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Research Article

Identification and Characterization of Phytochemicals in Ethanol Leaf-extracts of *Phyllanthus reticulatus* and *Anthocleista djalonesis*

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ABSTRACT

The study was carried out to identify and characterize the phytochemicals in ethanol leaf-extracts of *Phyllanthus reticulatus* and *Anthocleista djalonensis.* The crude extracts were prepared by soaking 350 g of pulverized sample of each plant into a 1000 mL of absolute ethanol and allowed for 48 hours. The mixtures were then filtered and the extracts concentrated with rotary evaporator. Basic phytochemicals were analyzed and the profiles of the bioactive components were further characterized using Gas Chromatography – Mass Spectroscopy (GC-MS). The results of the phytochemical contents revealed the presence of flavonoids, alkaloids, saponins, phenols, cardiac glycosides, tannins, anthocyanins and terpenoids at different concentrations. The GC-MS chromatograms of the two extracts showed several peaks indicating the occurrence of bioactive compounds in *P. reticulatus* and *A. djalonensis* respectively. Among the bioactive compounds detected in *P. reticulatus* extract, 9,12,15-octadecatrienoic acid (13.001%), was most abundant, followed by ethyl 9,12,15-octadecatrienoate (12.049 %), n-hexadecanoic acid (9.828 %) and tocopherol (5.599 %). Similarly, the major compounds found in *A. djalonensis* extract are hexadecanoic acid (14.351 %), 9, 12,13-octadecatrienoic acid (14.313 %), pentadecanoic acid (12.820 %), oxazole (7.505 %), and linoleic acid (7.353 %). The results of the analysis suggest that *Phyllanthus reticulatus* and *Anthocleista djalonensis* contain many bioactive compounds which may be linked to their medicinal properties.

Keywords: *Phytochemicals, Bioactive, Compounds, Gas-Chromatography, Mass-Spectroscopy, Characterization.*

INTRODUCTION

Medicinal plants are used all over the world and they are becoming increasingly popular in modern society as an alternative to orthodox medicine (Zaidan *et al.,* 2005). The applications of plants as medicines has been in existence for centuries and man still search for more medicinal plants to treat a particular disease. Herbal preparations are believed to be closer to nature and safer than conventional drugs because the bioactive components of plants are mostly compatible with the body system and hence less adverse effects (Sasidharan *et al.*, 2011). Plants of medical importance are used all over the world for curative and prophylactic reasons for various ailments, particularly in developing countries (Zaidan *et al.,* 2005).

Phyllanthus reticulatus is a shrub that grows up to 5 m high, with light reddish-brown or grey-brown with hairy stems at

earlier stage and turns smooth surface stem when it gets older. It belongs to the family *Phyllanthaceae* (Mandisa, and Walter, 2010). The fruits are black in colour before ripe and have bitter taste with pungent aroma. The leaves are 2.5-5.0 cm long and 0.7-1.5 broad with oblong and elliptic shape.

The plant's leaves and roots are all important in herbal medicine for the treatment of fracture and painful injuries (Bagchi *et al*., 2002). The leaves have cooling effect, astringent and serve as a treatment for asthma, diabetes and diarrhea (Sathasivampillai *et al*., 2017). It also has healing effect on sores, burns, suppurations and chafed area. The bark, leaves, aerial parts are used for treatment of intestinal hemorrhage, anaemia, and for solving fertility problems and for the production of natural dye (Indi and Chinta, 2007).

Anthocleista djalonensis also called "Fartanlafla" in Mali (Adiaratou *et al*., 2005), "Osu" in Bayelsa (Southern Nigeria), Ubo or Ekpu in Ebonyi (Southeast Nigeria) is used as a remedy of many ailments. The plant is used as antipyretic, stomachic, analgesic and purgative agents (Oliver-Bever, 1986). The herbal products from the roots are used for the treatment of diabetes mellitus (Amofo, 1977). Different concoctions made from the leaves are taken in Sierra Leone to cure jaundice. In Ivory Coast the roots are used as diuretic, emmenagogue, antidote to poison, treatment for leprosy, edemas and elephantiasis of the scrotum. The root also is use to relief chest pain, constipation and stomach pain (Okoli and Iroegbu, 2004).

The numerous medicinal properties of *Phyllanthus reticulatus* and *Anthocleista djalonensis* deserve more attention. In this study, the characterization of the bioactive components of these plants extracts were studied to maximize the applications of these plants to solve some health related challenges.

MATERIALS AND METHODS

Sample collection

The samples were collected from Mgbabor village in Ebonyi Local Government Area of Ebonyi State, identified and authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki. Nigeria.

Sample processing

The leaves of the plants were washed under running water and dried at room temperature for three weeks (21 days). The samples were then pulverized using a mechanical grinder and then sieved to remove the chaff. The powdered samples were used for the analysis.

Extract preparation

Exactly 350 g of the pulverized sample was soaked in 1000 mL of absolute ethanol, covered and allowed for 48 hours with intermittent stirring. The solution was then filtered using white muslin cloth and liquid contents in the extracts were removed using rotary evaporator and stored at 4^{o}C for further analysis.

Quantitative phytochemical analysis of the extracts

The alkaloids and flavonoids contents of the extracts were determined using the methods described by Harborne (1984), while the quantity of tannins was estimated with a modified method of Onwuka, (2005). The Salkowski test was adopted for the analysis of cardiac glycosides as described by Sofowora (1993), while terpenes were quantified using a method described by Indumathi *et al*., (2014). Moreover, total phenolic constituent of each of the extracts was determined according to the method of Trease and Evans (1989). The quantification of anthocyanin was conducted using a modified method of Giusti and Wrolstad (2001), while the quantity of saponins was deduced by a method described by Oloyed (2005).

GC-MS Analysis of the Extracts:

Exactly 10.0 g of the extract was dispersed into methanol to dissolve the volatile organic compound content. The injector temperature was set at 250 ^{o}C while the detector temperature was 310 ^{o}C. Samples were collected in glass containers with Teflon lined septa. The sample container was filled just to overflowing and was then sealed with a screw cap such that no air bubbles were entrapped in it. Purge and trap sampling was then performed via the GC auto-sampler. Transfer line was connected directly into the GC injector through a proper needle. The injection volume of standards and samples was 2 µL. Conventional injector mode was used in the split-splitless injector. Each eluate was captured and read using mass spectrometry compartment of the GC-MS machine. Peaks in the chromatogram were identified using the National Institute of Science and Technology (NIST) Library.

RESULTS

The result of the phytochemical content of *Phyllanthus reticulatus* and *Athocleista djalonensis* leaf extracts revealed the presence of flavonoids, alkaloids, saponins, phenols, cardiac glycosides, tannins, anthocyanins and terpenoids at different quantities as represented on Figures 1 and 2 respectively.

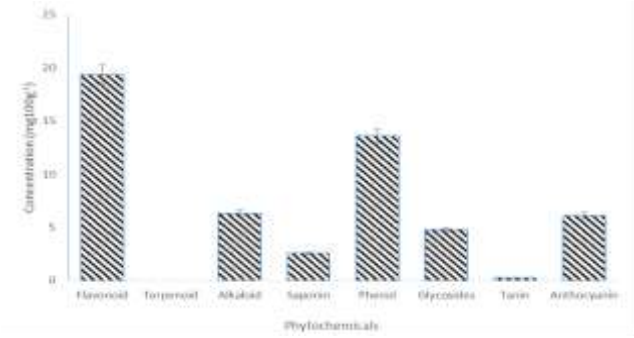

Figure 1. Phytochemical content of ethanol leaf-extract of *Phyllantus recticulatus* as expressed as mean standard deviation.

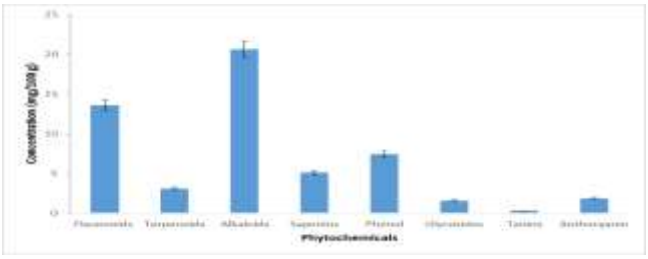

Figure 2. The phytochemical constituents of ethanol leaf extracts of *Athocleista djalonensis* as expressed as mean ± standard deviation.

The chromatograms of the GC-MS profiles of the *Phyllanthus reticulatus* and *Athocleista djalonensis* leaf extracts showed several peaks and the prominent phytocompounds were identified (Figures 3 and 4). Among the plant compounds detected from *Phyllanthus reticulatus* ethanol leaf extract include 9,12,15-octadecatrienoic acid, ethyl 9,12,15-octadecatrienoate, n-hexadecanoic acid, 2-coumaranone, tocopherol, squalene, phytol, hexadecanoic acid / eicosanoic acid, ethane, 1,1,2,2-tetraethoxy- silane, cyclohexanecarboxylic acid, benzofuran, 2,4-decadienamide, 7-methoxy-2-benzofuranyl methyl, D-allose, beta.-D-glucopyranose, 11,14-eicosadienoic acid, 4H-pyran-4-one, 9-Hexadecyn-1-ol, neophytadiene, 1,2,3-benzenetriol, cis-2-ethyl-2-hexen-1-ol/benzeneacetic acid, 2-hydroxy/methyl ester, 2-cyclohexylpiperidine and 9,12,15-octadecatrienoic acid (Table 1). While the major compounds identified from *Athocleista djalonensis* ethanol leaf extract include, hexadecanoic acid, 9,12,15-octadeca trienoic acid, pentadecanoic acid, oxazole, 9,12,15-octa decatrien-1-ol, linoleic acid, 3-octyn-1-o1, octadecanoic acid, squalene, phytol, bicydo(3,1,1) heptane, linolaidic acid and cholesta-2,8-dien-6-o1 (Tables 2).

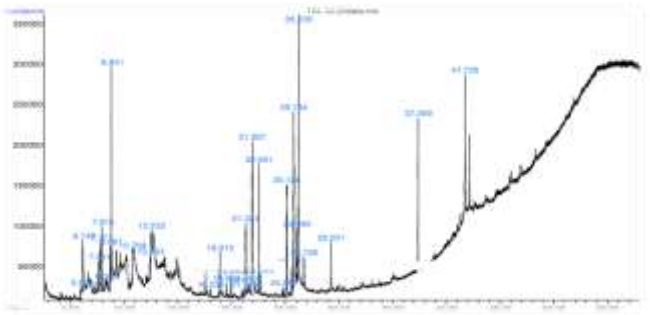

Figure 3. GC-MS chromatogram of ethanol leaf-extract of *Phyllantus recticulatus* showing their peaks.

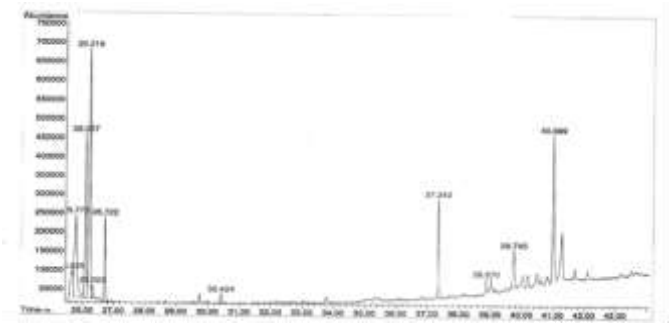

Figure 4. GC-MS chromatogram showing the peaks of ethanol leaf-extract of *Anthocleista djalonensis*

DISCUSSION

Analyzing from the above results, phytochemicals of *P. reticulatus* ethanol leaf extract showed most abundance of flavonoids (19.33±0.15 $mg100g^{-1}$) then, the phenols (13.57±0.01 $mg100g^{-1}$) while terpenoid was in trace quantity (Figure 2). This records showed some similarities to the reports of Jamal *et al.* (2008) which investigated also on the phytochemical contents of *P. reticulatus* extract. The tannins content of *P. reticulatus* may suggest for its applications in herbal medicine and –o-dyeing activity of the plant. The findings however are contrary to the records of Kumar *et al.* (2008) which recorded absence of alkaloids in *P. reticulatus*. The differences in the phytochemical contents might be as a result of how the samples were prepared or could be due to differences in the collection environments.

The results obtained from phytochemical analysis of *Anthocleista djalonensis* showed flavonoids, alkaloids, saponins, phenols, cardiac glycosides, tannins, anthocyanins and terpenoids at different quantities as presented in Figure 3 above. Majority of the bioactive constituents which include phenols, flavonoids, tannins and anthocyanins have been noted for their scavenging properties against reactive oxygen species (ROS) and inhibiting potentials against lipid peroxidation (LP) (Gbadamosi *et al*., 2001). Tannins are also antimicrobial, antiaging, anticancer and anti-inflammatory (Mamta *et al*., 2013).

Table 1. The GC-MS profile of the *Phyllanthus reticulatus* leaf extract

Peak No.	Chemical	R.T. (Min)	Peak height	corr. Area	corr.% max	% of total
1	9,12,15-Octadecatrienoic acid	25.784	214106	1210733	100.00	13.001
2	Ethyl 9,12,15-octadecatrienoate	26.230	326842	1122125	92.68	12.049
3	n-Hexadecanoic acid	21.927	189121	915241	75.59	9.828
4	2-Coumaranone	8.801	260487	622630	51.43	6.686
5	Tocopherol (Vitamin E)	41.725	166639	521398	43.06	5.599
6	Squalene	37.359	186208	512302	42.31	5.501
7	Phytol	25.126	138977	472955	39.06	5.079
8	Hexadecanoic acid / Eicosanoic acid,	22.551	157663	470518	38.86	5.052
9	Cyclohexane carboxylic acid	7.771	59808	317698	26.24	3.411
10	Benzofuran	8.681	55957	314397	25.97	3.376
11	2,4-Decadienamide	21.321	89061	291943	24.11	3.135
12	7-methoxy-2-benzofuranyl methyl	29.251	60088	245272	20.26	2.634
13	D-Allose.beta.-D-Glucopyranose	12.463	29192	188839	15.60	2.028
14	11,14-Eicosadienoic acid	25.618	36729	185865	15.35	1.996
15	4H-Pyran-4-one	7.914	80429	181635	15.00	1.950
16	9-Hexadecyn-1-ol	26.093	66396	178961	14.78	1.922
17	Neophytadiene	18.912	54574	177628	14.67	1.907
18	1,2,3-Benzenetriol	10.769	42525	145019	11.98	1.557
19	cis-2-Ethyl-2-hexen-1-ol/ Benzeneacetic acid, 2-hydroxy/ methyl ester	17.578	27400	132500	10.94	1.423
20	2-Cyclohexylpiperidine	22.648	25549	106849	8.83	1.147
21	9,12,15-Octadecatrienoic acid	26.728	36567	103850	8.58	1.115

Table 2: GC-MS Profile of *Athocleista djalonensis*

Peak	Compounds	Molecular weight	Retention time	% Total yield
1	Hexadecanoic acid	284	22.551	14.350
2	9,12,15-octadeca trienoic acid	292	26.219	14.313
3	Pentadecanoic acid	270	21.967	12.827
4	Oxazole	69	40.999	7.505
5	9,12,15-octa decatrien-1-01	264	25.772	7.266
6	Linoleic acid	308	26.087	7.253
7	3-octyn-1-01	126	20.085	6.963
8	Squalane	410	37.342	4.718
9	Phytol	296	25.105	4.225
10	Octadecanoic acid	372	26.772	3.908
11	Bicydo(3,1,1)heptane	194	18.906	2.378
12	Linoelaidic acid	280	25.635	2.942
13	Cholesta-2,8-dien-6-01	384	39.745	2.547

Their antimicrobial activity might be as a result of their interactions with extracellular and soluble proteins within the bacterial cell wall (Ekundayo, 1980). The plant contains saponins which are known for its foam formation in water, red blood cells lysing and cholesterol binding (Morebise and Fafunso, 1998). Alkaloids are used as stimulant and also for the medicinal applications such as optical anesthetic and also antipyretic, antispasmodic, analgesic and anti-bacterial activities (Nwaichi and Igbinaboro, 2012). Terpenoids is another bioactive compound which has some aromatic attributes and are actively involved in herbal treatments. The steroids and sterols which are found in animals are of terpenoids origin. Terpenoids can be anticarcinogenic as seen in perilla alcohol, antimalarial found in artemisinin or anti-ulcer, antimicrobial and also diuresis seen in glycyrrhizin (Dudareva, 2004).

The graph generated from the GC-MS analysis of *Phyllanthus reticulatus* ethanol leaf-extract contains various peaks of different heights revealing the emitted compounds at different percentages (Figure 4; Table 1). These compounds such as α-tocopherol and n-hexadecanoic acid have been identified with some healing and protective effects which include antioxidant properties as reported by Agomuo *et al.* (2017). It has been on records that 9,12,15-octadecatrienoic acid shows protections against arthritis, cancer and liver related problems (Agomuo *et al.*, 2017). Another constituent known as 2-coumaranone can effect positive changes on monoamines contents and so control the monoamine oxidase activities in the body system. The regulation of monoamine oxidase enzyme can be of medical importance which by implication reduces the by-products of the enzyme such as hydrogen peroxide and aldehyde species (Edmondson, 2014). Hence, 2-coumaranone could act as neuroprotective (Edmondson, 2014). *Phyllanthus reticulatus* ethanol leaf extract component like squalene could be used as anticancer, antioxidant, drug carrier, detoxifier, skin hydrating and emollient activities as recorded by Kim and Karadeni (2012). Phytol, an acyclic diterpene alcohol might also be of importance in the synthesis of vitamin E and vitamin K_1 (Netscher, 2007). Cyclohexane carboxylic acid and ethyl decadienoate were also among the identified and could function in metabolism of fatty acids (EU Food Improvement Agents, 2012).

Similarly, the GC-MS of *Anthocleista djalonensis* ethanol leaf-extract showed various peaks as a result of the presence of different bioactive compounds that possess diverse biological activities (Figure 5; Table 2). Some of the compounds are potential anti-inflammatory, antibacterial, antifungal and skin conditioning agents. Hexadecanoic can be anti-inflammatory, antioxidant, and nematicide (Rizvi *et al*., 2011). Another major component, 9,12-octadecadienoic acid also known as linoleic acid is a polyunsaturated omega-6 fatty acid. Linoleic acid is an essential fatty acid and it is consumed for proper health maintenance. Lack of linoleate in the body can lead to mild skin scaling and hair loss (Basak and Duttaroy, 2020). Linoelaidic acid, a conjugated form of linoleic acid was reported to be used as a remedy for cancer, atherosclerosis, obesity, immune dysfunction and diabetes (Basak and Duttaroy, 2020).

CONCLUSION

The study has revealed the presence of many prominent phytocompounds in *Phyllanthus reticulatus* and *Anthocleista djalonensis* which have been proven to be beneficial to human health and activities. Hence, the medicinal properties attributed to these plants might be as a result of the activities of the constituting compounds. Therefore, the findings of this study have thrown light on biological active compounds present in *Phyllanthus reticulatus* and *Anthocleista djalonensis* leaf extracts. The presence of these compounds might be attributed to the numerous applications of these plants in herbal medicine.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author, MCO designed the study. Authors FUA and LNE collected all data and performed the statistical analysis. Authors ICN, VUN and TNO did the sample collection and extract preparations. Authors ICN, MCO and TNO did the literature search. Authors FNN, MCO and MEO wrote the first draft of the manuscript. All authors collectively did the bench work, read and approved the final manuscript.

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None

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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