



Characterization of Chemical Composition of *Bryophyllum pinnatum* Leaf Ethyl Acetate Fraction

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Authors' contributions

There was total collaboration among all authors in the execution of this study. Authors CUI, ESA, VAO, KMEI and JNI designed the study and wrote the protocol, while authors CUI, ESA, JNI and KMEI performed the statistical analysis and interpretation of study data. Authors ESA and JNI did the literature searches, while author ESA wrote the first draft of the manuscript and incorporated all corrections from co-authors. Authors CUI, VAO and KMEI critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To characterize the chemical composition of *Bryophyllum pinnatum* leaf ethyl acetate fraction.

Methodology: Quantitative phytochemical composition was assessed using gas chromatography fitted with flame ionization detector (GC-FID), while chemical characterization was via gas chromatography-mass spectrophotometry (GC-MS) analysis. The mass spectra peaks were matched with those found in the National Institute of Standard and Technology (NIST) spectral database.

Results: Results revealed a rich presence of proanthocyanin, rutin, quinine, flavan-3-ol, anthocyanin, lunamarin, sapogenin, phenol, flavonones, steroids, epicatechin, kaempferol, phytate, oxalate, resveratol, catechin, flavones, tannin, ribalinidine, naringin, and spartein in varying proportion in *B. pinnatum* leaves ethylacetate fractions. GC-MS analysis of the fractions revealed

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the presence of at least 50 constituents. The major constituents were Hexadecanoic acid, methyl ester (24.88%), 10,13-Octadecadienoic acid, methyl ester (29.69%), Tetracosanoic acid, methyl ester (7.84%), Methyl stearate (6.97%), cis-Methyl 11-eicosenoate (6.26%), Methyl 18-methylnonadecanoate (4.99%), Docosanoic acid, methyl ester (3.71%) and 4,7-Methano-1H-indene, octahydro- (2.43%).

Conclusion: This rich array of chemical compounds presents *B. pinnatum* leaves as an important source of potential lead compounds with biological and pharmacological benefits and hence a potential candidate for drug discovery.

Keywords: *Phytochemicals; GC-MS; methyl esters; medicinal plant; Bryophyllum pinnatum.*

1. INTRODUCTION

Plants have been widely applied in traditional medicine for management of diseases or as adjuvants to promote drug action. They are identified as important source of phyto-active compounds with verifiable medicinal properties. Plants have the ability to synthesize a wide variety of chemical compounds used to perform important biological functions, defend against attacks from predators such as insects, fungi and herbivorous mammals, and as well serve as nutrient source for humans [1,2]. At least twelve thousand (12000) of such compounds have been isolated so far; a number estimated to be less than 10% of the total [3]. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies or where produced as analogues of plant products [4]. The World Health Organization (WHO) estimates that 80% of Asians and Africans living in the suburbs and rural communities depend largely on herbal medicine for their primary healthcare [5]. The importance of traditional system of medicine and of certain traditional medical practices are widely recognized all over the world. Recently, some authors have suggested that traditional medical system should be integrated into the mainstream of healthcare services due to their easy accessibility, availability, cultural acceptability, therapeutic potential and minimal cost [6,7]. Medicinal properties of plant and plant products are associated with their phytochemical composition.

Phytochemicals, also known as phytonutrients, are grouped into several classes according to their chemical structure and biological activity. They have been widely applied for their medicinal properties as pure compounds, fruit and vegetable crude extracts. Phytochemicals such as phenolic compounds, terpenoid compounds, and alkaloids have been investigated for their beneficial effect in various disease conditions [8].

Bryophyllum pinnatum (Crassulaceae) commonly known by names such as air plant, love plant, miracle leaves, life plant, etc. [9], grows primarily in the rain forest belt but is widely distributed worldwide [10]. Among the Igbos of southeastern Nigeria, it is known as 'odaa opue', and 'ewe abamoda' or 'odundun' by the Yorubas [11]. The leaves, stem and roots of the plant have been traditionally known to be beneficial in the treatment of a number of conditions in Africa, Asia, Australia and tropical America [12,13]. The leaves and stem of *B. pinnatum* are applied in ethno-medicine for management of a plethora of disease conditions; ranging from inflammation, analgesic, wound healing, facilitation of the dropping of placenta of newly born baby, diarrhea, coughs, bronchial infections, gastric ulcers etc [14,15]. Recent works have focused on beneficial properties such as free radical scavenging activity [16,17,9], hepatic-protective activity [18], and anti-ulcer activity [11,19]. The proximate composition, mineral, lipids and amino acid compositions of *B. pinnatum* have been reported [20-22]. Extensive understanding of the chemical compositions of medicinal plants is important in drug discovery and design. The present study is aimed at quantitation of phytochemicals and characterization of the specific bioactive compounds of *B. pinnatum* leaves ethyl acetate fraction by gas chromatography – mass spectrum (GCMS) analysis. This study will elucidate the active principles responsible for its numerous reported medicinal properties and provide a template for identification, isolation and purification of these compounds for further analysis and drug design.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *B. pinnatum* were collected from a garden at Umunam village, Ngor Okpala L.G.A., Imo State. The plant materials were authenticated by Prof. F. N. Mbagwu a plant

taxonomist at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State. Plant specimens were deposited in the institution's herbarium with voucher number IMSUH 0225.

2.2 Preparation of Extract

Fresh leaves of *B. pinnatum* were carefully shed from the stem; this was dried at 30°C and ground to fine powder using a blender (Kenwood BL357). The powder (800g) was extracted with 2 L of 80% ethanol using a Soxhlet extractor. The ethanol extract was partitioned between ethyl acetate and water to recover ethyl acetate soluble component of the extract. The extracts was recovered by distillation under reduced pressure at 49°C in a Buchi rotavapour (Switzerland), then dried to solid forms in vacuum desiccators, and stored in a freezer ($\leq 4^{\circ}\text{C}$) until when needed.

2.3 Quantitative Phytochemical Content Determination

One gram (1g) of processed sample was weighed into a test tube and 15ml ethanol and 10ml of 50% m/v potassium hydroxide were added. The mixture in the test tube was allowed to react in a water bath at 60°C for 60mins. Later, the reaction product was transferred to a separation funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which were all transferred into the funnel. The combined extracts were washed three times with 10ml of 10% v/v ethanol aqueous solution; and dried with anhydrous sodium sulfate. The extract was solubilized in 1000 μl of pyridine of which 200 μl was transferred to a vial for analysis [23]. The analysis of free steroids was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250 μm x 0.15 μm) was used. The injector temperature was 280°C with split less injection of 2 μl of sample and a linear velocity of 30cms⁻¹, Helium 5.0 Pa s was the carrier gas with a flow rate of 40 ml min⁻¹. The oven operated initially at 200°C, was heated to 330°C at a rate of 3°C min⁻¹ and was kept at this temperature for 5min. The detector operated at a temperature of 320°C; phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in $\mu\text{g/g}$.

2.4 Characterization of Bioactive Compounds

Characterization of plant extract bioactive compounds was carried out using GC-MS analysis on a Varian 450-GC and 240-MS system (Varian, Salt Lake City, USA) fitted with an electron impact mode injector (70 eV) and a Varian data system.

The compounds in the GC-MS chromatogram of *B. pinnatum* leaves ethyl acetate fraction analysed were identified using mass spectrometry. The interpretation of mass spectra peaks of detected unknown compounds was done by matching with database of known component stored in NIST. Major components were identified with authentic standards obtained from computerized libraries. The compound name, molecular formula, peak area, retention time and mass/charge ratio were ascertained. The relative percentage amount of each component was determined by matching its average peak area to the total area.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Content of *B. pinnatum* Leaves Ethyl Acetate Fraction

Results of quantitative phytochemical composition of *B. pinnatum* leaves ethyl acetate fraction showed a rich presence of important phytochemicals such as proanthocyanin, rutin, quinine, flavan-3-ol, anthocyanin, catechin, sapogenin, phenol, flavonones, steroids, epicatechin, kaempferol, phytate, oxalate, resveratol, flavones, tannin, ribalinidine, naringin, and spartein (Table 1). *B. pinnatum* leaves fraction contains 0.15 ± 0.01 , 5.13 ± 0.31 , 2.34 ± 0.19 , 1.68 ± 0.08 , 2.18 ± 0.26 , 23.65 ± 1.18 , 6.18 ± 0.25 , 10.25 ± 0.92 , 13.75 ± 0.96 , 20.65 ± 0.83 , and 6.86 ± 0.41 $\mu\text{g/g}$ of proanthocyanin, rutin, quinine, flavan-3-ol, anthocyanin, catechin, sapogenin, phenol, flavonones, steroids, and epicatechin respectively. Others include kaempferol, phytate, oxalate, resveratol, flavones, tannin, ribalinidine, naringin, and spartein in concentrations of 19.86 ± 1.59 , 25.78 ± 1.55 , 30.62 ± 1.84 , 28.91 ± 2.89 , 34.65 ± 3.12 , 40.44 ± 3.24 , 1.54 ± 0.06 , 0.03 ± 0.00 and 0.15 ± 0.01 $\mu\text{g/g}$ respectively.

Table 1 also shows that among other phytochemicals, *B. pinnatum* leaves ethyl acetate is relatively rich in Tannin (40.44 ± 3.24

µg/g), Flavone (34.65 ± 3.12 µg/g), Oxalate (30.62 ± 1.84 µg/g), Resveratrol (28.91 ± 2.89 µg/g), Catechin (23.65 ± 1.18 µg/g), Phytate (25.78 ± 1.55 µg/g) and Kaemferol (19.86 ± 1.59 µg/g).

3.2 Chemical compositions of *B. pinnatum* leaves ethyl acetate fraction analyzed by GC-MS

The GC-MS chromatogram (Fig. 1) of *B. pinnatum* leaves ethyl acetate fraction showed presence of about 50 bioactive compounds. The major constituents in the extract were quantified as Hexadecanoic acid, methyl ester (24.88%), 10,13-Octadecadienoic acid, methyl ester (29.69%), Tetracosanoic acid, methyl ester (7.84%), Methyl stearate (6.97%), cis-Methyl 11-eicosenoate (6.26%), Methyl 18-methylnonadecanoate (4.99%), Docosanoic acid, methyl ester (3.71%) and 4,7-Methano-1H-indene, octahydro- (2.43%) (Table 2).

Phytochemical analysis of ethyl acetate fraction of *B. pinnatum* leaves indicated the presence of important phytochemicals such as proanthocyanin, rutin, quinine, flavan-3-

ol, anthocyanin, lunamarin, sapogenin, phenol, flavonones, steroids, catechin, epicatechin, kaempferol, phytate, oxalate, resveratrol, flavones, tannin, ribalinidine, naringin, and spartein. *B. pinnatum* leaves was found to be relatively rich in flavan-3-ols (catechin), flavonols (kaemferol), flavone, resveratrol, tannin, oxalate, and phytate. Phytochemicals are produced as secondary plant products of predominantly teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals; normally inducible through physical or chemical micro environmental triggers, usually a stress factor [24]. These inducers elicit secondary product formation in nature, by activating otherwise dormant biochemical pathways [24]. Polyphenolic phytochemicals such as flavonoids are known for their important biological activities which include free radicals scavenging [25,26], free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, antioxidant effect [27,28], antimicrobial activity, antiulcer properties, antiviral [29],

Table 1. Quantitative phytochemical content of *B. pinnatum* leaves

Phytochemical components (µg/g)	<i>B. Pinnatum</i>
Proanthocyanin	0.15 ± 0.01
Rutin	5.13 ± 0.31
Quinine	2.34 ± 0.19
Flavan-3-ol	1.68 ± 0.08
Anthocyanin	2.18 ± 0.26
Sapogenin	6.18 ± 0.25
Phenol	10.25 ± 0.92
Flavonones	13.75 ± 0.96
Steroids	20.65 ± 0.83
Epicatechin	6.86 ± 0.41
Kaempferol	19.86 ± 1.59
Phytate	25.78 ± 1.55
Oxalate	30.62 ± 1.84
Resveratrol	28.91 ± 2.89
Flavones	34.65 ± 3.12
Tannin	40.44 ± 3.24
Ribalinidine	1.54 ± 0.06
Naringin	0.03 ± 0.00
Sparteine	0.15 ± 0.01
Catechin	23.65 ± 1.18

Results are presented as Mean ± standard deviation of triplicate determinations

Table 2. Chemical compositions of *B. pinnatum* leaves ethyl acetate fraction analyzed by GC-MS

Peak	Retention time	Area (%)	M/Z value	Compound name	Molecular formula
1	8.3056	0.0357	112	2-Heptenal, (E)-	C ₇ H ₁₂ O
2	10.9031	0.0307	144	Heptanoic acid, methyl ester	C ₈ H ₁₆ O ₂
3	12.4538	0.0232	98	1-Pentene, 2,3-dimethyl-	C ₇ H ₁₄
4	14.2371	0.0317	321	Sulfurous acid, nonyl 2-pentyl ester	C ₁₇ H ₃₆ O ₃ S
5	15.6328	0.8631	158	Octanoic acid, methyl ester	C ₉ H ₁₈ O ₂
6	21.9908	0.0232	98	3-Hexenal	C ₆ H ₁₀ O
7	23.6191	0.0577	152	2,4-Decadienal, (E,E)-	C ₁₀ H ₁₆ O
8	24.7821	0.1319	110	2,4-Heptadienal, (E,E)-	C ₇ H ₁₀ O
9	28.0387	0.0321	68	2,3-Pentadiene	C ₅ H ₈
10	30.4811	1.0557	186	Nonanoic acid, 9-oxo-, methyl ester	C ₁₀ H ₁₈ O ₃
11	33.7376	0.082	214	Dodecanoic acid, methyl ester	C ₁₃ H ₂₆ O ₂
12	36.8778	0.0722	172	7-Nonenoic acid, methyl ester	C ₁₀ H ₂₀ O ₂
13	38.0796	0.0352	114	2-Furfurylthiol	C ₅ H ₆ OS
14	38.7387	0.025	156	3-Octenoic acid, methyl ester, (E)	C ₉ H ₁₆ O ₂
15	40.9873	0.0402	96	cis-1-Methyl-2-(2'-propenyl)cyclopropane	C ₇ H ₁₂
16	41.6851	0.0375	242	Methyl tetradecanoate	C ₁₅ H ₃₀ O ₂
17	42.3441	0.029	110	1,5-Hexadiene, 2,5-dimethyl-	C ₈ H ₁₄
18	49.4	24.8795	270	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂
19	50.6793	0.0796	152	3-Oxatricyclo[3.2.1.0(2,4)]octane, (1.alpha.,2.beta.,4.beta.,5.alpha.)-(E)-2-Butenylcyclopropane	C ₁₀ H ₁₆ O
20	51.4547	0.0681	55	(E)-2-Butenylcyclopropane	C ₄ H ₇
21	53.2768	0.0418	256	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂
22	54.4786	29.6858	295	10,13-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂
23	56.0294	6.9745	299	Methyl stearate	C ₁₉ H ₃₈ O ₂
24	56.4946	0.483	283	trans-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂
25	56.8047	0.4115	295	9,11-Octadecadienoic acid, methyl ester, (E,E)-	C ₁₉ H ₃₄ O ₂
26	57.5801	0.0455	250	cis,cis,cis-7,10,13-Hexadecatriena	C ₁₆ H ₂₆ O ₂
27	57.7352	0.2071	279	Methyl 6-cis,9-cis,11-trans-octadecatrienoate	C ₁₈ H ₃₁ O ₂
28	58.0453	0.9202	293	Methyl 9.cis.,11.trans.t,13.trans. – octadecatrienoate	C ₁₉ H ₃₂ O ₂
29	58.2004	0.1044	156	3-Decen-1-ol, (E)-	C ₁₀ H ₂₀ O
30	58.8207	6.2636	325	cis-Methyl 11-eicosenoate	C ₂₁ H ₄₀ O ₂
31	59.092	1.0702	126	9-Oxabicyclo[6.1.0]nonane, cis-	C ₈ H ₁₄ O
32	59.3247	4.9873	327	Methyl 18-methylnonadecanoate	C ₂₁ H ₄₂ O ₂
33	59.8674	1.3813	282	(S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate	C ₁₈ H ₃₄ O ₂
34	60.1388	0.457	170	8-Nonenoic acid, 9-(1,3-nonadienyl oxy)-, methyl ester	C ₁₀ H ₁₈ O ₂
35	60.9142	0.3395	282	9 – Octadecenoic acid	C ₁₈ H ₃₄ O ₂
36	61.4569	2.4256	136	4,7-Methano-1H-indene, octahydro-	C ₁₀ H ₁₆
37	61.6508	0.1235	228	Dodecanoic acid, ethenyl ester	C ₁₄ H ₂₈ O ₂
38	62.1935	0.0247	244	1,6-Dibromo-2-cyclohexylpentane	C ₆ H ₁₂ Br ₂
39	62.5037	3.7073	355	Docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂
40	62.775	0.0546	255	Dimethylmalonic acid, 4-chlorophenyl decyl ester	C ₁₂ H ₁₁ ClO ₄
41	63.3178	0.4096	127	2H-Azepine, 3,4,5,6-tetrahydro-7-methoxy-	C ₇ H ₁₃ NO
42	63.8218	1.4687	268	9-Hexadecenoic acid, methyl ester,	C ₁₇ H ₃₂ O ₂

Peak	Retention time	Area (%)	M/Z value	Compound name	Molecular formula
43	64.0932	1.4704	116	(Z)-2-Ethylbutyric acid, 2-hexyl ester	C ₆ H ₁₂ O ₂
44	64.4033	0.1964	283	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂
45	65.295	7.8351	383	Tetracosanoic acid, methyl ester	C ₂₅ H ₅₀ O ₂
46	65.6439	1.0862	383	Lauric anhydride	C ₂₄ H ₄₆ O ₃
47	65.9153	0.0672	240	Oxirane, tetradecyl-	C ₁₆ H ₃₂ O
48	66.1479	0.0348	299	Stearic acid hydrazide	C ₁₈ H ₃₈ N ₂ O
49	66.458	0.066	649	Docosanoic acid, isobutyl ester	C ₄₄ H ₈₈ O ₂
50	67.2334	0.0241	285	Myristic acid isobutyl ester	C ₁₈ H ₃₆ O ₂

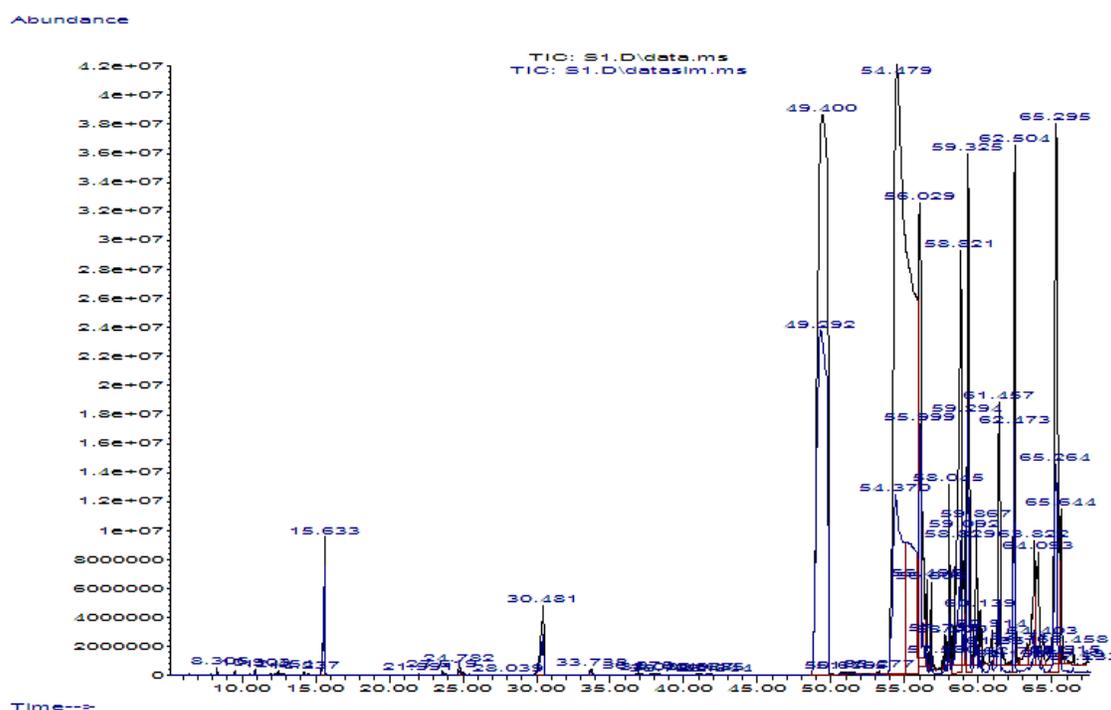


Fig. 1. Gas chromatography-mass spectrometry profile of *b. Pinnatum* leaves ethylacetate fraction

tumors suppressing effect and hepatoprotective effect [30-35]. Mechanisms by which flavonoids provide health benefits in addition to being direct chemical protectants involve modulatory effects on a variety of metabolic and signaling enzymes. Flavonoids have been shown to block the angiotensin converting enzyme that raises blood pressure [36,37]; inhibit cyclooxygenase, which forms prostaglandins [38,39] and they block enzymes that produce estrogen. The implications of these in vitro inhibitory actions are that certain flavonoids could prevent platelet aggregation

reducing heart disease and thrombosis [40-42] and inhibit estrogen synthase; thus decreasing the risk of estrogen related cancers [43]. Antiplatelet activity of red wine and grape juice reported in human and animal systems has been associated with synergy of flavonoids including anthocyanins [44,45]. Rutin pretreatment has been shown to protect against ethanol-induced cellular necrosis, restoration in the levels of glutathione peroxidase along with 'antiperoxidative effect' [46]. Flavan-3-ols (catechins, epicatechin gallate and epigallocatechin gallate) abundant in grapes,

berries, cocoa and green tea are considered to be responsible for part of the protective effect of red wine against atherosclerotic cardiovascular disease [31,47]. Resveratrol, kaempferol, naringenin, luteolin and quercetin [48-50] have been shown to exert anti-adipogenesis activity and improve insulin sensitivity/glucose intolerance, mediated by the adenosine monophosphate-activated protein kinase (AMPK) and mitogen-activated protein kinases signaling pathways (MAPK) respectively in pre-adipocytes and mature adipocytes [48].

Furthermore, the GC-MS analysis profile included highly complex glycosides, ketone, saturated and unsaturated fatty acids and esters in the fraction. About 8 major constituents were identified in the ethylacetate fraction of *B. pinnatum* leaves; quantified as Hexadecanoic acid, methyl ester (24.88%), 10,13-Octadecadienoic acid, methyl ester (29.69%), Tetracosanoic acid, methyl ester (7.84%), Methyl stearate (6.97%), cis-Methyl 11-eicosenoate (6.26%), Methyl 18-methylnonadecanoate (4.99%), Docosanoic acid, methyl ester (3.71%) and 4,7-Methano-1H-indene, octahydro- (2.43%). Other relatively abundant components included Nonanoic acid, 9-oxo-, methyl ester (1.06%), 9-Oxabicyclo[6.1.0]nonane, cis- (1.07%), (S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate (1.38%), 9-Hexadecenoic acid, methyl ester (1.47%), 2-Ethylbutyric acid, 2-hexyl ester (1.47%) and Lauric anhydride(1.09%). Hexadecanoic acid, methyl ester and 10,13-Octadecadienoic acid, methyl ester were predominantly present with a relative abundance of 24.88% and 29.69% respectively. Gas chromatography (GC) and Mass spectroscopy (MS) have been widely applied in the identification of bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, etc [51,52]. Most of the identified constituents of *B. pinnatum* leaves ethyl acetate fraction have been reported to possess beneficial biological and pharmacological activity. Hexadecanoic acid, methyl ester [53] and 9,12-octadecadienoic acid [54] have been reported to possess antioxidant, antibacterial/antifungal and anti-inflammatory/anticancer properties respectively. The presence of this characteristic array of compounds may be responsible for the usefulness of the plant in treatment of various ailments in ethno-medicine.

4. CONCLUSION

The present study has characterized the bioactive components of *B. pinnatum* leaves ethyl acetate fraction to include phytochemicals such as proanthocyanin, rutin, quinine, flavan-3-ol, anthocyanin, catechin, sapogenin, phenol, flavonones, steroids, epicatechin, kaempferol, phytate, oxalate, resveratrol, flavones, tannin, ribalinidine, naringin, and spartein. The GC-MS analysis indicated a predominance of Hexadecanoic acid, methyl ester and 10, 13-Octadecadienoic acid, methyl ester. The presence of these bioactive compounds presents *B. pinnatum* leaves as an important source of potential lead compounds with biological and pharmacological benefits and hence a potential candidate for drug discovery.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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