Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Plant Science and Biotechnology

Phytochemical Profiling and GC-MS Analysis of Extracts of Two Tropical Moss Species

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DOI: 10.36347/sajb.2023.v11i05.003 | **Received:** 05.10.2022 | **Accepted:** 14.11.2022 | **Published:** 30.05.2023

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Abstract **Original Research Article**

The crude extracts obtained from two tropical moss species namely: *Philonotis hastata* (Duby) Wijk & Margad and *Barbula lambaranensis* C. Mull were analysed with a view to identifying the bioactive compounds present in them. The mosses were collected from their natural population and air dried at ambient temperature in the laboratory for fourteen days. The aqueous extracts were subjected to phytochemical analysis using standard methods while the n- hexane extracts were subjected to gas chromatography-mass spectrometry (GC-MS) analysis. The results of the phytochemical screening showed the presence of alkaloids (0.189mg/g), saponins (0.047mg/g) and flavonoids (22.35QE/g) in *P. hastata* while the alkaloids, saponins and flavonoids content in *B. lambaranensis* were 0.184mg/g, 0.037mg/g and 14.18QE/g respectively. Steroids and reducing sugars were also present in the two moss species. The results of the GC-MS analysis showed the presence of 23 compounds in *P. Hastate* with 9-Octadecenoic acid methyl ester, 9,12-Octadecadienoic acid (Z,Z)- methyl ester, cis-Vaccenic acid, Hexadecanoic acid methyl ester and 1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethlidene)-1,4a,8-trimethyl-9-oxo-, methyl ester forming the major components while in *B. lambaranensis*, 31 compounds were present from which Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, 13-Tetradecenal, 9-Octadecenoic acid (Z)- methyl ester and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester formed the prominent components. Five compounds: Hexadecanoic acid methyl ester, n-Hexadecanoic acid, cis-Vaccenic acid, Octadecanoic acid and 9-Octadecanoic acid methyl ester were commonly identified in the two moss species. The identified compounds are considered to be biologically and pharmacologically important. Further investigation on the identified bioactive compounds from the studied moss species will be beneficial to formulate novel drugs for the treatment and management of diseases.

Keywords: moss, phytochemicals, GC-MS, bioactive compounds, drugs.

INTRODUCTION

Bryophytes represent the second largest group of land plants and pioneer in the evolution of land plants [1]. They are made up of three groups: liverworts (6000 species), the hornworts (300 species) and mosses (13000 species). They are distributed along the tropical and temperate ecosystems [2].

Bryophytes are one of the promising sources of antibiotics and biologically active compounds in nature [3]. The use of some bryophytes as medicinal plants in China to cure bruises, burns, snake bites, external wounds, pulmonary tuberculosis, fractures, convulsions, scalds, uropathy and pneumonia have been reported [4]. In the last several years, more than 400 lead molecules have been isolated and structurally elucidated [5]. The major compounds were flavonoids, bioflavonoids, terpenes, terpenoids, like diterpenoids, triterpenoids, lipophilic mono and disesquiterpenoids. Most of the phytochemicals in bryophytes are biologically active substances [5]. Phylogenetically, economically and ecologically, these phytochemicals are likely involved in defence and protection against pests and microbial infections [6]. As bryophytes growing on forest floor do so in close proximity to several biodegrading wastes and soil-borne organisms, the need for a defence mechanism against fungi and bacterial is inevitable to survive in such habitats.

It has been reported that bryophytes contain compounds with numerous kinds of biological activities [5]. For example, compounds isolated from *Plagiochasma japonica* and *Marchantia tosana* exhibited antitumour, antifungal and antimicrobial activities, inhibition of superoxide release, thrombin

activity and muscle relaxation [7]. Mosses contain polyunsaturated fatty acids that have been reported to be significant in human medicine such as in the prevention of atherosclerosis and cardiovascular disease, reduction of collagen-induced thrombocyte aggregation and lowering triacylglycerols and cholesterol in plasma [8].

Relatively, little is known about the secondary metabolites of bryophytes particularly at structural level and the information is scattered [5]. Major limitations in this regard include the difficulties in proper identification, limited number of the same species available for subsequent analyses due to inconspicuous position in the field as well as the sophistication of the equipment required.

The present study was carried out to identify the bioactive constituents of two tropical moss species with a view to providing information on their significance in the development of novel drugs.

MATERIALS AND METHODS

Plant Materials

Fresh and young samples of *Philonotis hastata* and *Barbula lambaranensis* were collected from their natural population in Ado-Ekiti, Nigeria in March, 2020. Taxonomic identification and authentication were carried out at the herbarium of the Ekiti State University, Ado-Ekiti. Voucher specimens were deposited there. The samples were separated from adhering particles and washed separately in different bowls of distilled water. They were then air dried at ambient temperature in the laboratory for fourteen days.

Phytochemical Screening

Phytochemical screening of the crude extracts of each of the moss species was carried out using standard procedures [9, 10].

Preparation of Sample for GC–MS Analysis

Air dried plant samples were separately pulverized into fine powder using an electric blender (ModelExcella QTY LPC). The powder was then sieved using sieve number 20 mesh to remove unwanted debris. 2 gram of the powdered sample was weighed into 250 ml conical flask and 10 ml of n-hexane was added to sonicate for two hours. It was then filtered by packing a column with silica gel and fibre glass wool. Anhydrous sodium sulphate was added to remove the water present in the extract. The extract was then concentrated with nitrogen concentrator to 2 ml for GC-MS analysis.

GC–MS Analysis

GC–MS analysis of the extract was performed using Agilent technologies model 7890A coupled with a mass spectrometer Agilent technologies 6975. The principle for the analysis was separation techniques. The mobile phase was helium gas while the stationery phase was the column of model Agilent technologies HP-5MS with length 30 m, internal diameter of 0.32 mm with thickness of 0.25 microliter. The oven temperature was programmed from 80^0C (isothermal for 2 min) with an increase of 10^0C /min to the final temperature of 240^0C and held isothermally for 6 min. The volume of sample injected was 10 microliter. The mode of analysis was split-less. The scan range was 50-550 Da. The mass spectrometer interphase temperature was 250^0C. Mass spectra were taken at 70Ev. The total GC running time was 23.154 min. The library used for the identification of compounds was National Institute Standard and Technology (NIST)-version Year 2014.

RESULTS

Phytochemial screening of aqueous extracts of the two moss species: *P. hastata* and *B. lambaranensis* revealed the presence of alkaloids, saponins, flavonoids, steroids and reducing sugar (Table 1). The quantities of these phytochemicals are shown in Table 2. *P. hastata* had 0.189 mg/g of alkaloids, 0.047 mg/g of saponins and 22.35 QE/g of flavonoids while *B. lambaranenensis* had 0.184 mg/g of alkaloids, 0.037 mg/g of saponins and 14.18 QE/g of flavonoids.

The results of the GC-MS analysis of the n-hexane extract of *P. hastata* revealed the presence of 23 compounds (Table 3) among which were five prominent compounds namely: 9-Octadecenoic acid methyl ester, 9,12-Octadecadienoic acid (Z,Z)- methyl ester, cis-Vaccenic acid, Hexadecanoic acid methyl ester and 1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethlidene)-1,4a,8-tri methyl-9-oxo-, methyl ester totalling 65.40% of the mixture. The chromatogram is presented in Fig. 1 while the GC-MS spectra of the prominent compounds are shown in Fig. 2-6. The GC-MS analysis of n-hexane extract of *B. lambaranensis* revealed the presence of 31 compounds (Table 4) out of which five compounds namely: Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, 13-Tetradecenal, 9-Octadecenoic acid (Z)- methyl ester and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester formed the major compounds amounting to 49.72% of the total mixture. The chromatogram is presented in Fig. 7 while the GC-MS spectra of the prominent compounds are shown in Fig. 8-12. Five compounds: Hexadecanoic acid methyl ester, n-Hexadecanoic acid, cis-Vaccenic acid, Octadecanoic acid and 9-Octadecanoic acid methyl ester were commonly identified in both moss species. However, the concentrations of Hexadecanoic acid methyl ester, n-Hexadecanoic acid and Octadecanoic acid were higher in the extract of *B. lambaranensis* than that of *P. hastata* (Fig. 13).

Table 1: Qualitative Phytochemical composition of *P. hastata* and *B. lambaranensis*

Phytochemicals	Occurence	
	P. hastata	***B. lambaranensis***
Alkaloids	+	+
Saponins	+	+
Tannins	-	-
Phenols	-	-
Flavonoids	+	+
Cardiac glycosides	-	-
Terpenoids	-	-
Anthraquinone	-	-
Steroids	+	+
Reducing sugar	+	+

(+): Present; (-): Absent

Table 2: Quantitative Phytochemical composition of *P. hastata* and *B. lambaranensis*

Phytochemicals	Composition	
	P. hastata	***B. lambaranensis***
Alkaloids mg/g	0.189± 0.01	0.184±0.01
Saponin mg/g	0.047±0.00	0.037±0.00
Total Phenol mg GAE/g	-	-
Tannin mg TAE/g	-	-
Flavonoids mg QE/g	22.35±0.09	14.18± 0.06

*Results are means of three replicates

Table 3: Phytoconstituents identified from n-hexane extract of *P. hastata*

S/N	Retention Time (Min)	Name of Compound	Area (%)
1	14.568	Hexadecanoic acid, methyl ester	7.79
2	14.844	n-Hexadecanoic acid	2.63
3	15.944	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.52
4	16.025	9-Octadecenoic acid, methyl ester	24.10
5	16.230	9,12-Octadecadienoic acid (Z,Z)-	8.22
6	16.301	cis-Vaccenic acid	9.52
7	16.482	Octadecanoic acid	0.75
8	16.787	n-Propyl 11-octadecenoate	0.73
9	17.549	Methyl 9-eicosenoate	0.74
10	17.763	Eicosanoic acid, methyl ester	1.54
11	18.239	N-Desmethyltapentadol	0.36
12	18.977	Docosanoic acid, methyl ester	3.85
13	19.139	Estra-1,3,5(10)-trien-17.beta.-ol	0.46
14	19.597	Bicyclo[10.1.0]tridec-1-ene	0.40
15	19.630	2-Methyl-Z,Z-3,13 octadecadienol	0.59
16	19.906	Tetracosanoic acid, methyl ester	2.77
17	20.044	trans-Z-.alpha.-Bisabolene epoxide	0.99
18	20.097	MDMA methylene homolog	1.36
19	20.301	1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoeth lidene)-1,4a,8-trimethyl-9-oxo-, methyl ester	6.47
20	20.397	3-Keto-isosteviol	0.75
21	20.444	3-Methoxy-4-nitrobenzyl alcohol, n-butyl ether	5.44
22	20.606	3-Keto-isosteviol	2.25
23	20.720	Methyl 18-methylnonadecanoate	0.78

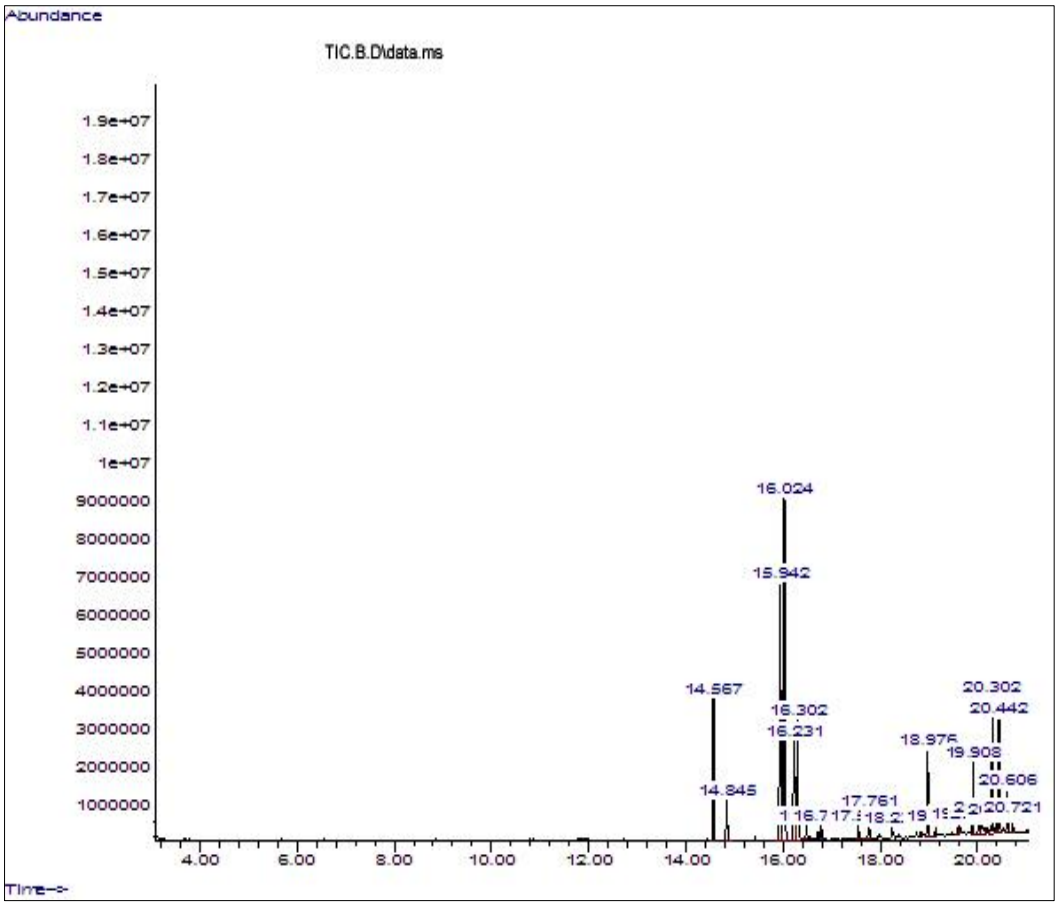

Figure 1: GC-MS Chromatogram of n-hexane extract of *P. hastata*

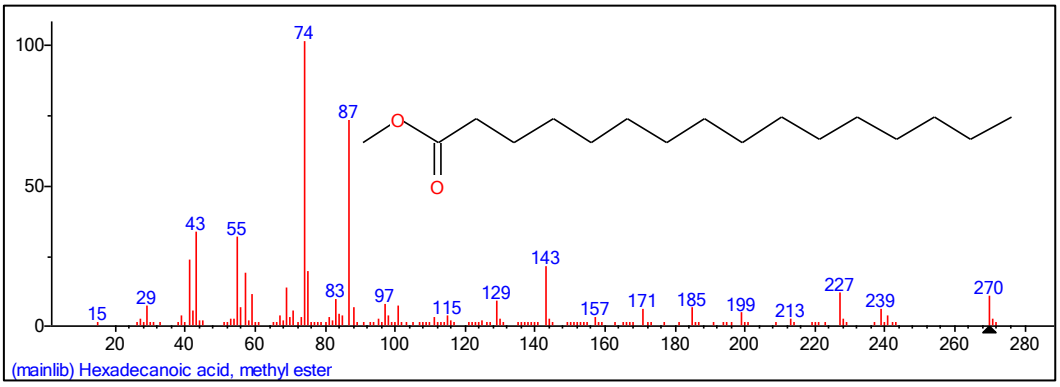

Figure 2: GC-MS spectra of Hexadecanoic acid, methyl ester (7.79%; RT: 14.568) from n-hexane extract of *P. hastata*

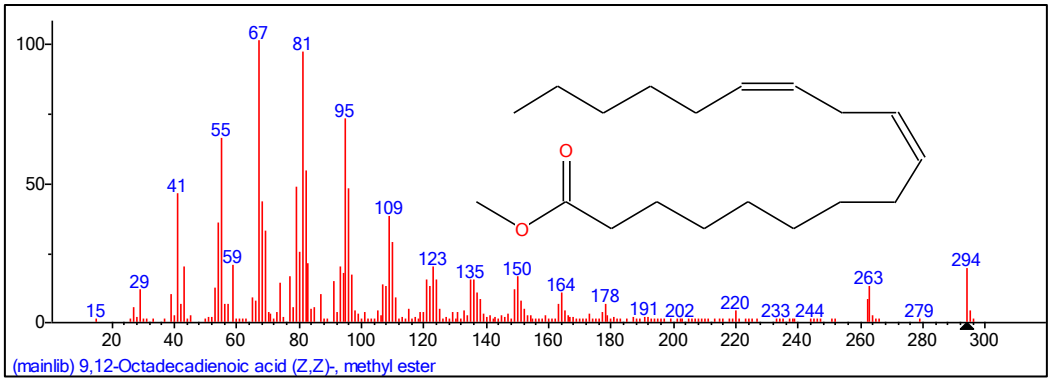

Figure 3: GC-MS spectra of 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester (17.52%; RT: 15.944) from n-hexane extract of *P. hastata*

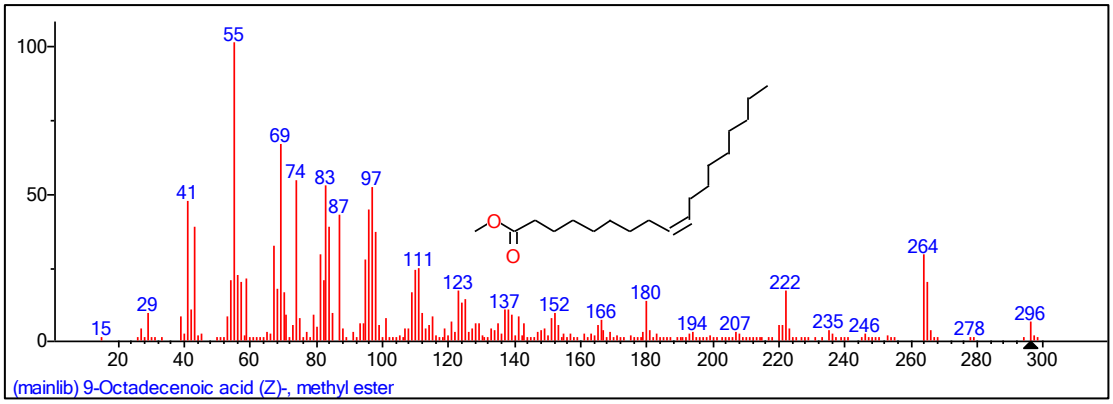

Figure 4: GC-MS spectra of 9-Octadecenoic acid (Z)-, methyl ester (24.10%; RT: 16.025) from n-hexane extract of *P. hastata*

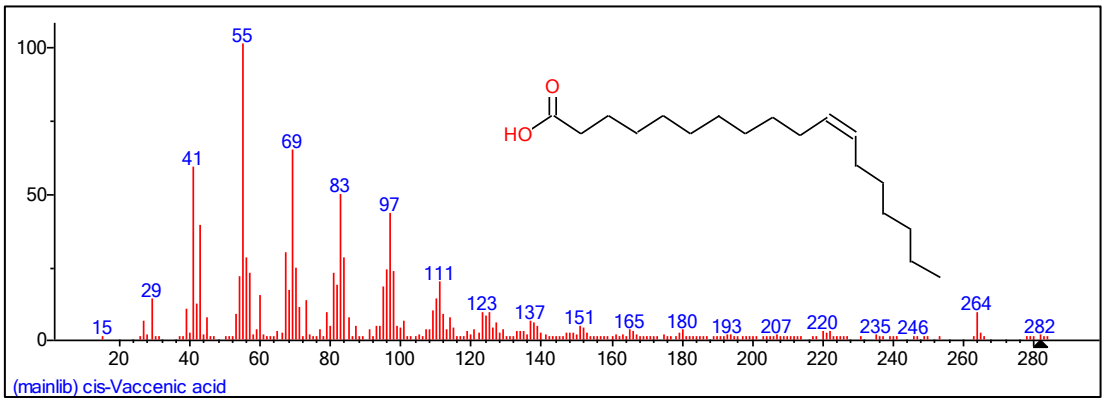

Figure 5: GC-MS spectra of cis-Vaccenic acid (9.52%; RT: 16.301) from n-hexane extract of *P. hastata*

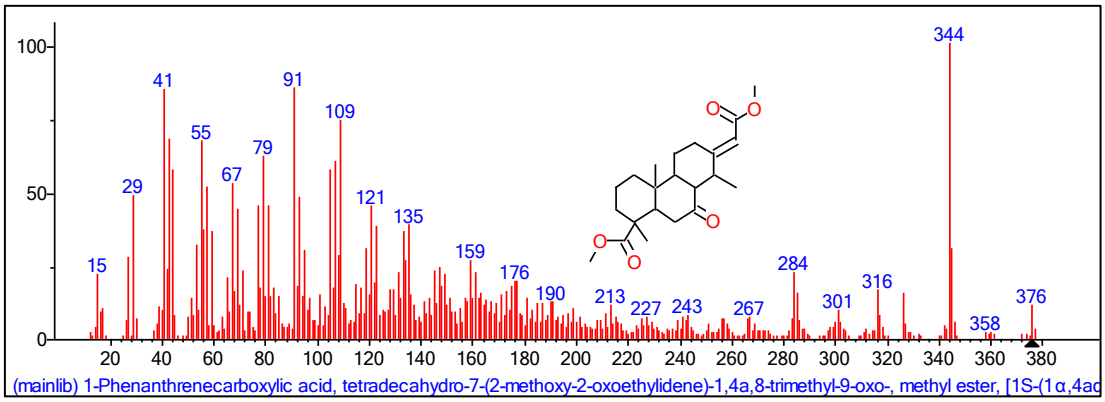

Figure 6: GC-MS spectra of 1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethylidene)-1, 4a,8-trimethyl-9-oxo-,methyl ester[1S-(Iα,4ao (6.47%; RT: 20.301) from n-hexane extract of *P. hastata*

Table 3: Phytoconstituents Identified from n-hexane extract of *B. lambaranensis*

S/N	Retention Time (Min)	Name of Compound	Area (%)
1	3.220	Pentanoic acid	3.16
2	4.911	Butanal, 3-methyl-	1.14
3	4.949	Ethanol, 2-(methylamino)-	1.52
4	5.120	Hexanal	1.51
5	6.282	2-Piperidinone	1.25
6	7.792	Indole	1.25
7	10.139	1-(1-Butyny)cyclopentanol	4.57
8	10.206	Methanesulfonamide, N,N dimethyl-	0.33
9	11.120	Dodecanoic acid	1.71
10	11.473	Methanesulfonamide, N,N-dimethyl-	1.10
11	11.777	Phenylephrine	0.51
12	11.835	Acetamide, N-(aminocarbonyl)-2-chloro-	0.33

S/N	Retention Time (Min)	Name of Compound	Area (%)
13	12.092	3,3-Dimethyl-4-methylamino-butan-2-one	0.44
14	13.154	Benzeneethanamine, 4-methoxy-.alpha.-methyl-	0.67
15	14.577	Hexadecanoic acid, methyl ester	11.76
16	14.858	n-Hexadecanoic acid	3.79
17	15.944	9,12-Octadecadienoic acid, methyl ester	10.65
18	16.016	9-Octadecenoic acid (Z)-, methyl ester	9.81
19	16.058	9-Octadecenoic acid (Z)-, methyl ester	0.86
20	16.244	Methyl stearate	4.38
21	16.287	cis-Vaccenic acid	4.28
22	16.458	Tetradecanamide	0.65
23	16.492	Octadecanoic acid	1.42
24	17.444	Glycidylpalmitate	5.47
25	17.806	9-Octadecenamide, (Z)-	0.91
26	18.558	Cyclooctene, 4-ethenyl-	2.69
27	18.606	13-Tetradecenal	9.92
28	18.763	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	7.58
29	19.639	9,17-Octadecadienal, (Z)-	4.56
30	19.763	Propanamide, N-(aminocarbonyl)-	1.42
31	20.320	2,6,10-Dodecatrien-1-ol, 3,7,11-tr imethyl-, (Z,E)-	0.40

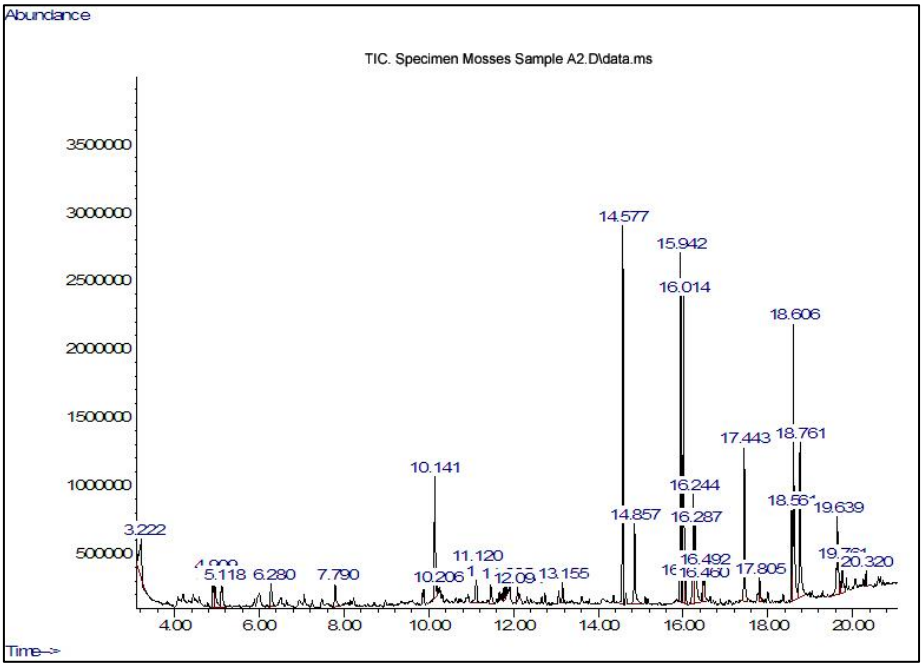

Figure 7: GC-MS Chromatogram of n-hexane extract of *B. lambaranensis*

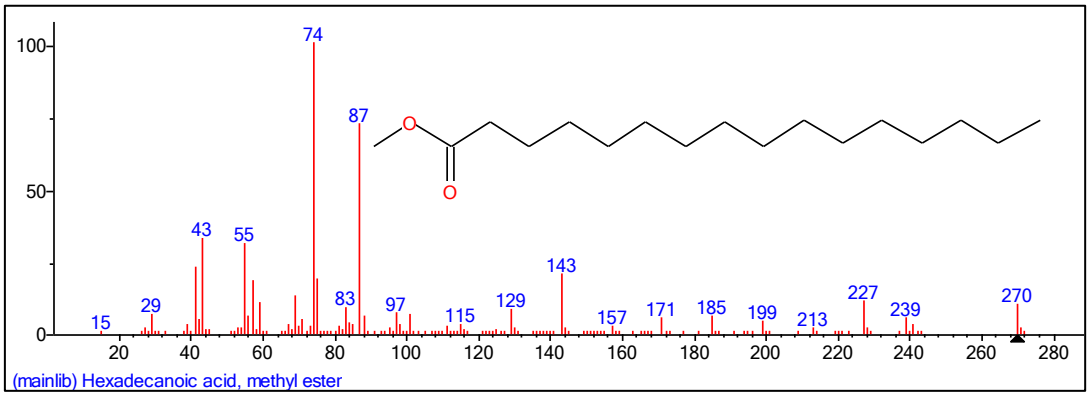

Figure 8: GC-MS spectra of Hexadecanoic acid, methyl ester (11.76%; RT: 14.577) from n-hexane extract of *B. lambaranensis*

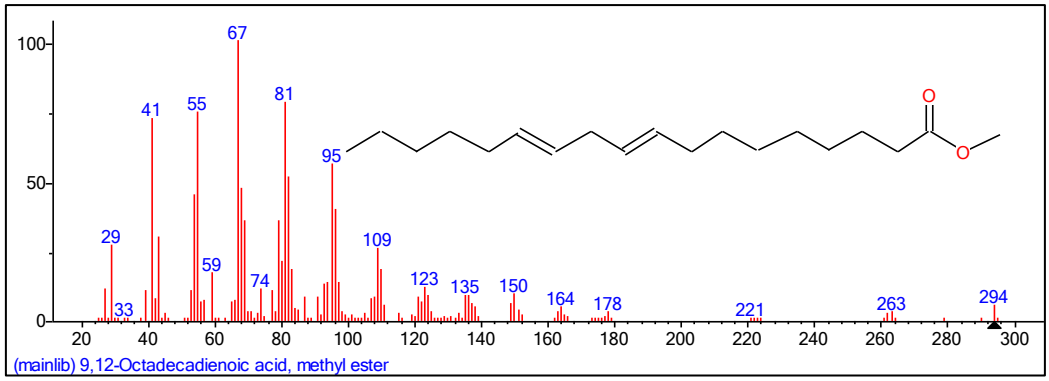

Figure 9: GC-MS spectra of 9, 12-Octadecadienoic acid, methyl ester (10.65%; RT: 15.944) from n-hexane extract of *B. lambaranensis*

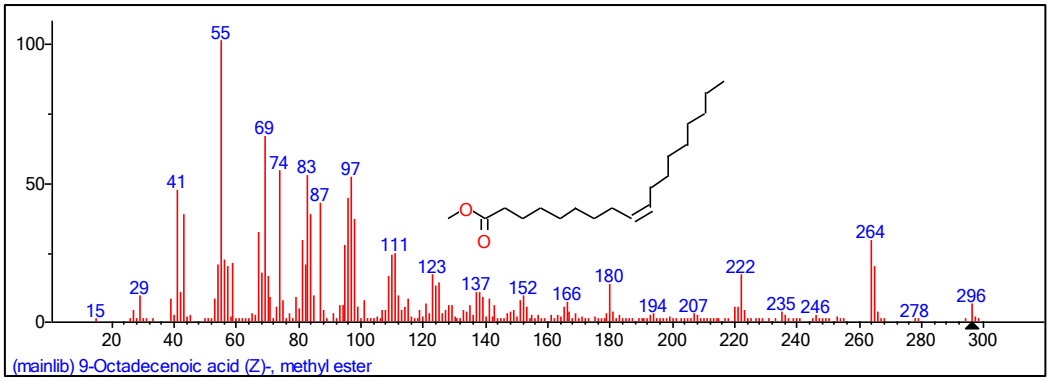

Figure 10: GC-MS spectra of 9-Octadecadienoic acid (Z), methyl ester (9.81%; RT: 16.016) from n-hexane extract of *B. lambaranensis*

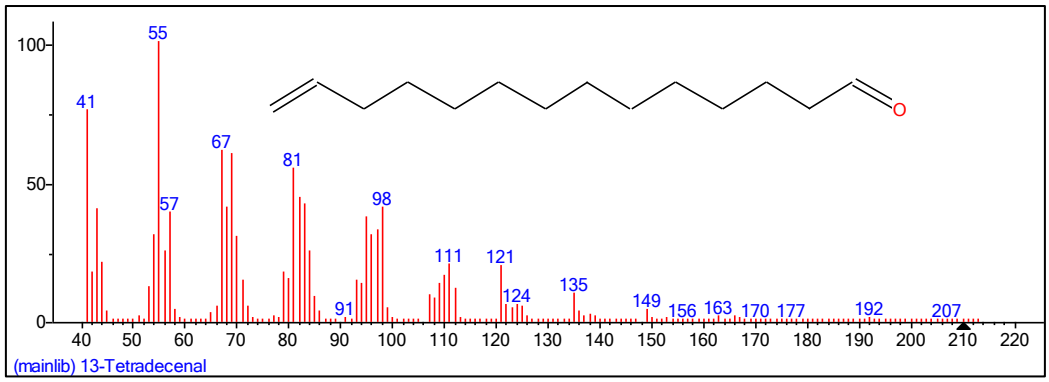

Figure 11: GC-MS spectra of 13-Tetradecenal (9.92%; RT: 18.606) from n-hexane extract of *B. lambaranensis*

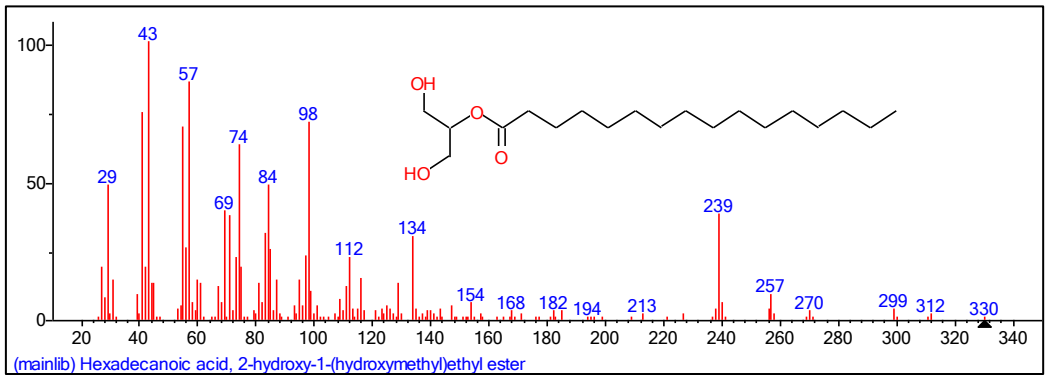

Figure 12: GC-MS spectra of Hexadecanoic acid, 2-hydroxyl-1-1(hydroxymethyl)ethyl ester(7.58%; RT: 18.763) from n-hexane extract of *B. lambaranensis*

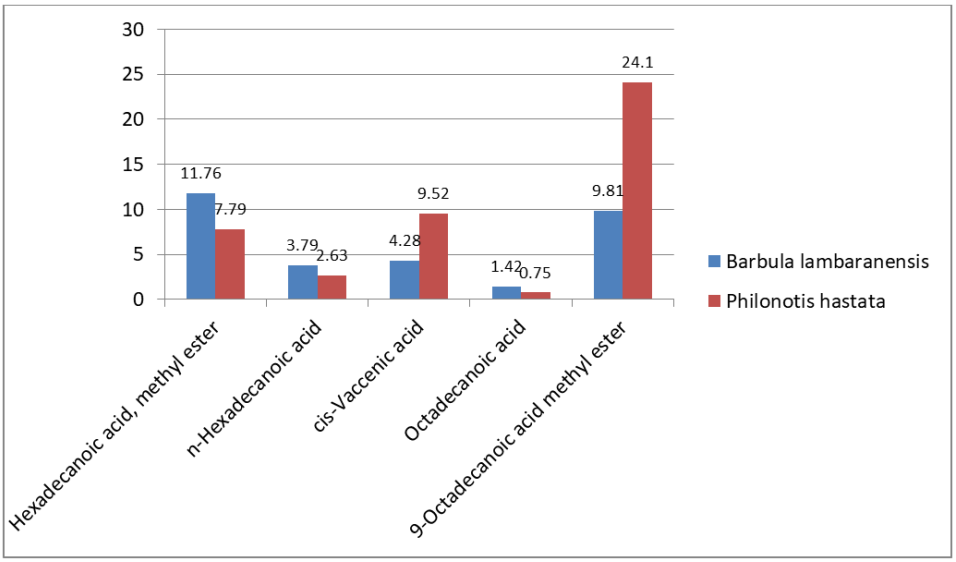

Figure 13: Distribution of the compounds common to both moss species

DISCUSSION

The results of the present study indicated the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and reducing sugar in the aqueous extracts of the studied moss species. This is an indication of their medicinal potentials. Several researchers have reported the occurrence of phytochemicals in bryophytes [11-13]. The result of the present study is comparable to that of these works. Some of these phytochemicals have been shown to have useful applications. Alkaloids have a wide range of pharmacological activities which include their actions on the autonomic nervous system, blood vessels, promotion of dieresis, respiratory system, gastrointestinal tract, malignant infection and malaria diseases [14]. Saponins are among various secondary metabolites with potent antifungal, anti- bacterial, anti-inflammatory and phytoprotectant properties which form barriers to microbial attack and in plant defence against herbivores [15]. Flavonoids have been reported to be highly effective scavengers of most oxidizing molecules including singlet oxygen and various free radicals implicated in several diseases [16]. Flavonoids have anti-oxidative and mucosal protective effect [17]. Vegetables rich in flavonoids are widely used functional foods since they can be used to treat cardiovascular diseases [18]. They are characterized by their good bioavailability and hence, constant dietary consumption of flavonoids has been reported to give pharmacologically relevant plasma concentrations in humans [19]. Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds such as sex hormones [20].

Previous researches have characterized several bioactive compounds in bryophytes using GC-MS analysis [21- 23]. The use of Gas Chromatography-Mass Spectrometry (GC-MS) is an established technique for reliable identification of some bioactive compounds in medicinal plants. Most of the identified compounds in the present study have been reported to possess varying pharmacological relevance. 9, 12-Octadecadieonoic acid, methyl ester has anti-inflammatory, anti-arthritic, hepatoprotective, anti-androgenic, hypocholesterolemic, anti-histaminic, anti-coronary, insectifuge and anti-eczemic properties [24]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Palmitic acid ester) acts as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, and hemolytic-5-alpha reductase inhibitor [25]. Hexadecanoic acid, a fatty acid commonly called palmitic acid was reported to have antioxidant, antibacterial, nematicide, anti-inflammatory, hypocholesterolemic, pesticide, lubricant, anti-androgenic, antitumor, flavour, chemopreventive, haemolytic-5-α reductase inhibition, lipo-oxygenase inhibition, cancer preventive and immunostimulant properties [25, 26]. Cis-vaccenic acid is an Omega-7-fatty acid known for its antibacterial activities and its hypolipidemic effect in rats has been reported [27]. 9-Octadecenoic acid (Z)-methyl ester has been found to be effective against the fungi *Aspergillus flavus* [28].

CONCLUSION

The findings of this study revealed the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and reducing sugar in the studied moss species. GC-MS analysis of the n-hexane extract of the mosses revealed the presence of 23 and 31 bioactive compounds in *P. hastata* and *B. lambaranensis* respectively. The major compounds identified in this study have been reported to be bioactive compounds of varying pharmacological relevance. Further studies are required to isolate and purify the lead molecules to evaluate their biological potentialities. This may lead to a new platform for novel drugs.

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