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Full Length Research Paper

Antimicrobial Analysis and Structural Elucidation of Chloroform-Methanol Extract of *Adenia Cissanpeloides*

AO Aboh[1*], VIE Ajiwe[2] and PAC Okoye[2]

[1] Department of Chemistry, Nwafor Orizu College of Education Nsugbe, Anambra State, Nigeria
[2] Department of Chemistry, Nnamdi Azikiwe University Awka, Anambra State, Nigeria

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The stem of Adenia cissanpeloides known as Nkochi Ngwu or Uturu among the Igbos was studied to elucidate the structure of the pharmaceutical active ingredients present in the stem extract of the plant. Thin layer chromatography showed a spot with Rf values of 0.79. The extract was subjected to spectroscopic analysis using FTIR, UV-visible, H1 NMR C13 NMR, GCMS and the structure elucidated was 2-methyl-5-nonanone. Antibacterial and Antifungal Activities of the pure extract using 10 bacterial 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 flavius, Aspergillus niger and candida albican showed that the chloroform-methanol extract had zones of inhibitions ranging from 12 to 24 respectively. These antimicrobial results were compared with that of some standard antibiotics and it was discovered that the fraction was less potent than the standard antibiotics.

Keywords: Stem, Chloroform-methanol Extract, Structural Elucidation, anti-Microbial.

INTRODUCTION

Plants represent an enormous reservoir of biologically active molecules and so far only a small fraction of plants with medicinal activity have been assayed (Kirk-Patrick, 1995). 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 (Ajibota, 2004). They include quinine obtained from cinchona bark, a specific and effective remedy for malaria as well as satonin found in species Artemisia in Asia (Finar, 2005). Willow bark contains salicylates used to control fever and pains (Bruice, 2007). Medicinal or herbal plants play vital roles which are beneficial to health care. More research is being carried out on herbal plants because much has been discovered in them that are of great importance to healthcare (Harbourne 1973). A plant species can only be labeled a “Medicinal plant” when the medicinal properties have been proven by Western research, (Soejarto and Farnsworth, 1991). Hence the increasing encouragement by the World Health Organization (WHO), Africa Union (AU) and Federal Government of Nigeria to scientifically investigate the medicinal plant in our zones (Morah 2007 Sofowora, 1993).

Adenia cissanpeloids is an annual or perennial plant. It is a creeping grass with a narrow greenish yellow stem which bear alternate smooth woolly heart shaped green leaves. *Adenia cissanpeloides* which is locally called “Nkochi ngwu”, or “Uturu” by the Igbos is used as a dermatological drug for the treatment of skin infections. 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.

Materials and Methods

The stem samples of *Adenia cissanpeloides* were collected from Nsugbe in Oyi Local Government of Anambra State, Nigeria. The stem samples were cut into little pieces and air dried for about eight days and ground into the powdered form using an electric blender.

Extraction and Fractionation of the Crude Extracts into Different Classes According to Polarity

The active ingredients present in the pulverized stem were extracted into different classes according to polarity using the method adopted by Harbonne[9].

Procedure

Fig 1 showed the flow diagram of the procedure employed in the extraction of the active ingredient from the pulverized stem sample. The sample was fractionated into classes according to polarity. The classes are: Moderately polar extract ($CHCl_3$ extract) which are terpenoids and phenolic, basic extract,

Corresponding Author: ujuaboh@yahoo.com

($CHCl_3/CH_3OH$ extract) which are mostly alkaloids. Polar extract (CH_3OH extract) which are quaternary alkaloids and N-oxides. Neutral extract, ($CH_3COOCH_2H_5$ extract) which are fats, waxes or lipids Residue, which are fibres (mainly polysaccharide. The crude chloroform- methanol extract from the stem was dried by evaporation and analyzed directly by thin layer chromatography to determine the number of components in the fraction and Rf value was recorded. The fraction was subjected to FTIR, UV-Visible, H1 NMR, C_{13}NMR, GCMS to elucidate the structure of the components in the stem extract. Antimicrobial Analysis was also determined and the results were summarized in Tables 1-5.

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 in 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 Inhibitory Concentration, Maximum Bactericidal Concentration and Minimum Fungicidal Concentration were determined using Punched Agar Diffusion Method (Bryant 1972). The results of the antimicrobial and antifungal activities of the pure extracts was compared with that of Funbact-A cream, Dermocare soap and Gentamicine ointment and recorded in the Tables 4 and 5.

Results and Discussion

The FTIR spectrum of the chloroform -methanol extract was summarized in Table 1. The extract showed absorption bands at 3366.86cm-1 and 3366cm-1 which corresponded to the O-H stretch of alcohols. The sharp peak at 2961.80 cm-1 corresponded to C–H stretch for alkyl groups attached to an aromatic ring. The absorption band at 2526.83 cm-1 corresponded to C=C for aromatic. The absorption band at 1654.98cm-1 showed the presence of C=O stretch for ketones. C=C stretch for alkene was indicated by the absorption at 1435.09. The C–O deformation bond for alcohols and phenols was indicated by the absorption band at 1035.81 cm-1. The C-H deformation bonds for alkyl and methyl groups were indicated by the absorption bands at 876.68 cm-1 and 462.93 cm-1 respectively.

The UV-Visible spectrum summarized in Table 2 showed absorption maxima at 739nm and 657nm which indicated the presence of some chromophores like C=O ($n \longrightarrow \pi^*$) and C=C ($\pi \longrightarrow \pi^*$) transitions.

The proton NMR spectrum summarized in Table 3 showed chemical signals at 4.9ppm which corresponded to the presence of a keto proton (–C=O). The signal at 3.6ppm suggested the presence of a hydroxyl proton O–H. The presence of a methine proton, methylene proton and a methyl proton were indicated by the chemical signals at 3.3ppm, 1.3ppm and 0.9ppm.

The C^{13}–NMR summarized in Table 3 recorded nine signals corresponded to 9 distinct carbon atoms in the compound. The chemical shifts at 105ppm indicated a keto carbon (position 1). The chemical shifts at 48.861ppm corresponded to a carbon bonded to a hydroxyl group (Position 2). The presence of two methine carbon atoms was indicated by the chemical shifts at 48.436ppm and 48.011ppm (position 3 & 4). The chemical shifts at 47.587ppm, 47.162ppm and 46.737ppm indicated the presence of three methylene carbon atoms (positions 5-7). The two terminal methyl groups present in the molecule were indicated by the signals at 46.313ppm and 29.327ppm (position 8 and 9).

When these results FTIR, UV-Visible, H1-NMR and C^{13}-NMR were correlated with the major fragments obtained from the GC-MS results the proposed structure assigned to the compound was obtained (fig.2) with molecular formula $C_{10}H_{20}O$. The results of the antibacterial and antifungal assay of the stem chloroform-methanol extract (Table 4) using the punched agar diffusion method showed that the extract was active on all the ten test bacteria and on one fungus *Candida albican*. It was 24mm for *Enterobacter aerogenes*, 20mm for *S.aureus* and *S.albus*, 16mm for *E.coli*, *Streptococcus sp*. and *Pseudomonas pyocyania*, 14mm for Proteus vulgaris, 12mm for *Kelbsiella aerogenes*, *Salmonella sp*, and fungus *Candida albican* and 10mm for *Bacillus sp*. Table (4). *E.coil* causes food borne diseases. Most strains are harmless and live in the intestine of healthy humans and animals. *E.coli* 0157:117 strains produce a powerful toxin that can cause illness through food infections (Karch et al., 1994). This may lead to hemorrhagic diarrhea and kidney failure. This study suggests that potent drugs that could cure these ailments might be produced from this plant extract the extract was less active than the standard antibiotics but compared fairly with Dermocare on Enterobacter aerogenes and streptococcus sp. with zones of inhibition of 28mm and 18mm respectively. It also compared fairly with the standard Gentamicine on *S.albus* with zone of inhibition of 24mm (Table 4). This indicated that the extract possessed useful medicinal values.

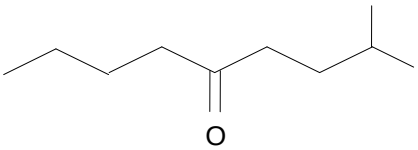

Fig 2: 2- methyl-5-nonanone (C10H20O)

The MIC and MBC results of the extract (Table 5) showed that *E.areogene* was inhibited and completely destroyed at very low concentrations of 0.0312mg/ml (MIC) and 0.0625mg/ml (MBC) respectively. *E.coli*, *Streptococcus sp, P.vulgaris, P.pyocyania* and *S.albus* was inhibited at concentrations of 0.125mg/ml each and completely destroyed at concentrations 0.25mg/ml each. *Klesiella aerogene*, *Salmonella sp., Candida albican* and *Bacillus sp*. were inhibited at concentrations of 0.25mg/ml each and completely destroyed at concentrations of 0.5mg/ml each. These results showed that the sample was active to the ten test bacteria and one fungus at low concentrations. This suggested that the extract was highly bactericidal and fairly fungicidal. The results are also comparable to the MIC and

MBC results of the test antibiotics. This supported the use of *Adenia cissanpeloides* in treatment of various ailments.

Conclusion

This result justified the use of the stem of *Adenia cissanpeloides* in ethno medical practices provided it would be administered within toxicity levels of human and animals. The antimicrobial results showed that the plant is highly bactericidal and fungicidal and of broad spectrum activities. It also suggested that the extract could be a potent raw material for pharmaceutical applications.

Table1: Results of the FTIR spectroscopic analysis of $CHCl_3$-MeOH Stem Extract

Wave number cm-1	Description
3366 .86	OH stretch of alcohols
3336	
2961.8	C–H stretch of alkyl group attached to a benzene ring
2526.83	C–H stretch for alkanes
1654.98	C= O stretch for ketones and aldehydes
1435.09	C =C stretches for alkene and arenes
1035.81	C– O deformation bond for alcohols, estersphenols
876.68	C –H deformation bond for alkyl and aryl groups
462.93	C –H deformation bond for methyl groups

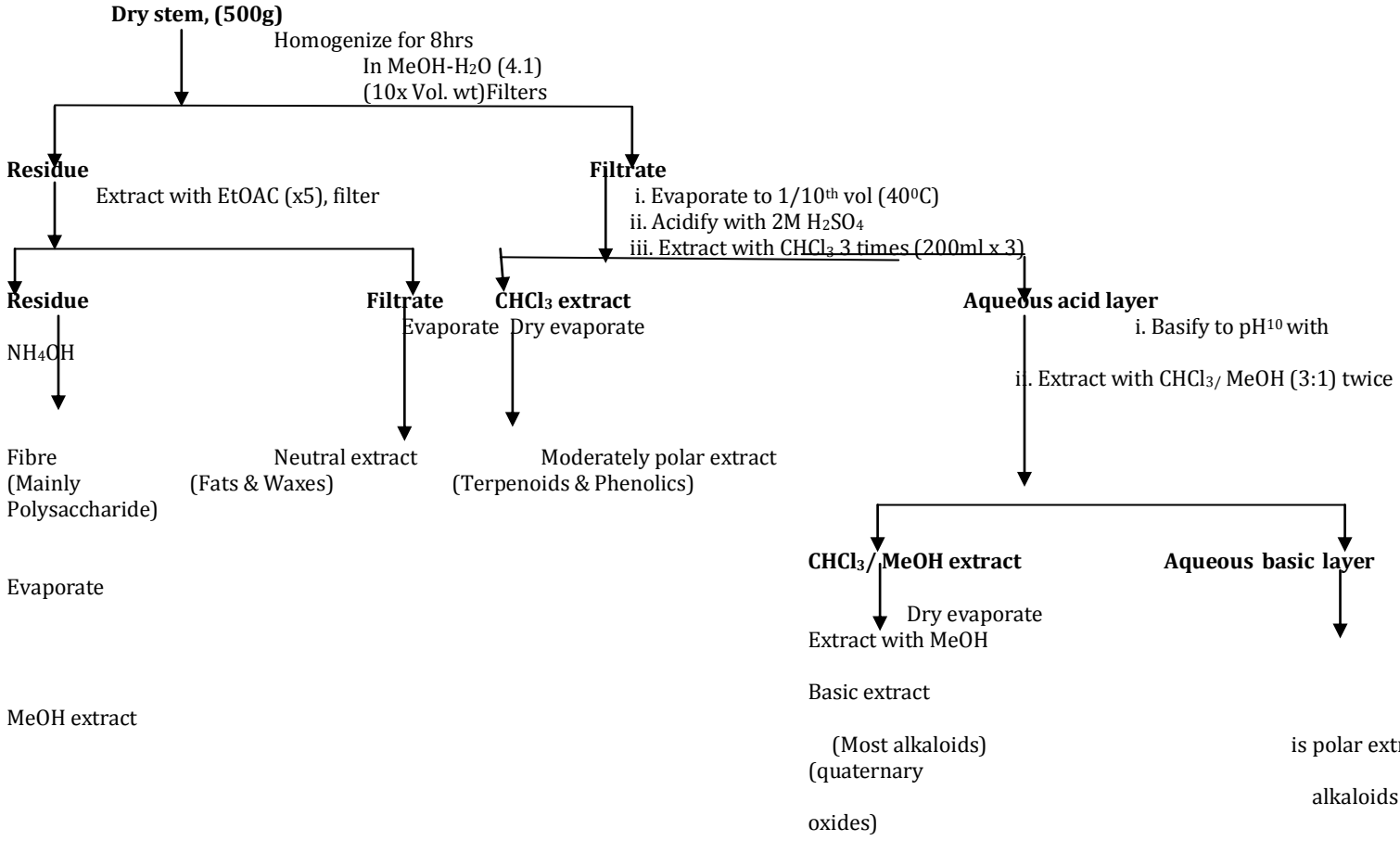

Fig 1: General Procedure for extraction of active ingredients of plant tissue and fractionating them into different classes according to polarity (Harbonne, 1998)

Table 2: Result of the UV-Visible spectroscopic analysis of the stem$CHCl_3$MeOH extract

Wavelength (nm)	Chromophores/Description
739 .00	C=O n → π*
657. 00	C=O n → π*

Table 3: Summary of the H^1 and C^{13} NMR results of the stem chloroform methanol extract S_{19}

H1 δ (ppm) and multiplicity	Coupling Constant J(H_Z)	Type of Proton	C^{13} δ ppm	Type of Carbon	Position of Carbon
4.9 (d)	66. 94	C =O	105 . 000	C – C=O	1
3.6 (s)		OH	48. 861	C – OH	2
3.3 (d)	9. 83	CH	48. 436	CH_2	3
1.3 (t)	13.53	CH_2	48 . 011	CH_2	4
0.9 (d)	9. 70	RCH_3	47. 587	CH_2	5
			47 . 162	CH_2	6
			46 . 737	CH_2	7
			46 . 313	CH_2	8
			29. 327	CH_3	9

Table 4: Results of antibacterial and antifungal activities of the extract S_{19} including Funbact-A cream, Gentamicine ointment and Dermocare soap

Extract S_1	Antibiotics	Dilution Conc mg/ml	*S.aureus*	*E. coli*	*Strep. specie* Lci	*P.vulgaris* Lci	*P.pyocyania*	*K.aerogenes Lci*	*Salmonella* specie Lci	*E.aerogenes* Lci	*Bacillus* specie Lci	*S.albus* Lci	*A.flavus*	*A.flavus*	*Candida Albical*
9	*Funbact –A Cream*	0.05	20	16	16	14	16	12	12	24	10	20	NA	NA	12
	Dermocare soap 80g+ cold water	0.05	38	36	30	35	28	24	22	40	20	38	26	24	36
		0.05	30	24	18	20	22	26	20	28	16	32	20	18	28
	Gentamicine oitment 15g+ chloroform	0.05	35	30	26	30	26	26	20	38	18	34	NA	NA	NA
	Gentamicine ointment. 15g + hot water	0.05	30	26	24	28	24	26	18	34	14	28	NA	NA	NA
	Control 50% methanol Chloroform	0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		0.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Note: NA= No action
E. coli (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 Fungal Concentration (MFC) of the Chloroform-methanol Stem Extract- S_{19}

Extract S_{19}	Dilution	Conc mg/ml	*S.aureus*	*E.coli*	*Strep. specie* Lci	*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*
	Neat	2.00	–	–	–	–	–	–	–	–	–	–	++	++	–
	1:2	1.00	–	–	–	–	–	–	–	–	–	–	++	++	–
	1:4	0.50	–	–	–	–	–	+	+	–	+	–	++	++	+
	1:8	0.25	–	+	+	+	+	++	++	–	++	–	++	++	+
	1:16	0.125	+	++	++	++	+	++	++	–	++	+	++	++	++
	1:32	0.0625	++	++	++	++	++	++	++	+	++	++	++	++	++
Control Tubes	1:64	0.0312	++	++	++	++	++	++	++	++	++	++	++	++	++
	1:128	.0156	++	++	++	++	++	++	++	++	++	++	++	++	++
	8		++	++	++	++	++	++	++	++	++	++	++	++	++
	9		–	–	–	–	–	–	–	–	–	–	–	–	–
	10		–		–	–	–	–	–	–	–	–	–	–	–
	MIC		0.0625	0.12	0.12	0.12	0.12	0.25	0.25	0.0312	0.25	0.125			0.25
	MBC		0.125	0.25	0.25	0.25	0.25	0.50	0.50	0.0625	0.50	0.250			
	MFC														0.50

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 organism.
Tube 10 = ½ strength solvent and broth control

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