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Research Article

Antimicrobial analysis and structural elucidation of chloroform leaf extract of *Adenia cissanpeloides*

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Abstract

The leaf of *Adenia cissanpeloides* known as Nkochi ngwu or Uturu among the Igbos was studied to elucidate the structure of the pharmaceutical active ingredient present in the chloroform extract of the leaf. Thin layer chromatography showed a spot with Rf value of 0.85. The extract was subjected to spectroscopic analysis using FTIR, UV-visible, H^1 NMR, C^{13}NMR, GCMS and the structure elucidated was 1, 2-Benzene dicarboxylic acid dioctyl ester. Antibacterial and Antifungal activities of the pure extract using 10 bacteria species gram positive and gram negative such as *S.aureus*, *E.coli*, *Streptococcus species*, *Proteus vulgaris*, *Enterobacter earogenes*, *Bacillus species*, *S.albus* and fungal cultures such as *Aspergilles flavus*, *Aspergillus niger and candida albican* showed that the chloroform extract had zones of inhibition ranging from 16 to 36mm.These antimicrobial results were compared with that of some standard antibiotics and it was discovered that the chloroform fraction was more potent than the standard antibiotics.

Keywords: Drugs, Antibiotics, phytochemicals, Antibacterial

1. Introduction

Drugs have been used by humans for thousands of years to alleviate pains and illness. Naturally occurring pharmaceutical are found in some plants and animals. The knowledge about natural medicine aided in treatment of human ailments using various parts of plants or extracts, a culture which was passed down from generation to generation without actually understanding how the drugs work[1]. Herbs are small plants used to make medicines or flavour foods. Medicinal or herbal plants play vital roles which are beneficial to health care. One of the best known medicinal plants is opium poppy *(Papaver Sominferum)* obtained from the fruit of an annual plants. It is an unrivalled pain reliever but very addictive[2]. Quinine obtained from cinchona bark is a specific and effective remedy for malaria as well as satonin found in the species Artemisia in Asia[3]. Many plants having medicinal properties abound in Africa and other developing countries and had been reported to possess useful activity both on traditional and pharmaceutical aspects[4]. They include *Picralima nitida*[5], *pterocaprus soyauxi* (Oha ocha) and *Dissetis rotundifolia*[6]. A plant species can only be labeled a "Medicinal plant" when the medicinal properties have been proven by Western research[7]. Many plant species have been recognized as having medicinal values and properties which may be present in one or all their parts; roots, stem, bark, fruits, leaf, flower or seed. Scientists still search the world for plants and berries and the oceans for flora and fauna that might yield new medicinal compounds**.** Medicinal plants contain compounds that induce pharmacological reactions in human body[8]. These classes of compounds have inhibitory effects on micro-organisms. They are derivative of natural products consisting of secondary metabolites such as *alkaloids*, *Saponins*, *tannins*, *flavonoids*, *Steroids*, *terpenes*, and *Glycosides.* Phytocompounds or phytochemicals are natural bioactive compounds found in plant that work with the nutrient and dietary fibre to protect the body against disease. According to Gordon[9], about 25 percent of the prescription drugs dispensed in the United State contain at least one active ingredient derived from plant material. There are reports of antibiotics losing their potency due to increasing resistance of causative micro-organism to existing antibiotics[10],

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hence the need to search for new organic molecules with antimicrobial properties from available sources. The phyto-compound can serve as raw materials for new and more efficacious drugs. *Adenia cissanpeloides***,** a perennial plant which is locally called "Nkochi ngwu", or "Uturu" by the Igbos is used as a dermatological drug in the traditional healing of various forms of skin disease-infections- viral, bacterial and fungal origin. There seemed to be no recorded research on *Adenia cissanpeloides* as a medicinal plant, this study is therefore carried out to assess the active ingredients present and establish its pharmaceutical potentials.

2. Materials and Methods

The leaves were air-dried under laboratory conditions for six days and ground into powdered form using an electric blender. The phytochemical tests were carried out using Harborne's method[11].

2.1 Extraction and Isolation of Different Classes of Natural Products:

500g of the pulverized leaves were measured into a container, methanol and water were added in the ratio of 4:1 and the mixture homogenized for 5 minutes, allowed to stand for 24 hours and filtered. The filtrates were concentrated to one tenth volume in water bath at temperature below 40^0C acidified with 2M H_2SO_4 and extracted subsequently with chloroform to obtain chloroform and aqueous acid layer. The aqueous acid layer was basified to pH of 10 with NH_4OH and extracted with chloroform-methanol in the ratio of 3:1 twice to obtain the chloroform-methanol and aqueous basic layer. The chloroform extract was dried by evaporation to give Phenolic and Terpenoids while the aqueous basic layer was evaporated and extracted with methanol.

2.2 Antimicrobial Analysis of the fractions:

Stock cultures of selected bacteria species both Gram-positive and Gram- negative such *as Staphylococcus aureus, Bacillus sp, Salmonella sp, Klebsiella aerogenes, Pseudomonas pyocyania, Enterobacter aerogenes, Proteus vulgaris, Streptococcus sp* including fungal cultures such as *Aspergillus niger, Aspergillus flavus and Candida albicans* were obtained from Glanson Laboratory, Awka and the assay done at Assurance Biotechnology Laboratory, Nimo, Anambra State. The spectral Analyses of the Bio active Plant Extract FTIR, UV-Visible, Proton and C^{13} NMR and GC-MS were done at the National Research Institute for Chemical Technology, Zaria (NARICT) and the Central Instrument Laboratory University of Ife Nigeria. Minimum Inhibition Concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration were determined using Punched Agar Diffusion Method[12]. The results of the antimicrobial and antifungal activities of the pure extracts were compared with that of Funbac-A cream, Dermocare soap and Gentamicine ointment and recorded in the Tables 4 and 9.

3. Results and Discussion

The FTIR spectrum of leaf $CHCI_3$ extract L_7 summarized in Table 1 showed absorption band at 3363cm-^{1} which corresponded to the O–H stretch of alcohols. The peak at 2941.54cm^{-1} corresponds to C–H stretch for alkenes attached to benzene ring. The absorption at 1657.87cm-^{1} corresponds to C=O stretch for ketones and acid anhydrides attached to benzene ring, the band at 1439.84cm-^{1} corresponds to C=C stretch for alkene groups. The absorption band at 1026.16cm-^{1} represent the alkyl deformation bonds for alcohols while the presence of methyl group was indicated by the absorption band at 463.9cm$^{-1.}$

Table 1: Result of the FTIR Spectroscopic analysis of $CHCl_3$ Leaf extract

Wave number cm-^{1}	Description
3363	O – H stretches for alcohols
2941.54	C – H stretch for alkenes attached to benzene ring
1657.87	C – O stretch for amides, ketones and acid anhydrides attached to benzene Ring.
1438.94	C – C stretch for alkene group
1026.16	C – O Deformation bonds for alcohols and phenols
463.9	C – H Deformation bonds of methyl group.

The UV spectrum summarized in Table 2 of this leaf $CHCI_3$ extract showed high absorption maxima which indicated high degree of conjugation. This indicated the presence of chromophores such as C=C–OH ($\pi \rightarrow \pi^*$) of alcohol, C=C ($\pi \rightarrow \pi^*$) of alkene and C=O ($n \rightarrow \pi^*$) of a lactone and an ester moiety.

Table 2: Results of the UV – Visible spectroscopic analysis of the $CHCl_3$ Leaf extract

Wave length (nm)	Chromophores/Description
654.00	C = C ($\pi \rightarrow \pi^*$)
594.00	C = C ($\pi \rightarrow \pi^*$)
537.00	C = O ($n \rightarrow \pi^*$)
437.50	C = C ($\pi \rightarrow \pi^*$) of pyrole
413.50	C = O of an ester ($n \rightarrow \pi^*$)
396.00	C = C ($\pi \rightarrow \pi^*$)
383.50	C = C ($\pi \rightarrow \pi^*$)
378.00	C = C ($\pi \rightarrow \pi^*$)
358.50	C = C ($\pi \rightarrow \pi^*$)
294.50	C = O ($n \rightarrow \pi^*$)
289.00	C = O ($n \rightarrow \pi^*$)
282.50	C = O ($n \rightarrow \pi^*$)

The H^1–NMR. Spectrum (200 MH_z) $CDCI_3$ of the sample L_7 (Table 3) showed an aromatic proton indicated by a singlet at 7.3ppm and a hydroxyl proton indicated by a singlet at 5.3ppm. The doublet at 2.3ppm indicated an olefinic proton. Two doublets at 1.2ppm and 0.8ppm indicated a methylene proton and a methyl proton respectively.

Table 3: Summary of the H^1 and C^{13}-NMR Results of the leaf chloroform extract

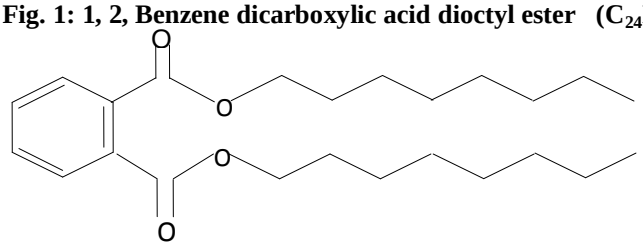
H^1 $^{\delta}$ ppm and multiplicity	Coupling Constant $J(H_Z)$	Type of Proton	C^{13} $^{\delta}$ ppm)	Type of Carbon	Position of Carbon
7.3 (s)	1. 81	C – AR	77 .648	C OO	1
5.3 (s)		O – H	77 .018	C=O	2
2.3 (d)	60.10	RC – CH	76 .374	C=O	3
1.2 (d)		CH_2	45 .375	C H	4
0.8 (d)	38. 09	R – CH_3	37 .439	C H	5
			37 .102	C H	6
			33 .837	CH_2	7
			32. 768	CH_2	8
			31 .934	CH_2	9
			30. 045	CH_2	10
			29 .708	CH_2	11
			29 .371	CH_2	12
			27 .438	CH_2	13
			27 .116	CH_2	14
			25. 213	CH_2	15
			24 .466	CH_2	16
			22 .694	CH_2	17
			21. 362	CH_2	18
			20 .542	CH_2	19
			19. 722	CH_2	20
			14. 128	CH_3	21

The C^{13}-NMR spectrum Table 3 indicated a total of twenty one (21) distinct carbon atoms as revealed from 21 carbon signals. The signal at 77.648ppm was a carboxyl carbon atom (position 1). Two carbonyl carbon atoms from a ketone had signals at 77ppm and 76.374ppm (positions 2 and 3 respectively). The signals at δ 45.375 to 37.102ppm are three methine carbon atoms of aromatic nucleus positions (4, 5, and 6). Fourteen methylene carbon atoms were indicated by the siganls at 33.837pmm to 19.722ppm (positions 7- 20). The signal at δ 14.128ppm indicated the presence of a terminal methyl carbon atom (position 21).

Based on FTIR, UV, NMR and GC-MS spectrum results the suggested structure was an ester derivative with molecular formula $C_{24}H_{38}O_4$ (fig.1).

Leaf Chloroform Extracts

Fig. 1: 1, 2, Benzene dicarboxylic acid dioctyl ester ($C_{24}H_{38}O_4$)



The level of antimicrobial activities exhibited by this chloroform leaf extract as shown in the antibacterial and antifungal screening results Table 4 with the test bacteria and fungi justified the traditional uses of the plant in ethnomedical practices. This was because the compound (a phthalate) derivative was strongly active against staphylococcus *aureus* with a zone of inhibition of 35mm and this pathogen causes skin and wound infections, abscesses, osteomyelitis and some infertility problems[13]. The extract was very active on *Pseudomonas pyacyania* with a zone of inhibition of 30mm which is more active than all the three standard antibiotics. For instance Funbact–A had a zone of inhibition of 28mm, Dermocare 22mm and Gentamicine 26mm suggesting that the extract could be used to produce more potent drugs that could cure diseases caused by this bacterium. The extract displayed stronger activities on test bacteria *Enterobacter aerogene* and *staphylococcus albus.* It had an average zone of inhibition of 38mm on *E.aerogene* which compared well with that of standard drug Funbact–A of 40mm and was higher than that of Dermocare of 28mm and was equally as active of Gentamicine (34mm). The average zone of inhibition of the extract on *S.albus* was 36mm which was comparable to the standard drug Funbact-A and was more active than Dermocare and Gentamicine with zones of inhibitions of 32mm and 28mm respectively. The extract was also active on *P.vulgaris* with a zone of inhibition of 28mm and *E.coli* (Zone of inhibition of 26mm). *P.vulgaris* causes urinary tract infections. The extract also was active on *streptococcus species* (minimum zone of inhibition 20mm which causes pneumonia, meningitis in neonates and the elderly. *P.vulgaris and Streptococcus sp*. colonise the intestines and female reproductive tract, increasing the risk of premature rupture of membranes during pregnancy and transmission of the organism to infants[14]. The activity of the extract on *Bacillus Sp.* (zone of inhibition. 16mm) suggested that the plant could be used in treatment of venereal diseases since *Bacillus* is a causative agent of anthrax. Anthrax is a disease associated with hemorrhage and serious effusion of various organs and body cavities with extreme prostration[15]. The extract was also active on *klebsiella aerogene* with a zone of inhibition of 24mm and *Salmonella species* with a zone of inhibition of 18 mm.

The extract was active on only one of the three test fungi - *Candida albican* with a zone of inhibition of 24mm. These findings therefore, scientifically justified the use of the leaf of this plant in the traditional medical practice.

The MBC results for this $CHCI_3$ leaf extract (Table 5) ranged from 0.0312 mg/ml for *S.aureus, Enterobacter aerogenes* and *S.albus* through 0.0625mg/ml for *E.coil, Pseudomonas Pyacyania, proteus vulgaris* and *klebsiella aerogenes* to 0.125mg/ml for *streptococcus sp.* and *salmonella sp* and 0.25mg for *Bacillus sp.* These are indications that the compound was active on test organisms even at low concentrations. These results were comparable to the MBC results of Funbact-A, Dermocare and Gentamicine used as positive controls.

The MIC results (Table 5) showed that the compound had MIC range of 0.015mg/ml to 0.125mg/ml for all test organisms which compared well with the MIC of the standard antibiotics which was 0.0156 to 0.125mg/ml each for Funbact-A and Dermocare while Gentamicine was 0.0156 to 0.25mg/ml. The MFC and MIC results for the fungus *Candida albicans* were 0.0625mg/ml and 0.0312 mg/ml respectively. This was comparable to the MFC of 0.0312 mg/ml and the MIC of 0.0156mg/ml each for the standard antibiotics Funbact-A and Dermocare soap. The MBC, MFC and MIC results showed activity to the test organisms at low concentration confirming that the extract was highly bactericidal and fungicidal. The findings of this study therefore support the fact that this compound could serve as a potent raw material for the production of new or known antibiotics.

Table 4: Results of antibacterial and antifungal activities of the chloroform extract including Funbact-A Cream, Gentamicine ointment and Dermocare soap.

S/N	Extract	Antibiotics	Volume used cm^3	*S.aureus* 6571	*E.coli* NTCT 10418	Streptococcus specie	P. vulgaris *Lci*	P. pyocyania	K. aerogenes *Lci*	*Salmonella* specie Lci	*E.aerogenes* Lci	*Bacillus specie Lci*	S.albus Lci	*A.flavus*	*A. niger*	*Candida albican*
1		L_7	0.05	35	26	20	28	30	24	18	38	16	36	NA	NA	24
2		*Funbact –A Cream*	0.05	38	36	30	35	28	24	22	40	20	38	26	24	36
3		*Dermocare soap 80g+ cold water*	0.05	30	24	18	20	22	26	20	28	16	32	20	18	28
4		*Gentamicine oitment 15g+ chloroform*	0.05	35	30	26	30	26	26	20	38	18	34	NA	NA	NA
5		*Gentamicine oitment. 15g + hot water*	0.05	30	26	24	28	24	26	18	34	14	28	NA	NA	NA
6		*Control 50% methanol*	0.05	NA	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7		*Chloroform*	0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Note: NA= No action; (NCTC 0418) = *Escherichia coli* (National culture type collection 10418); *S.aureus* (NCTC 6571) = *Staphylococcus aureus* (National culture type collection 6571).

Table 5: Results of Tentative Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the $CHCl_3$ Leaf Extract

Extract	*Dilution*	*Concentration g*	*Staphylococcus aureus*	*Escherichia coli*	*Streptococcus specie*	*Proteus vulgaris*	*Pseudomonas Pyocyania*	*Klebsiella aerogenes*	*Salmonella Specie*	*Enterobacter aerogenes*	*Bacillus specie*	*Staphylococcus albus*	*Aspergilus flavus*	*Aspergilusniger*	*Candida albican*
L_7	Neat	2.00	–	–	–	–	–	–	–	–	–	–	++	++	–
	1:2	1.00	–	–	–	–	–	–	–	–	–	–	++	++	–
	1:4	0.50	–	–	–	–	–	–	+	–	–	–	++	+	–
	1:8	0.25	–	–	–	–	–	–	++	–	+	–	++	++	–
	1:16	0.125	–	–	+	–	+	+	++	–	++	–	++	++	++
	1:32	0.0625	–	+	++	+	++	++	++	+	++	–	++	++	++
	1:64	0.03125	+	++	++	++	++	++	++	+	++	+	++	++	++
	1:128	0.0156	++	++	++	++	++	++	++	++	++	++	++	++	++
Control	8		++	++	++	++	++	++	++	++	++	++	++	++	++
Tubes	9		–	–	–	–	–	–	–	–	–	–			
	10		–	–	–	–	–	–	–	–	–	–			
	MIC		0.0625	0.0312	0.0625	0.0312	0.0312	0.0312	0.0625	0.0156	0.125	0.0156			0.0312
	MBC		0.0312	0.0625	0.125	0.0625	0.0625	0.0625	0.125	0.0312	0.25	0.0312			
	MFC		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			0.0625

Notes: - = No growth on subculture (MBC); + = Growth on subculture MIC; + + = Visible growth in media and control; Tube 8 = Media and culture: This is the ability to support growth; Tube 9 = Broth cum extract control: To check sterility of broth without organis; Tube 10 = ½ strength solvent and broth control.

4. Conclusion

The finding of this study has scientifically justified the use of the leaf of *Adenia cissapeloides* in the ethno medical practices when administered within the appropriate toxicity levels for humans and animals. The antimicrobial results showed that the extracts were highly bacterial and fungicidal and of broad spectrum activities.

It therefore suggested that the extracts could serve as potent raw materials for the production of new or known antibiotics.

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