	++	++		++	++				++	
0.0625	++	++		++	++				++	
0.63125	++	++		++	++				++	
0.015625	++	++		++	++				++	
0.007813	++	++		++	++				++	
Control										
Tube 8	++	++		++	++				++	
Tube 9	-	-		-	-				-	
Tube 10	-	-		-	-				-	
MIC mg/ml	0.25	0.25		0.25	0.25				0.25	
MBC mg/ml	0.5	0.5		0.125	0.125				0.5	

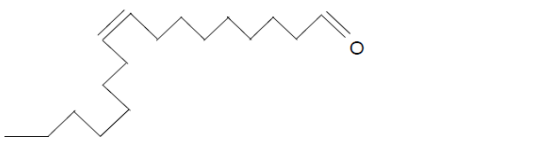
KEY: NA = No activity.

A = E.Coli (NCTC 1048) B = Staphylococcus aureus (NCTC 6571)
C = Bacillus specie D = Protens Vulgaris
E = Enterobacter aerogenes F = Klebsiella aerogenes
G = Streptococcus specie H = Pseudomonas pyocyania
I = Salmonella specie J = Streptococcus specie



Hexadecanoic acid, ethyl ester $C_{18}H_{36}O_2$ mol weight = 284

Figure 1:



Cis - 9 - Hexadecenal $C_{16}H_{30}O$ mol weight 238

Figure 2:

CONCLUSIONS

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs as a result of the presence of various compounds that are vital for good health. The Cis – 9 – Hexadecenal which was found in the plant is an aldehyde. Aldehydic compounds are known to have anti – bacterial activity and would therefore be effective in the management of bacterial infections. Aldehydes also impart the benefits of anti – inflammatory, anti- viral and sedative properties.

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