

## Structural Elucidation and Antimicrobial Analysis of Chloroform Leaf Extract of *Acanthus montanus*

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**Abstract:** Leaf extract of *Acanthus montanus* of the family *Acanthaceae* which is widely used traditionally for medicinal purposes was worked on to elucidate the structure of the pharmaceutical active ingredients. Thin layer chromatography of chloroform leaf extract showed one spot with R<sub>f</sub> value of 0.78. The extract was subjected to spectroscopic analysis using FTIR, Uv-Visible, <sup>1</sup>H NMR, <sup>13</sup>C NMR and GCMS instruments. The suggested structure for the chloroform leaf extract was 4-butyl acrylate (3,5-Di-tert-butyl-4-hydroxy phenyl). Antibacterial and Antifungal activity of the pure extract was carried out extract using 10 bacteria species (gram positive and gram negative) such as *Salmonella* sp, *Staphylococcus aureus*, *Bacillus* sp, *Proteus vulgaris*, *S. albus*, *Enterobacter aerogenes*, *Pseudomonas pyocyania*, *Streptococcus* sp, *Klebsiella aerogenes* and fungal cultures such as *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* with zone of inhibition ranging between 8 and 22mm. This antimicrobial result was compared with that of some standard antibiotics and was discovered to be potent and effective in both fungi and bacteria.

**Keywords:** Fungi, Bacteria, Antimicrobial, Antibiotics and Structural elucidation.

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### I. Introduction

Since the dawn of human civilization, medicinal plant constitutes the basis of primary health care for the majority of the population. Many plants synthesize substances that are useful in human and animals nutrition. The use of plants as medicine predates written human history [1]. All plants produce chemical compounds as part of their normal metabolic activities. The development in the field of modern medicine temporarily subdued the traditional herbal medicine but has now stayed a comeback and herbal renaissance is blooming across the world [2, [3]. Green medicine is healthier, safer and harmless than synthetic ones. The acceptance of traditional medicine as an alternative form of health care and development of microbial resistance to the available antibiotics led researchers to investigate the antimicrobial activity of plant [4,5]. In recent years there has been renewed interest in natural medicines that are obtained from plant parts or extracts. On the order of 40% or more of the pharmaceuticals currently used in western countries are derived from natural sources [6]. Medicinal plant in addition to providing the basic for between 30 and 40% of today's conventional drugs, the medicinal and curative properties of various plants are also employed in herbal supplements, botanical, nutraceuticals and teas [7]. Medicinal plants are particularly important to rural residents who are not well served by formal health care systems. Studies on the medicinal values of some Nigeria plants have attracted so much attention from various professionals all over the world including Nigeria. This has lead the Federal Government of Nigeria to adopt the National Policy on traditional medicine in 1997 [8,9]. The plant studied in this research (*Acanthus montanus*) a perennial herb from the family *Acanthaceae* also known as mountain thistle or alligator plant is a striking small shrub with sparse branches and soft stem [10]. Native of West Africa and very popular in Southern Nigeria where it is used ethnomedicinally for the treatment of cough, inflammatory and infectious diseases. The root is acclaimed highly by various society of Africa as an effective remedy for abscesses while the leaf extract had been used to treat cases of threatened abortion, abdominal pains, pains of acute gastritis and believe to be antacid [11].

The objective of this study was to extract the phytochemical constituents in the leaf of the plant and elucidate the structure using spectroscopic techniques. The antimicrobial activity of each fraction was determined and compared with some existing antibiotics.

### II. Materials And Methods

The leaves were air dried under laboratory conditions for seven days, ground into powdered form using manual blender. Phytochemical tests were carried out by methods outlined by Jiofack *et al.* [7].

#### EXTRACTION AND ISOLATION OF DIFFERENT CLASSES OF NATURAL PRODUCTS

500g of the pulverized leaves were measured into a container, chloroform was added and the mixture was homogenized for 5 minutes, allowed to stand for 24h and filtered. The filtrates were concentrated to one tenth volume in water bath at temperature below 40<sup>0</sup>C acidified with 2ml H<sub>2</sub>SO<sub>4</sub> and extracted subsequently

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with chloroform. The chloroform extract was dried by evaporation giving rise to moderately polar extract (terpenoids and phenolics). The obtained extract was analyzed directly by thin layer chromatography to determine the number of components and it recorded one spot with R<sub>f</sub> value of 0.78. The fraction was further subjected to FTIR, UV-Visible, H<sup>1</sup>NMR, C<sup>13</sup>NMR and GCMS to elucidate the structure of the component in the CHCl<sub>3</sub> leaf extract. The antimicrobial activity of the chloroform leaf extract was then carried out with 10 bacteria and three fungi. The microbial activity of the Chloroform extract was further compared with existing commercial antibiotics. The method used for microbial analysis was the punch agar diffusion method [3].

### III. Results

Table 1. Results of the FTIR Spectroscopic Analysis of the Pure CHCl<sub>3</sub> Leaf Extract.

Wave band cm <sup>-1</sup>	Description
3244.38	OH stretch of aromatics
2921.29	CH stretch of alkanes and aromatics
2856.67	
1725.38	C=O stretch of esters and ketones
1459.20	C=C stretch of aromatics and alkanes
1375.29	C-H in plane bend of alkenes
1251.84	C-O stretch of esters and ketones
1183.30	
1101.39	C-O deformation of ketones and esters
1061.85	CH deformation of alkyl groups
976.01	CH bend of alkene
753.23	CH bend (ortho) of aromatics
721.40	CH bend of disubstituted alkenes

Table 2. UV- Visible Spectrum Results for Pure CHCl<sub>3</sub> Leaf Extract

Wave length(λnm)	Bond	Transition
891.80		
883.00	-C=O	n→π*
807.50	-C=O	n→π*
739.00	C=C	n→π*
656.50	C=C	π→π*

Table 3. Summary of the H<sup>1</sup> and C<sup>13</sup> NMR results of the CHCl<sub>3</sub> Leaf Extract

H <sup>1</sup> δ(ppm) and multiplicity	Coupling constant	Types of proton	C <sup>13</sup> δ (ppm)	Types of carbon	Position of carbon
7.22(s)	-	ArOH	77.66	C=O	1
3.90 (t)	10.27	R-COO-C-H	77.22	C-O	2
2.29(d)	9.47	Ar-C-H	77.02	C-OH	3
1.64(d)	8.40	C=C-CH <sub>3</sub>	76.39	C	4
1.34(d)	55.52	R <sub>2</sub> CH <sub>2</sub>	33.94	C=C	5
0.82(d)	16.34	RCH <sub>3</sub>	31.92	C=C	6
			31.42	C=C	7
			30.16	C=C	8
			29.69	CH	9
			29.61	CH	10
			29.44	CH	11
			29.37	CH <sub>2</sub>	12
			29.25	CH <sub>2</sub>	13
			29.12	CH <sub>2</sub>	14
			29.06	CH <sub>2</sub>	15
			24.89	CH <sub>2</sub>	16
			24.70	CH <sub>3</sub>	17
			22.69	CH <sub>3</sub>	18
			20.70	CH <sub>3</sub>	19
			14.13	CH <sub>3</sub>	20

FTIR spectrum (Table 1) supported the structure of CHCl<sub>3</sub> leaf thus, OH stretch of aromatic was observed at 3244.38cm<sup>-1</sup> while CH stretch of alkanes and aromatics appeared at 2921.29 and 2856.67cm<sup>-1</sup>. C=O stretch of esters and ketones appeared at 1725.38cm<sup>-1</sup> whereas C=C stretch of aromatics and alkenes appeared at 1459.20cm<sup>-1</sup> while CH in plane bend of alkenes was observed at 1375.29cm<sup>-1</sup>. C-O stretch of esters and ketones occurred at 1251.84cm<sup>-1</sup>. C-O deformation of ketone and esters appeared at 1101.39cm<sup>-1</sup>, CH deformation of alkyl groups appeared at 976.01cm<sup>-1</sup> while CH bend of aromatics occurred at 153.23cm<sup>-1</sup> and CH bend of disubstituted alkenes was observed at 721.40cm<sup>-1</sup>.

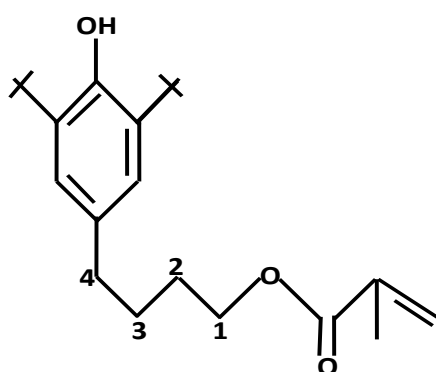
*Structural Elucidation and Antimicrobial Analysis of Chloroform Leaf Extract of Acanthus montanus*

UV- Visible Spectrum (Table 2) supported the structure of CHCl<sub>3</sub> pure extract with high λ<sub>max</sub> indicating high degree of unsaturation corresponding to phenolics and their analogues that exhibit bathochromic shift. The absorption in visible region was very prominent.

<sup>1</sup>H NMR spectrum (Table 3) showed a phenolic proton at 7.22 ppm while a triplet ester hydrogen was observed at 3.90 ppm. A duplet benzyl proton appeared at 2.29 ppm whereas a duplet alkene hydrogen occurred at 1.64 ppm, equally a duplet methylene proton appeared at 1.34 ppm while a duplet methyl hydrogen was observed at 0.82 ppm.

<sup>13</sup>C NMR spectrum (Table 4) recorded 20 signals corresponding to 20 distinct carbon atoms. A carbonyl carbon atom occurred at 77.66 ppm due to double bond while a carboxyl carbon was observed at 77.22 ppm. A hydroxyl carbon appeared at 77.02 ppm whereas four alkene carbon – carbon atoms appeared at 33.94, 31.92, 31.42 and 30.16 ppm respectively. Three methine carbon atoms were observed at 30.16, 29.69 and 26.61 ppm while four methylene carbon atoms appeared at 29.44, 29.37, 29.25 and 29.12 ppm respectively. Four methyl carbon atoms occurred at 24.70, 22.69, 20.70 and 14.13 ppm respectively.

GCMS results gave one major fragment with butyl acrylate as the parent compound. The molecular mass is 332 with formula as C<sub>21</sub>H<sub>32</sub>O<sub>3</sub> and suggested structure from instrument library is shown below.



**4- (3,5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate**

These results suggested that CHCl<sub>3</sub> leaf extract was a phenolic compound and a derivative of benzene. The result supported the ethnomedicinal use and antimicrobial results because butyl acrylate and their derivatives are used pharmaceutically as anticancer drugs [12].

Table 4. Result of Antibacterial and Antifungal Activities of the Pure Extract (B8) including Lincocin, Ammoxil and Augmentin

S / N	Extract/ Antibiotic use	Volume cm <sup>3</sup>	Average Diameter (mm) of zones of inhibition of Test Organisms												
			<i>E. coli</i> NCTC 10418	<i>S. aureus</i> NCTC 6571	<i>Bacillus</i> sp L.C.I	<i>Prot</i> <i>eus</i> <i>vulg</i> <i>aris</i> <i>L.C.</i> <i>I</i>	<i>Enteroba</i> <i>cteraeorg</i> <i>enes</i>	<i>Klebsi</i> <i>ellaae</i> <i>rogen</i> <i>es</i> <i>L.C.I</i>	<i>Strept</i> <i>ococc</i> <i>us</i> <i>sp</i> <i>L.C.I</i>	<i>Pseudom</i> <i>onas</i> <i>pyocyani</i> <i>a</i> <i>L.C.I</i>	<i>Salmon</i> <i>ella</i> <i>sp</i> <i>L.C.I</i>	<i>S.</i> <i>albus</i> <i>L.C.I</i>	<i>Aspergillus</i> <i>niger</i> <i>L.C.I</i>	<i>Aspergillus</i> <i>flavus</i> <i>L.C.I</i>	<i>Candida</i> <i>albican</i>
2	CHCl <sub>3</sub>	0.05	16	14	10	20	22	8	11	10	12	12	NA	NA	NA
	Extract Leaf Root Spot A														
3	Lincocin 500g + Distilled Water	0.05	NA	30	24	NA	18	16	28	NA	NA	NA	NA	NA	NA
4	Ammoxil 500g + Distilled Water	0.05	12	NA	NA	24	28	20	NA	24	28	NA	NA	NA	NA
5	Augmentin 625mg + Distilled Water	0.05	NA	14	14	28	30	NA	16	18	22	NA	NA	NA	NA

NA= No Actions

Table 5. Result of Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration(MFC)

PRESENCE OR ABSENCE OF GROWTH OF TEST ORGANISM					
Extract	Dilution	Mg/ml	<i>Aspergillusniger</i> L.C.I	<i>Aspergillusflavus</i> L.C.I	<i>Candida albicans</i>
CHCl <sub>3</sub> —MeOH Leaf	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	0.0313	++	++	++
	1:128	0.0156	++	++	++
Control Tube 8			—	—	—
MIC			0.0625	0.1250	0.2500
MFC			0.1250	0.2500	0.5000

L.C.I =Local Clinical Isolates

The sensitivity test on CHCl<sub>3</sub> leaf extract showed that *Enterobacteraerogenes* was the most inhibited organism with diameter zone of inhibition [22.00mm] which was higher than that of Lincocin[18.00mm] but lower than that of Ammoxil [28.00mm] and Augmentin [30.00mm]. The diameter zones of inhibition of the extract for *E. coli* [16.00mm] was higher than that of Lincocin (no action), Ammoxil [12.00mm] and Augmentin [no action]. CHCl<sub>3</sub> leaf extract was equally active against organisms like *Pseudomonas pyocyania*, *Salmonella sp*, *S.albus*, *Streptococcus sp*, *S.aureus*, *E. coli*, and *Klebsiellaerogenes*, whereas Lincocin, Ammoxil and Augmentin recorded no action. The extract was not active on fungi like *Aspergillusniger*, *Aspergillusflavus* and *Candida albicans* just like Ammoxil, Augmentin and Lincocin. These results confirmed that the CHCl<sub>3</sub> leaf extract might be effective in treatment of diseases caused by test bacteria thus supporting the use of the plant in the cure of some diseases by trado-medical practitioners [11,12]. The MIC range of the extract in the ten test bacteria was 0.0625mg/ml to 0.2500mg/ml showing that even at a very low concentration, the extract was highly bactericidal.

#### IV. Conclusion

This study scientifically validates the use of the CHCl<sub>3</sub> leaf extract of *Acanthus montanus* as a potent Antimicrobial agent. The CHCl<sub>3</sub> extract has been shown to be effective against some test organism signifying that the plant could be used in the cure and management of the various diseases caused by the test organism and can serve as potential raw material for new or known antibiotics.

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