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Evaluation of Bioactive Components of Leaf and Stem bark of *Anthocleistadjlonensis*

Ali Fredrick U[1], Orinya O.F[3], Ominyi M.C[1]., Ebenyi L.N[1]. , Ezeh, E. O[1]., Ogbanshi ME[2] and Ezenwali M. O [4].

[1]**Biotechnology Department Ebonyi State University Abakaliki ,Nigeria**

[2]**Biochemistry Department Ebonyi State University Abakaliki ,Nigeria**

[3]**Medical Biochemistry Department Ebonyi State University Abakaliki ,Nigeria**

[4]**Applied Biochemistry Department Enugu State University of science and Technology, Nigeria**

Email:alifredrik13@gmail.com; fredrickali.@ebsu-edu.net

ABSTRACT

The objective of the present study was to investigate the presence selective phytochemicals in the ethanol extract of stembark and leaves of *Anthocleistadjonensis*. Crude ethanol extract of the whole plant powder was evaluated for bioactive components using standard methods of phytochemical screening, gas chromatography and mass spectrophotometry (GC-MS).The results of phytochemical screening inboth leaves and stembark extract of *Anthocleistaadjonensis* revealed: alkaloid (90%, 60%), flavonoid (25%,05%), saponin (25%, 25 %),and tannin (0.00%,15 %) respectively. The major compounds found in leaves using GC-MS were, 11-octadecenoic acid 9.54 %, oleic acid 9.12 %, 3,11-tetradecendien-1-ol 8.91 % squalene 8.69 %,and E-9-tetra decenoic acid 6.89 %. whereas that of stembark were Octadecenoic acid 16.34%,9,12-octadeca-dienoic acid 10.21%,n-hexadecanoic acid 9.48 %,11-octadecanoic acid 8.46 %,and 4-hexen-3-one 8.17 % . Important secondary metabolites were identified, the bioactive compounds revealed will be isolated and may be used for medicinal purposes in future.

Key words: phytochemicals, medicinal, screening, taxonomist and metabolites.

INTRODUCTION

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since the ancient times till date. According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis WHO (2003)[1]. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active Adiaratou *et al.,* (2005)[2]. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian Aggarwal and Shishodia(2006)[3]. Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs[4]. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 percent of the world's inhabitants, who rely mainly

on traditional medicine for their primary health care. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-'from Greek - phyto meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests Oko *et al.,* (2013)[4]. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources [5].

The science of application of these indigenous or local medicinal remedies including plants for treatment of diseases is currently called ethno pharmacology but the practice dates back since antiquity Ali *et al.,* (2015)[6]. Ethno pharmacology has been the mainstay of traditional medicines the entire world and currently is being integrated into mainstream medicine Ali *et al.,* (2014)[7]. Different catalogues including *De Materia Medica*, *Historia Plantarum*, and Species Plantarum have been variously published in attempt to provide scientific information on the medicinal uses of plants Ali *et al.,* (2015) [8]. The types of plants and methods of application vary from locality to locality with 80 % of rural dwellers relying on them as means of treating various diseases. For example, the use of bearberry (*Arctostaphylosuvaursi*) and cranberry juice (*Vacciniummacrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleucaalternifolia*) are described as broad-spectrum antimicrobial agents Ali *et al.,* (2015)[9]. A single plant may be used for the treatment of various disease conditions depending on the community. The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal (smoking, snoffing or steaming), topical (lotions, oils or creams), bathing or rectal (enemas).*Anthocleistadjalonensis* commonly called "Fartanlafla" in Mali Ali *et al.,* (2016)[10], "Osuo" in Bayelsa (Southern Nigeria) **Common name:**"Fartanlafla" "Ovo" (Izzi, Ebonyi state) "Cabbage tree" (English), "Ekpu"(Ezzangbo, Ebonyi State Nigeria), is used traditionally for the treatment of various diseases.The plant is known for antipyretic, stomachic, analgesic and purgative actions Ali *et al.,* (2016)[11], the aqueous extract was reported to produce a rise in blood pressure of cats and an increase in tone and amplitude of movement of rabbit duodenal preparations. The root decoction has been used in the treatment of diabetes mellitus. Herbalists claim a high percentage of cures in their diabetic patients treated with it [12].

Traditionally, the leaves are reputed to be used for the treatment of malaria and jaundice Anderson and Teube(2001)[13]. The bark is used as a purgative in small doses as large doses are considered toxic. According to the Mendi-ethnomedicine, when the tree is used as firewood, the people sitting around the fire become sick [14].

The decoction of the leaves is drunk in Sierra Leone as treatment for jaundice, in Ivory Coast the root is used as a diuretic, vigorous purgative, poison antidote, treatment for leprosy, as an emmenagogue and in the treatment of edemas and elephantiasis of the scrotum. The root decoction is taken against chest pain, constipation and stomach pain Baba and Usifoh(2013)[15]. The study was carried out to evaluate the bioactive components of *Anthocleistadjalonensis* using quantitative phytochemical and GC-MS analysis.

MATERIALS AND METHODS

REAGENTS/CHEMICALS

All reagents and chemicals were of analytical standard.

PLANT MATERIALS

Fresh leaves and bark of *Anthocleistadjalonensis* were collected from their natural habitat in AgbajaUnuphuIzziAbakalikiEbonyiState,Nigeria.They were authenticated and

identified by Prof. S.E Okafor, a plant taxonomist of the Department of Biological Science, Ebonyi State University, Abakaliki Nigeria .

Plate 1: picture of a cut stem bark of *Anthocleistadjalonensis*

Plate 2: picture of leaves of *Anthocleistadjalonenensis*

METHODS

A. Preparation of samples

Fresh leaves and bark of *Anthocleistadjalonensis* collected from Agbaja UnuphuIzzi Abakaliki Ebonyi State and brought to Department of Biotechnology laboratory Ebonyi State University, Nigeria; Destalked and Dried under room temperature for two weeks.After which the leaves and bark were pulverised into coarse forms with a milling machine and sieved with a 2 mm-mesh sieve and the fine powder particles were stored in an airtight bottle till used.

B. Extraction of plant materials

Fifty grams (50g) each of the fine leaves and stem bark powder was macerated in 150 ml of absolute methanol and left to stand for 24hours, thereafter the extractive was filtered out with the help of a filter cloth.The methanol was allowed to evaporate and the resulting extracts were screpted into clean bottle kept in a refrigerator for further use.

C. Quantitative phytochemical analysis of extracts

The freshly prepared crude extracts of the leaves and bark of *Anthocleistadjalonensis* were quantitatively tested for the presence of phytochemical constituents using Beare-Rogers *et al.,* (2001)[16] method.

D. Gas chromatography and mass spectroscopy (GC-MS) analysis of extracts

GC-MS analysis 2ul of the leaves and stem bark extracts of *Anthocleistadjalonensis* was employed for GC-MS analysis of different compounds, Instruments and chromatographic conditions of GC-MS analysis were carried out on a Gc-clarus 500 perkin Elmer system comprising an AOC-20i Auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30×0.25mm×1D×1um of capillary column,composed of 100% Dimethyl polysiloxane) operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1mlmin and an injection volume of 0.5EI was employed (split ratio of 10:1)injector temperature 250°c;ion-source temperature 280°c.The oven temperature was programmed from 110°c(isothermal for 2min) with an increase of 10°c/min to 200°c/min,then 5°c/min to 280°c/min, ending with a 9min isothermal at 280°c.Mass spectra were taken at 70eV;scan interval of 0.5sec and fragments from 45 to 450 Da.Identification of phytochemicals and interpretation on mass spectrum GC-MS was conducted using the database of National Research Institute Technology (NARICT), Zaria, Kaduna state ,Nigeria, having more than 62000 patterns. The spectrum of unknown components were compared with the spectrum of the known components using computer searches on a NARICT version 2.1Ms data library. Thename, molecularweight, retention time and structure of the components of the test materials were ascertained and results shown in chapter four.

RESULTS

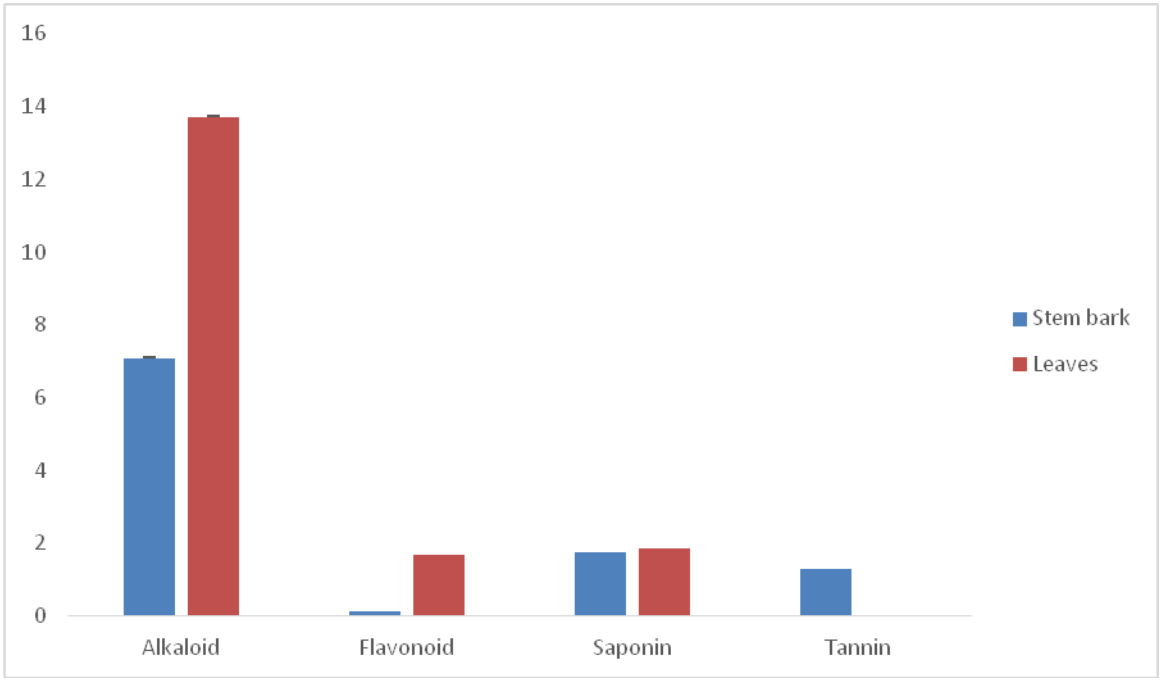

Figure 1: The result of the quantitative phytochemical constituents in methanol stem bark and leaves extracts of *Anthocleistadjalonensis* expressed as Mean± Standard deviation phytochemical.

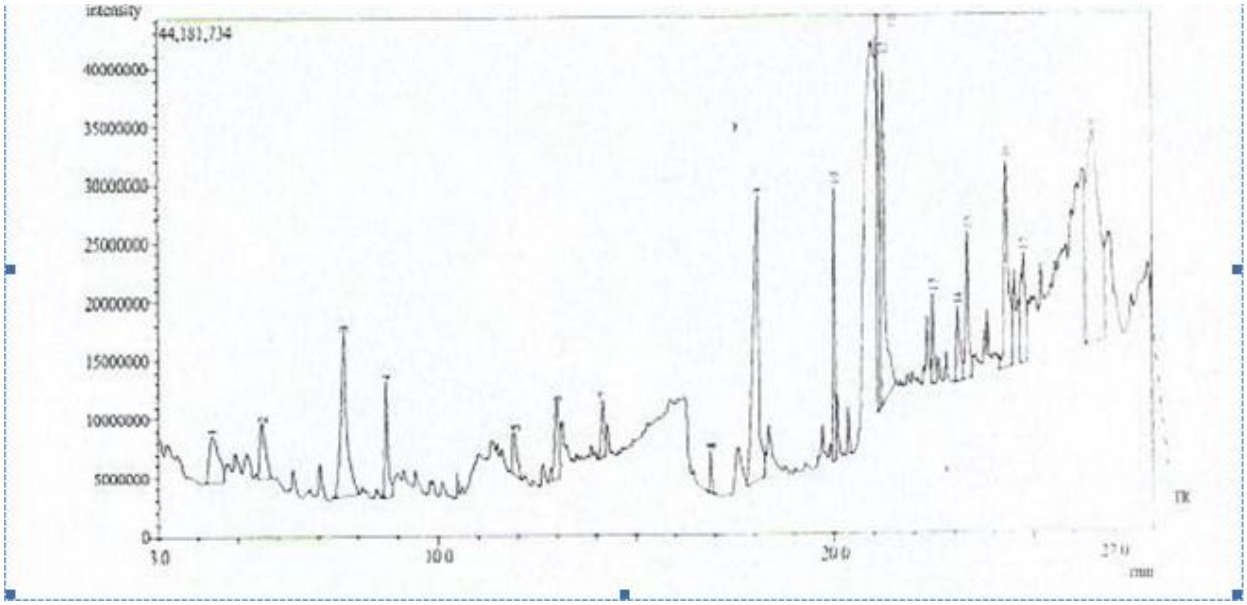

Figure 2: Chromatogram of methanol Stem-bark extract of *Anthocleistadjalonensis* indicating 18 peaks representing bioactive components obtained from GC-MS analysis.

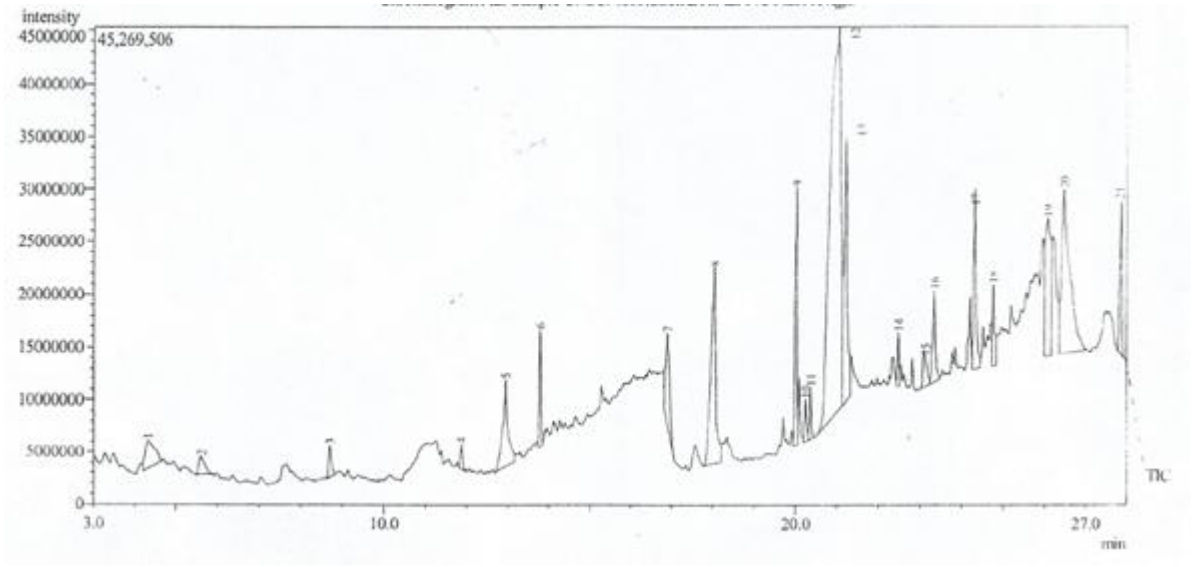

Figure 3: Chromatogram of methanol Leaf extract of *Anthocleistadjalonensis* indicating the 21 peaks representing the bioactive components obtained from GC-MS analysis.

Table 1**:** Qualitative phytochemical constituents in methanol stem bark and leaves extracts of *Anthocleistadjalonensis*percent (%)

Phytochemical	LeavesStem bark		
Alkaloid		9060	
Flavonoid		25	05
Saponin	25	25	
Tannin		-	15

The legends : the presence (+) or the absence (-) of phytochemicals

Table 2: GC-MS evaluation of bioactive Constituents extracts of stem bark of *Anthocleistadjalonensis*

Peak	Compound	Molecular formula	Molecular weight	Retention time	Base peak	Mass peak	Percentage (%) yield
1.	1-penten-3-ol	$C_6H_{12}O$	100	4.35	57	61	2.84
2.	Butyraldehyde	C_4H_8O	72	5.61	44	73	2.19
3.	4-hexen-3-one	$C_8H_{14}O$	126	7.65	41.1	70	8.17
4.	9,7-dioxa-tricyclo(4,4,0,0,3,8)deca-4,9-diene	$C_8H_8O_2$	136	8.71	77	75	3.21
5.	Benzoic acid	$C_7H_8N_2O_2$	152	11.93	121.1	73	2.18
6.	1,4-cyclohexadiene-1,2-dicarboxylic anhydride	$C8H_9NO_3$	150	12.99	78.1	146	2.91
7.	5-methyl-2-nitrobenzyl alcohol	$C_8H_9NO_3$	167	14.15	91.1	136	2.77
8.	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	16.89	74	143	1.75
9.	Octadecanoic acid	$C18H_{36}O_2$	284	18.09	43.05	209	3.35
10.	11-octadecanoic acid	$C_{19}H_{36}O_2$	296	20.03	55.05	244	8.46
11.	9,12-octadeca-dienoic acid	C18H32O2	280	21.13	69.05	384	10.21
12.	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.67	43.05	142	9.48
13.	1-octanol	$C_{12}H_{26}O$	186	22.49	57.05	310	4.81
14.	Erucic acid	$C_{22}H_{42}O_2$	338	23.1	55.05	318	2.33
15.	Eicosanoic acid	$C_{20}H_{40}O_2$	312	23.35	43.05	268	6.13
16.	9-tetradecenal	$C_{14}H_{26}O$	210	24.32	55.05	66	7.29
17.	Docossanoic acid	$C_{23}H_{46}O_2$	354	24.75	74.05	270	5.54
18.	Octadecenoic acid	$C_{21}H_{46}O_4$	356	26.48	41.05	148	16.34

Table 3: GC-MS evaluation of bioactive Constituents extracts of Leaves of *Anthocleistadjalonensis*

Peak	Compound	Molecular formular	Molecular weight	Retention time	Mass peak	Base peak	Percentage (%) yield
1	Glycidyl propyl ether	$C_6H_{12}O_2$	116	4.33	62	57.1	1.06
2	Glutaraldehyde	$C_5H_8O_2$	100	5.60	63	44	0.28
3	2,7-Dioxa-tricyclo (4,4,0,0(3,8) deca-4,9-diene	$C_8H_8O_2$	136	8.69	82	77	0.95
4	1,2-Dimethyl-3 nitro-4- nitro-benzene	$C_8H_8N_2O_3$	180	11.88	104	180	2.33
5	1,2-Diphenyl-1-propane	$C_{15}H_{16}$	196	12.94	116	92.1	0.64
6	1,2-Diphenyl-1 isocyano ethane	$C_{15}H_{13}N_20_7$	207	13.78	103	91.1	5.30
7	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	16.9	162	74.1	5.30
8	Octadecanoic acid	$C_{18}H_{36}O_2$	284	18.05	187	43.1	6.79
9	11-Octadecenoic acid	$C_{19}H_{36}O_2$	296	20.0	218	55	9.54
10	2-Hexadecen-1-ol	$C_{20}H_{40}O$	296	20.2	205	71.05	1.59
11	Heneicosanoic acid	$C_{22}H_{44}O_2$	340	20.4	231	74.1	1.7
12	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	226	21.1	251	55.1	6.89
13	Octadecanoic acid	$C_{22}H_{44}O_4$	372	21.2	306	43.1	5.41
14	2-hydroxy-1,3-propane diyl ester	$C_{39}H_{76}O_5$	624	22.5	251	57.1	2.65
15	9 -Hexadecenoic acid	$C_{16}H_{30}O_2$	254	23.1	256	55.1	4.24
16	Eicosanoic acid	$C_{20}H_{40}O_2$	312	23.3	290	43.1	6.57
17	3,11-Tetradecadien-1-ol	$C_{14}H_{26}O$	210	24.3	362	55.1	8.91
18	Docosanoic acid	$C_{23}H_{46}O_2$	354	24.8	330	74.1	3.29
19	Cyclododecanemethanol	$C_{13}H_{26}O$	198	26.1	261	55.1	8.48
20	Oleic acid	$C_{20}H_{38}O_3$	326	26.5	303	55.1	9.12
21	Squalene	$C_{30}H_{50}$	410	27.9	269	69.1	8.69

DISCUSION

Plants have always been an exemplary source of drugs and many currently available drugs have been directly or indirectly obtained from botanicals, which is the foundation of modern drugs and mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date Beckett and Stenlake(1988)[17]. Natural products have provided considerable impetus to the discovery of drugs .In particular,therapeutic areas of infectious diseases and oncology have benefited from numerous drug classes derived from natural product sources Benoit *et al.,* (2009)[18]. Results of this study revealed phytochemicals of importance among of which include alkaloid that has many pharmacological activities: Leonurine analkaloid is a prominent pharmacologically active guanidine, being commonly regarded as the predominant active principle of leonurus and leonotis drugs. Alkaloids have played an important role in the development of several clinically useful anticancer agents. The catharanthus alkaloids are established as antimitotic agent ,inhibiting the polymerization of tubulin, like vinblastine Cai and Sun2003)[19]. Oxymatrine an alkaloid extracted from *Sophoraflavescens* posseses anti inflammatory, anti-oxidative and anti-apoptotic properties,and has been used for the chronic viral hepatitis and many other diseases [19].Report have also showed that the benzophenanthridine alkaloid effectively inhibited the growthof *Mycocystisaeruginosa* Caterm *et al.,* (2001)[20]. Furthermore, alkaloids exhibit hypoglycemic effect onblood glucose level in alloxan-induced diabetic mice. Hence ,stembark and leaf extract under study revealed high concentration of alkaloids with the stembark having highest percentage (Table 1.) it may be inferred that the test plant is a potential source of drug and this is in line with the work of [21].

Flavonoids enhance a lot of drug therapeutic effect and are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation.Some flavonoids provide stress protection, for example, acting as scavengers of free radicals, thus the stembark of the test plant have high percentage of flavonoids and will provide protections against free radicals thereby ameliorating diseases associated with antioxidant imbalance. Report have shown that crude plant extracts protect albino rats exposed to toxin against oxidative stress Chen *et al.,* (2006)[22]. Flavonoids chelate metals that generate ROS via the Fenton reaction Choi and Frisv(2006)[23]. Flavonoids are also involved in the resistance to aluminum toxicity in maize [24].

Saponins are glucosides with foaming characteristics, it consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, is also known assapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a

hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin. In this study, 25% saponin were identified in both stembark and leaf extracts.(Table 1).Hence,administration of the extract to disease conditions will provide health benefits. Saponins have many health benefits, research have shown it beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system Cunnane and Anderson(1997)[25]. It reduce Cholesterol by binding with bile salt and cholesterol in the intestinal tract Dahziel, (1954)[26], as a result bile salts form small micelles with cholesterol facilitating its absorption. Saponins cause a reduction of blood cholesterol by preventing its re-absorption Dalziel (1937)[27]. The amount of saponin in the test plant suggest the use of the extract in disease management .Report by other authors showed antitumor , anti-mutagenic activities and reduction in the risk of human cancer cellsof animals treated with plant extracts Dalziel(1995)[28], which was attributed to saponin Darmstadt *et al.,* (2002)[29]. Saponin also provide immunity-booster, in *Anthocleistaadjalonsis* to fight infections by parasites David(2011)[30]. However, when ingested by humans, saponins also seem to help our immune system and to protect against viruses and bacteria D'Incalci *et al.,* (2005)[31]. Hence, both the leaves and stembark contain saponin that may serve both curative and protective against diseases in man and animals[32].

The food and feed are generally rich in antinutritionalfactors, particularlytanins Diplock *et al.,* (1998)[33]. The results of this study showed the presence of tannin in stembark while no trace of tannin was detected in leaf extract.The effect of tannin on animals range from beneficial to toxicity and death Doughari and Obidah(2008)[34]. This results suggest that consumption of stembark by animals may affect metabolism of other nutrients in their body since tinins is an anti-nutrient [35].

The GC-MS analysis of the stem bark of *Anthocleistadjalonensis* revealed the presence of 18 compounds. The major compounds in the leaf extracts include: oleic acid,11-octadecennoic acid,3,11-tetradecendien-1-ol,squalene and E-9-tetradecenoic acid while 4-hexan-3-one, 11-octadecanoic acid, 9,12-octadecadienoic acid, n-hexadecanoic acid and octadecenoic acid were found as the major components in the methanol extract of stem bark (Figue 2, and Table 2). Whereas that of stembark showed 21 compounds with octadecenoic acid having high percentage,followed by 9,12-octadecadiennoic acid,11-octadecanoic acid and hexen-3-one as the major compounds. However, these compounds possess biological properties such as antiapoptosis,antiaging,anticancer,anti-inflammatory,cardiovascular protection and improvement of endothelic function, as well as inhibition of angiogenesis and cell proliferation activity Firn(2010)[36]. Example: Oleic acid restores proper fuel metabolism in failing hearts , supercharges muscles and brain by making myelin

Francis *et al.,* (2002)[37]. Oleic acid alleviates type 2 diabete thus, it reverses the symptoms caused by type 2 diabetes in miceand reduces blood pressure Friesen(2006)[38]. Oleic acid regulates the activity of adrenoreceptor signaling pathways which direct the adrenergic receptors (α- and β-adrenoceptors) that help regulate blood pressure Gbadamosi *et al.,* (2001)[39]. Eating a diet high in oleic acid for four weeks reduced blood pressure and increased good HDL cholesterol in women Gibson *et al.,* (1998)[40]. Oleic acid may help in burning offat. Hence, administration of the plant extracts to animals with abnormal fat metabolism may reverse it significantly. It also normalizes or increases fat oxidation (burning) Girao *et al.,* (1986)[41], by increasing the expression of genes involved in fat burning. Furthermore,administration of the extract on patience having high blood pressure will reduce high blood pressure. Diets rich in oleic acid prevent ulcerative colitis[42].

Squalene protects healthy and smooth skin against wrinkles by feeding and guarding important processes in the skin cells Gunstone *et al.,* (2007)[43]. The unsaturated fatty acids of squalene playscrucial role in the moisture regulation of the skin Hansen *et al.,* (1954)[44]. About 10 percent of our skin surface (skin lipids) is made of squalene, it promotes health at the cellular level Harborne(1973)[45]. It can help prevent cancer, lowers "bad cholesterol" level, enhances skin, and boosts our body's immune systems Abo *et al.,* (1991)[46]. Palmitic acid (PA) has been widely used as useful and effective additive to pulmonary surfactants (PS) such as Survanta and Surfaxin.However,9-octadecenoic acid is an unsaturated fatty acid , most widely distributed and abundant in nature. It is used commercially inpreparation of oleates, lotions, and as a pharmaceutical solvent[46].

The presence of these bioactive components confirms the application of *Anthocleistadjalonensis* for treatment of ailments such as cancer,diabetes,infection ,high blood pressure, e.t.c by traditional practitioners.

CONCLUSION

Anthocleistadjalonensis is rich in selected secondary metabolites investigated and other bioactive components most especially fatty acids which could serve as healthy source of oil for soap and biodiesel. Many of the compounds identified have medicinal merits and thus confirms the folkloric use this plant parts in the treatment of diseases in Abakaliki.

RECOMMENDATION

Although, *Anthocleistadjalonensis* is a novel plant, individuals should endaevour to use both its leaf and stem bark for diseases management as it has a wide range of phytochemicals.

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