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GC-MS ANALYSES OF YOUNG AND MATURE *Archidium ohioense* SCHIMP EX. C. MULL AND *Philonotis hastata* (DUBY) WIJK & MARGAD EXTRACTS

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ABSTRACT

Analyses of the constituents of the crude extracts obtained from young and mature moss species namely: *Archidium ohioense* and *Philonotis hastata* were conducted with a view to investigating the effects of maturity stages on their bioactive constituents. The mosses collected from their natural population were air dried at ambient temperature in the laboratory, extracted with methanol and the crude extracts subjected to gas chromatography-mass spectrometry (GC-MS) analysis. The results of the analyses showed the presence of 20 compounds in young *A. ohioense* with n-hexadecanoic acid [26.60%], bis(2-ethylhexyl) phthalate [12.47%], bicyclo (3.1.1) heptane 2,6,6-trimethyl-[1r-(1.alpha.,2.beta.,5.alpha.)]- [11.59%] and phytol [9.69%] forming the prominent components while in the mature *A. ohioense*, 13 compounds were present, from which n-hexadecanoic acid [51.25%], hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)-ethyl ester [9.90%] and n-propyl 9-octadecenoate [7.47%] formed the prominent components. In *P. hastata*, 20 compounds were identified in the young stage sample with n-hexadecanoic acid [22.46%], bis(2-ethylhexyl) phthalate [20.95%] and phytol [18.14%] as the prominent components while 9 compounds were identified in the mature sample with n-hexadecanoic acid [51.84%], hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester [18.12%] and bis(2-ethylhexyl) phthalate [9.11%] which formed the prominent components. The study indicated that, maturity stages at collection of the mosses affected their bioactive compositions, with the young stage mosses showing more bioactive compounds than the mature ones.

Keywords: *A. ohioense*, *P. hastata*, Mosses, Maturity stages, Crude extract, GC-MS.

INTRODUCTION

Bryophytes are one of the promising sources of antibiotics and biologically active compounds in nature (Fatoba and Akolo, 2010; Remya *et al*., 2012). Historically, a large number of medicinal plants were discovered and used by the aboriginal people to treat illnesses (McCutcheon *et al*., 1992). A number of bryophytes have also been reportedly used as medicinal plants in China to cure bruises, burns, snake bites, external wounds, pulmonary tuberculosis, neurasthenia, fractures, convulsions, scald, uropathy and pneumonia (Ritika *et al*., 2014). Similarly, in Nigeria, insecticidal activities of solutions of four mosses' powders namely: *Calymperes afzelii* Sw., *Thuidium gratum* (P. Beauv.) Jaeg., *Bryum coronatum* Schwaegr. and *Barbula lambarenensis* (Hook) Spreng., against maize stem borers in the field were reported, and found to be better or just as good as 'Tricel' an inorganic insecticide (Ande *et al*., 2010).

Many pharmaceutical inventions were developed from a starting point of knowledge derived from the biological activities of natural organisms (Raj *et al*., 2011). Screening of biologically active molecules particularly the phytochemicals, is an important aspect in bryology. This is because metabolism of the plants has an evolutionary composition that offers a variety of secondary metabolites such as flavonoids, biflavonoids, terpenes, diterpenoids, fatty acid derivatives, aromatic compounds and non-aromatic compounds, e.t.c. (Asakawa, 2007; Krzaczkowski *et al*., 2008), with a natural balance (minimum amount necessary in the defense) and synergistic activity between compounds (Liu, 2004; Xie and Lou, 2009). The presence of bioactive compounds in plants indicates their medicinal potential for pharmacological as well as pathological discovery of novel drugs (Talukdar *et al*., 2010; Bode and Oyedapo, 2011). However, the discovery of the actual value of a traditional plant as well as discovery of a therapeutic agent solely depends upon the knowledge about the biologically-active compound composition of the plant (Mehta *et al*., 2017). Numerous synthetic drugs are currently available for use in the management of various ailments, disorders, pains,

and even control of pests but, being effective, natural, environmental friendly and easily affordable have increased the consumption and demand for medicinal plants (Jayashree and Maneemegalai, 2008; Fatoba and Akolo, 2010; Anuradha *et al*., 2013; Adaramola *et al*., 2018). Scientists have also found numerous kinds of biological activity in compounds isolated from bryophytes (Alam, 2012). Even in a single bryophyte species, there could be multiple kinds of biochemical activities. For example, phytochemicals from the liverworts- *Plagiochasma japonica* and *Marchantia tosana* exhibit antitumor, antifungal and antimicrobial activities, inhibition of superoxide release, thrombin activity, and muscle relaxation (Lahlou *et al*., 2000). The use of mosses to lower the body cholesterol had been reported (Radwan, 1991). Mosses contain polyunsaturated fatty acids that are already known to have important potentials in human medicine, such as preventing atherosclerosis and cardiovascular disease, reducing collagen-induced thrombocyte aggregation, and lowering triacylglycerols and cholesterol in plasma (Radwan, 1991).

Several factors have been reported to affect functional and biological compounds of medicinal plants and examples include environment (Maja *et al*., 2018), solvent of extraction, temperature and cultivation conditions (Burris *et al*., 2012; Chukwunonso and Macmanus, 2017). However, not much is known about the influences of maturity stages on the bioactive compounds of mosses. Hence in this study, GC-MS analyses of young and mature samples of selected mosses on the Obafemi Awolowo University Campus, Ile-Ife, Nigeria were carried out. This was with a view to providing information on this knowlege gap and guiding researchers as they search for phytopharmaceuticals for product development from mosses.

MATERIALS AND METHODS

Plant Materials

Young samples of *Archidium ohioense* Schimp ex. C. Mull and *Philonotis hastata* (Duby) Wijk & Margad., were collected in April 2019 and the mature stage samples were collected in August 2019, both from their natural population on Obafemi Awolowo University Campus, Ile-Ife, Nigeria, within Latitudes 7° 3' and 7° 34' N and Longitudes 4° 30' and 4° 32' E. Taxonomical identification and authentication of the samples were carried out at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, with herbarium numbers 633 and 634 for *Archidium ohioense* and *Philonotis hastata* respectively. Each of the samples was carefully sorted, separated from dirt, washed separately in different bowls of distilled water and air dried at ambient temperature in the laboratory for 48 hours.

Preparation of Extract

Extracts of the moss samples were prepared according to the method of Akinpelu *et al*., (2017). Air-dried milled sample of each species (50 g) was soaked separately in 700 ml of methanol at ambient temperature in the laboratory for 72 hours. The resulting suspension of each sample was filtered using Whatman No. 1 filter paper and the filtrate was evaporated to dryness on rotatory evaporator at 35 °C to obtain the respective crude extracts.

Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The Gas Chromatography -Mass Spectrometry (GC-MS) analyses of the crude extracts were carried out in the Department of Chemical Engineering, University of Ilorin, Ilorin, Nigeria, on Agilent 19091S Gas Chromatograph (GC) interfaced to a Mass Spectrometer 433HP-5MS instrument, employing the following conditions: silica capillary column fused with 5% phenyl methyl silox (30 m x 250 µm, film thickness 0.25 µm). The carrier gas used was Helium gas at a constant flow rate of 1.5 ml/minute, 10 µl syringe size was used and an injection volume of 1.0 µl. The split ratio was 50:1, split flow 75 µl/minute, septum purge flow 3 µl/minute and total flow of 79.5 µl/minute. Ionization energy of 70 eV was used as electron ionization system, injector temperature and pressure were 300 °C and 11.962 psi respectively. Average velocity was 44.411 cm/sec. The oven equilibration time of 15 seconds was observed and thereafter the temperature was programmed at 40 °C for 5 minutes followed by 4 °C to 150 °C for 2 minutes and then 20 °C/minute to 250 °C for 5 minutes. The total GC running time was 44.5 minutes.

Identfication of Compounds

The interpretations of the chromatogram of each of the samples based on the mass spectrum of each compound from the GC-MS was conducted using the database of National Institute of Standard and Technology 11 Library (NIST11.L) in order to ascertain the quality and identify the phytocompounds in the moss extracts. The spectra of the unknown components were compared (head to tail) with the spectrums of known components in the database of NIST11 Library. The name and molecular structure of the components of the test materials were also ascertained using the fragmentation patterns they exhibited and the information available in the Library.

RESULTS

Identified compounds from the young and mature methanol extracts of *A. ohioense* and *P. hastata* by the GC-MS analyses are presented in tables 1 – 4, while, the GC-MS chromatograms of the analyses are presented in figures 1, 3, 4 and 6 respectively. The results of the analyses showed the presence of different fatty acids, cyclic and heterocyclic compounds in the mosses extracts with significant variations in the bioactive compounds identified in the young and mature samples. Young *A. ohioense* revealed the presence of 20 compounds in its extract among which were four prominent compounds namely: [1R-(1.alpha.,2.beta.,5.alpha.)]-2,6,6-trimethyl-bicyclo[3.1.1]heptane (11.59%), n-hexadecanoic acid (26.60%), phytol (9.69%) and bis(2-ethylhexyl) phthalate (12.47%), totalling 60.35% of the mixture, while the mature *A. ohioense* extract revealed the presence of 13 compounds with three (3) prominent compounds viz. n-hexadecanoic acid (51.25%), n-propyl-9-octadecenoate (7.47%) and hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)-ethyl ester (9.90%), totalling 68.62% with n-hexadecenoic acid constituting highest percentage in both the young and mature samples (Figure 2). The GC-MS analysis of the methanolic extract of young *P. hastata* revealed the presence of 20 compounds out of which three (3) compounds namely: n-hexadecanoic acid (22.46%), phytol (18.14 %) and bis (2-ethylhexyl) phthalate (20.95%) formed the major components amounting to 61.55% of the total. On the contrary, the GC-MS analysis of the mature methanol extract of *P. hastata* revealed the presence of only nine (9) compounds but contained three (3) prominent compounds which were n-hexadecanoic acid (51.84%), bis (2-ethylhexyl) phthalate (9.11%) and hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester (18.12%) adding up to be 79.07% of the total mixture with n-hexadecenoic acid constituting the highest proportion (Figure 5). Three compounds: n-hexadecanoic acid, methyl palmitate and hexadecanoic acid-2-hydroxy-1-(hydroxymethyl)ethyl ester were commonly identified in both the young and mature extracts of the two species. However, the concentrations of n-hexadecanoic acid (the most abundant constituent of the young and mature samples of the studied mosses) and hexadecanoic acid- 2-hydroxy-1-(hydroxymethyl)ethyl ester, were higher in the mature mosses extracts than in the young mosses extracts while methyl palmitate concentration was higher in the young samples than in the mature samples (Figure 7). Phytol was identified only in the young stage extracts of the two studied moss species and bis (2-ethylhexyl) phthalate was identified in both the young and mature crude extracts of *P. hastata* as well as in the young *A. ohioense* extract but with higher concentration in the young stage extract of *P. hastata*. Highlights on the proportion of n-hexadecanoic acid, the most abundant compound in both the young and mature *A. ohioense* and *P. hastata* methanol extracts is presented in figure 8.

Table 1: Phytoconstituents Identified in Methanol Extract of Young *A. ohioense*

S/n	RT (Min)	Area%	Name	Molecular Formula	Percentage Quality (%)
1	16.597	1.43	2-methoxy-Phenol	$C_7H_8O_2$	55
2	20.168	0.98	*Octanoic acid	$C_8H_{16}O_2$	35
3	21.176	2.91	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	$C_6H_8O_4$	43
4	24.791	1.33	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	94
5	31.528	3.41	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	95
6	36.451	0.82	Heptadecanal	$C_{17}H_{34}O$	93
7	37.152	1.58	Hepta-2,4-dienoic acid, methyl ester	$C_8H_{12}O_2$	38
8	37.446	0.94	Dodecane	$C_{12}H_{26}$	35
9	37.614	0.94	Oxirane, tetradecyl-	$C_{16}H_{32}O$	91
10	37.821	11.59	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	$C_{10}H_{18}$	70
11	37.877	3.48	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydro-thiophene1,1-dioxide	$C_{14}H_{16}O_2S_2$	38
12	38.034	3.30	9-Octadecyne	$C_{18}H_{34}$	53
13	38.102	0.98	Phthalic acid, butyl hept-4-yl ester	$C_{19}H_{28}O_4$	78
14	38.177	3.86	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	78
15	38.509	5.87	*Methyl palmitate	$C_{17}H_{34}O_2$	99
16	38.828	26.60	*n-Hexadecanoic acid	$C_{16}H_{32}O_2$	99
17	39.579	1.83	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	95
18	39.691	9.69	Phytol	$C_{20}H_{40}O$	93
19	42.788	5.97	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl-ester	$C_{19}H_{38}O_4$	91
20	43.244	12.47	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	91

Key:
S/n = Serial number, RT = Retention time
* = Compound common in both the young and mature *A. ohioense* samples

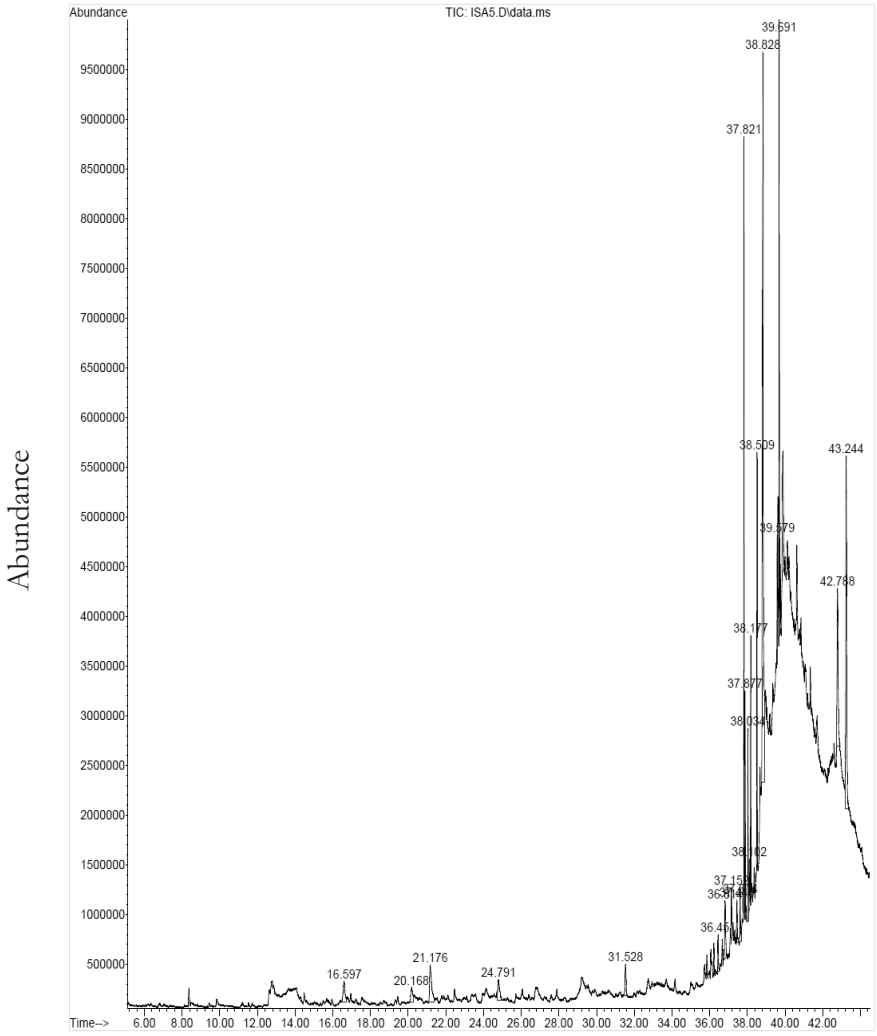


Figure 1: GC-MS Chromatogram of Methanol Extract of Young *A. ohioense*

Table 2: Phytoconstituents Identified in Methanol Extract of Mature *A. ohioense*

S/n	RT (Min)	Area%	Name	Molecular Formula	Percentage Quality (%)
1	12.850	2.47	Hexanoic acid	$C_6H_{12}O_2$	40
2	20.300	2.59	*Octanoic acid	$C_8H_{16}O_2$	94
3	23.409	4.06	3-Hexanol, 4-ethyl-	$C_8H_{18}O$	64
4	23.990	1.54	3-Heptanol, 4-methyl-	$C_8H_{18}O$	59
5	32.760	3.19	2-Butenoic acid, 2-methyl-, propyl ester, (E)-	$C_8H_{14}O_2$	38
6	37.821	3.53	Phytol, acetate	$C_{22}H_{42}O_2$	72
7	37.877	3.13	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	99
8	38.033	1.75	Octadecanal	$C_{18}H_{36}O$	53
9	38.509	4.24	*Methyl palmitate	$C_{17}H_{34}O_2$	98
10	38.834	51.25	*n-Hexadecanoic acid	$C_{16}H_{32}O_2$	99
11	39.866	7.47	n-Propyl 9-octadecenoate	$C_{21}H_{40}O_2$	89
12	42.781	9.90	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)-ethyl ester	$_{19}H_{38}O_4$	76
13	43.244	4.88	Phthalic acid, decyl 2-ethylhexyl ester	$C_{26}H_{42}O_4$	64

Key:
S/n = Serial number, RT = Retention time
* Compound common in both the young and mature *A. ohioense* samples

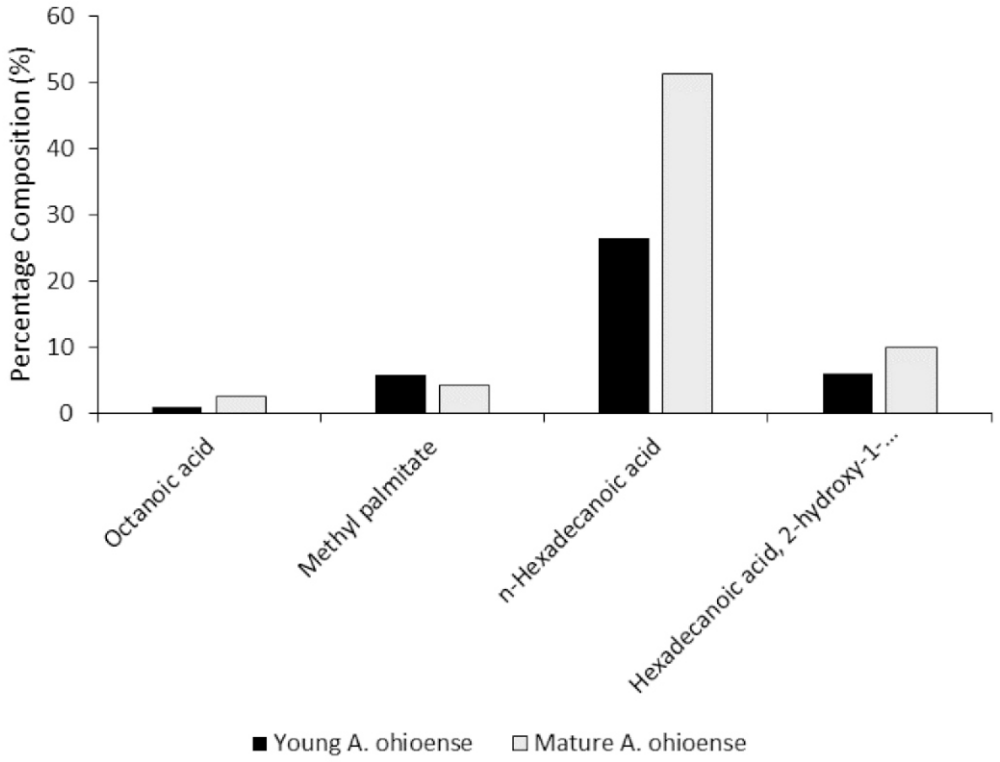


Figure 2: Compounds Common in both the Young and Mature *A. ohioense* Samples

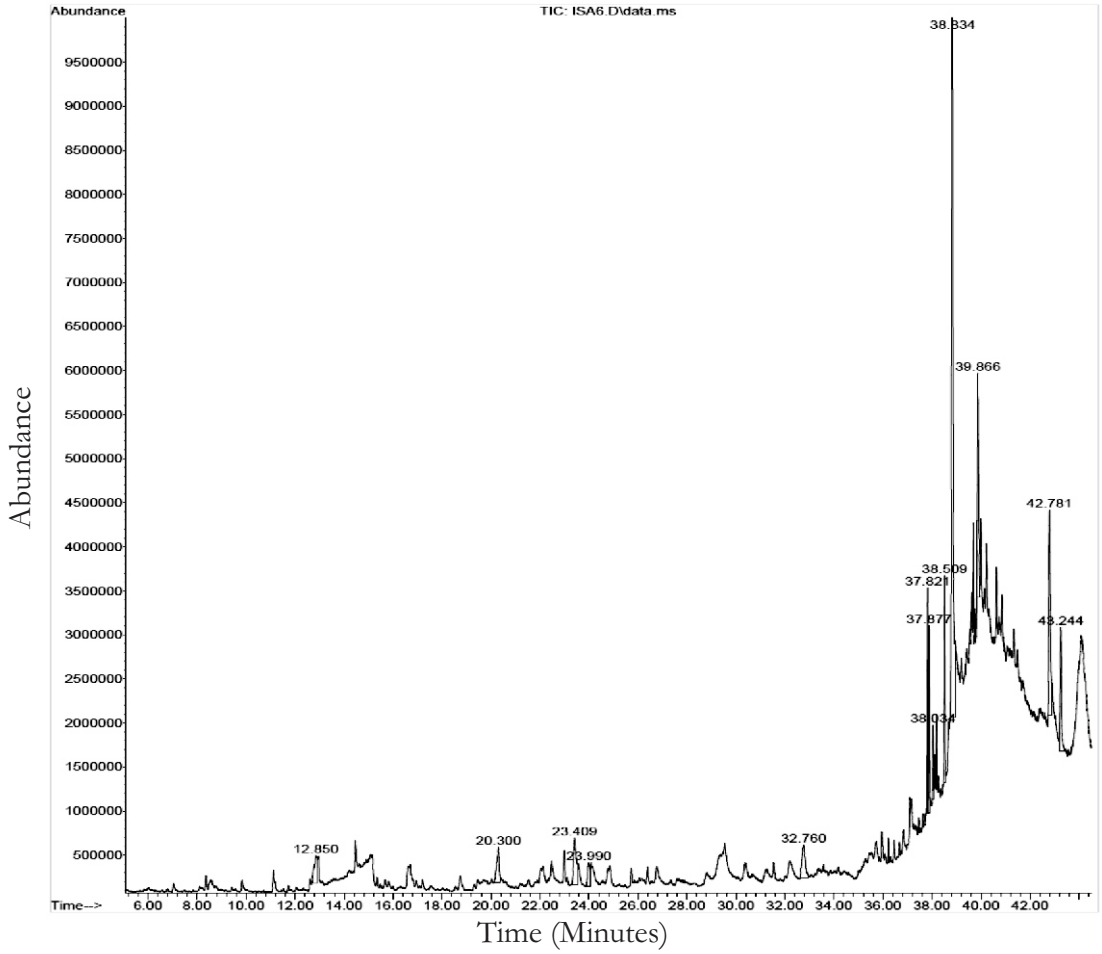
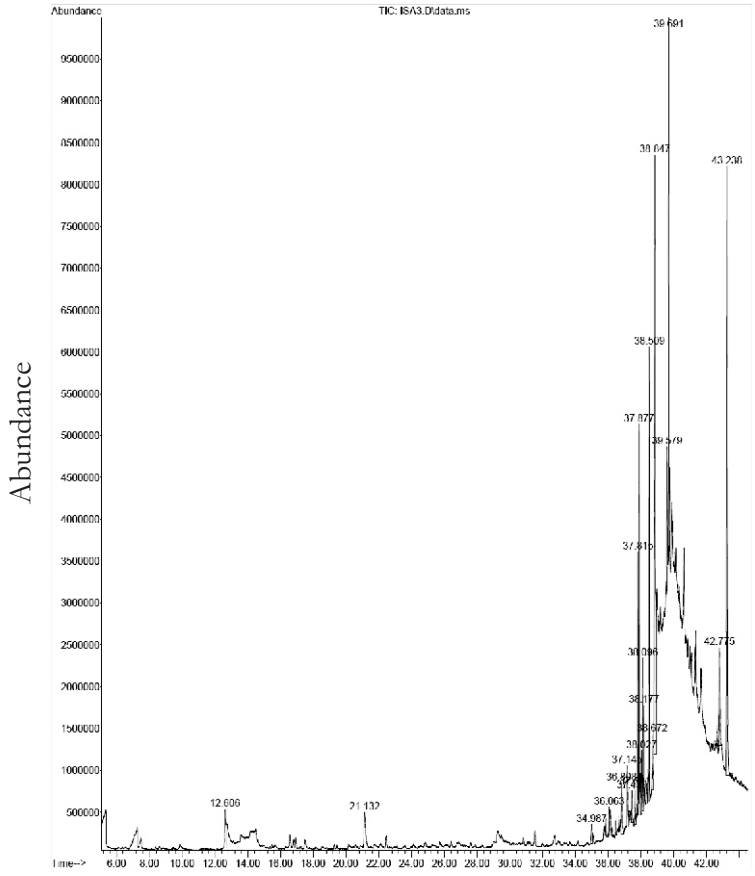


Figure 3: GC-MS Chromatogram of Methanol Extract of Mature *A. ohioense*

Table 3: Phytoconstituents Identified in Methanol Extract of Young *P. hastata*

S/n	RT (Min)	Area%	Name	Molecular Formula	Percentage Quality (%)
1	12.606	2.01	Phenol	C_6H_6O	91
2	21.132	2.29	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	$C_6H_8O_4$	55
3	34.987	0.73	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	$C_{14}H_{24}O$	50
4	36.063	0.75	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	$C_{13}H_{20}O_3$	86
5	36.808	1.75	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	38
6	37.145	1.97	Thiophene, 2-methyl-5-propyl-	$C_8H_{12}S$	27
7	37.439	0.77	Orcinol, monoacetate	$C_9H_{10}O_3$	46
8	37.621	0.83	11-Hexadecen-1-ol, acetate, (Z)-	$C_{18}H_{34}O_2$	83
9	37.815	3.85	*Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	64
10	37.877	5.40	*2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	91
11	38.027	0.96	3-Octadecyne	$C_{18}H_{34}$	58
12	38.096	2.36	Phthalic acid, isobutyl nonyl ester	$C_{21}H_{32}O_4$	83
13	38.177	1.57	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	$C_{14}H_{26}O$	62
14	38.509	6.43	*Methyl palmitate	$C_{17}H_{34}O_2$	99
15	38.672	1.00	*Isophytol	$C_{20}H_{40}O$	64
16	38.847	22.46	*n-Hexadecanoic acid	$C_{16}H_{32}O_2$	99
17	39.579	2.00	9,12-Octadecadienoic acid, methylester	$C_{19}H_{34}O_2$	99
18	39.691	18.14	Phytol	$C_{20}H_{40}O$	93
19	42.775	3.75	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	87
20	43.238	20.95	*Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	91

Key:
S/n = Serial number, RT = Retention time



* = Compound common in both the young and mature *P. hastata* samples

Figure 4: GC-MS Chromatogram of Methanol Extract of Young *P. hastata*

Table 4: Phytoconstituents Identified in Methanol Extract of Mature *P. hastata* Leaf

S/n	RT (Min)	Area%	Name	Molecular Formula	Percentage Quality (%)
1	32.742	2.00	Decan-2-yl (E)-2-methylbut-2-enoate	$C_{15}H_{28}O_2$	43
2	37.821	4.66	*Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	64
3	37.877	4.31	*2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	91
4	38.177	1.82	Cyclohexanol, 1-ethynyl-	$C_8H_{12}O$	43
5	38.515	4.59	*Methyl palmitate	$C_{17}H_{34}O_2$	99
6	38.884	51.84	*n-Hexadecanoic acid	$C_{16}H_{32}O_2$	99
7	39.697	3.55	*Isophytol	$C_{20}H_{40}O$	83
8	42.794	18.12	*Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	81
9	43.244	9.11	*Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	91

Key:

S/n = Serial number, RT = Retention time

* Compound common in both the young and mature *P. hastata* samples

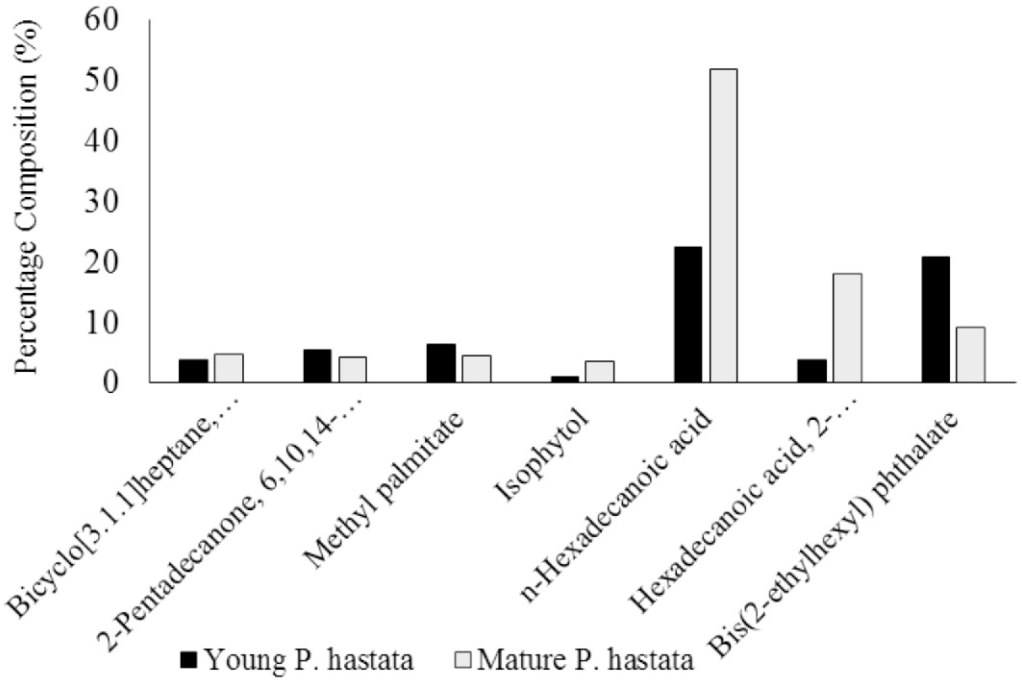


Figure 5: Compounds Common in both the Young and Mature *P. hastata* Samples

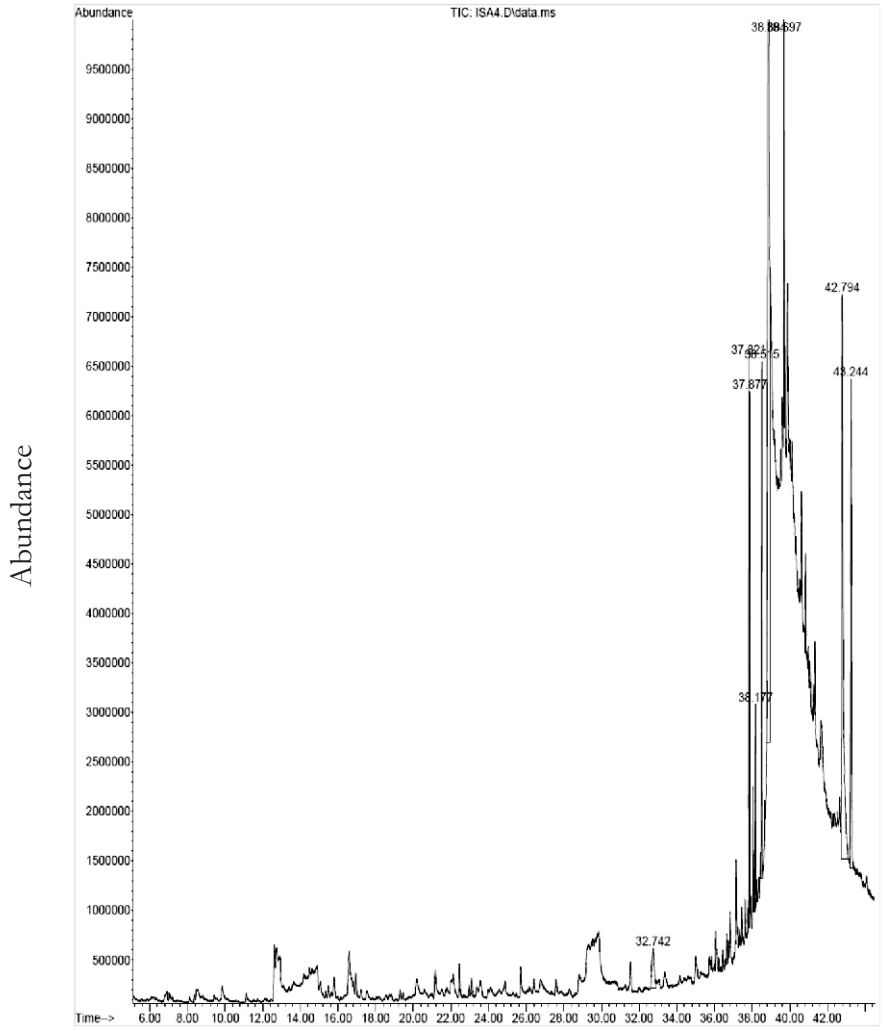


Figure 6: GC-MS Chromatogram of Methanol Extract of Mature *P. hastata*

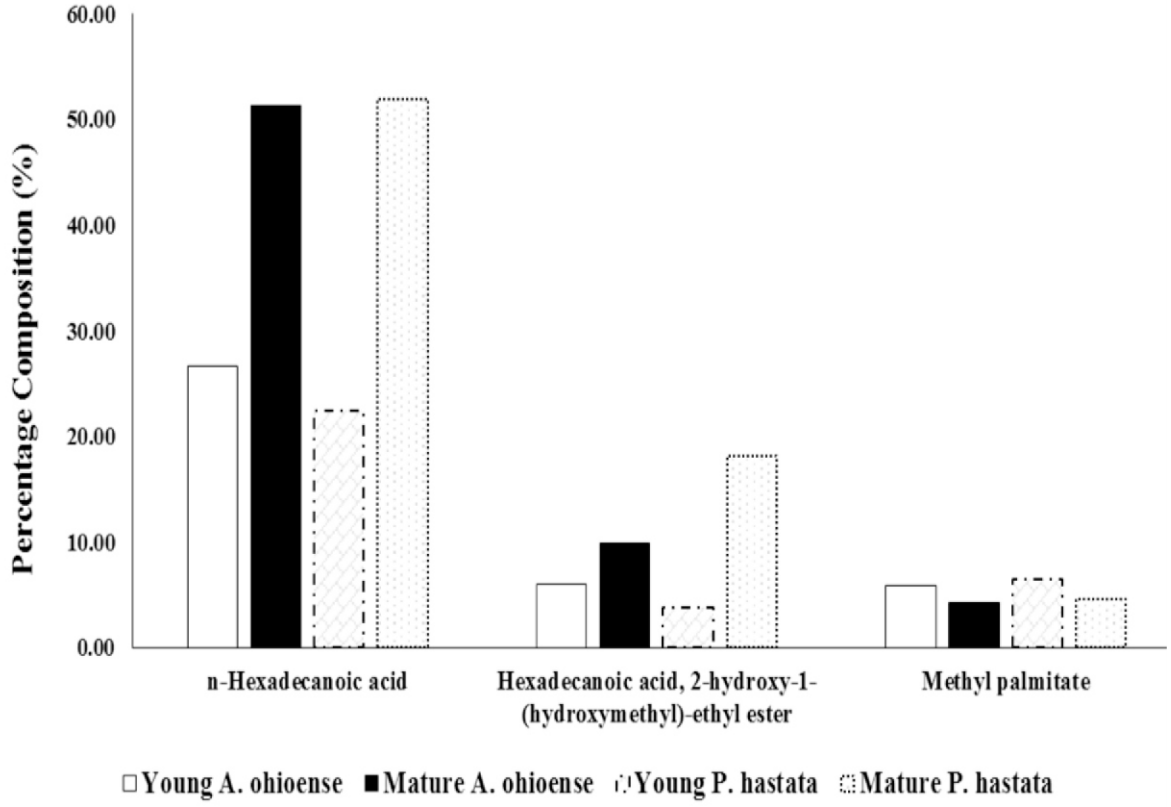


Figure 7: Compounds Common in both the Young and Mature *A. ohioense* and *P. hastata* Methanol Extracts

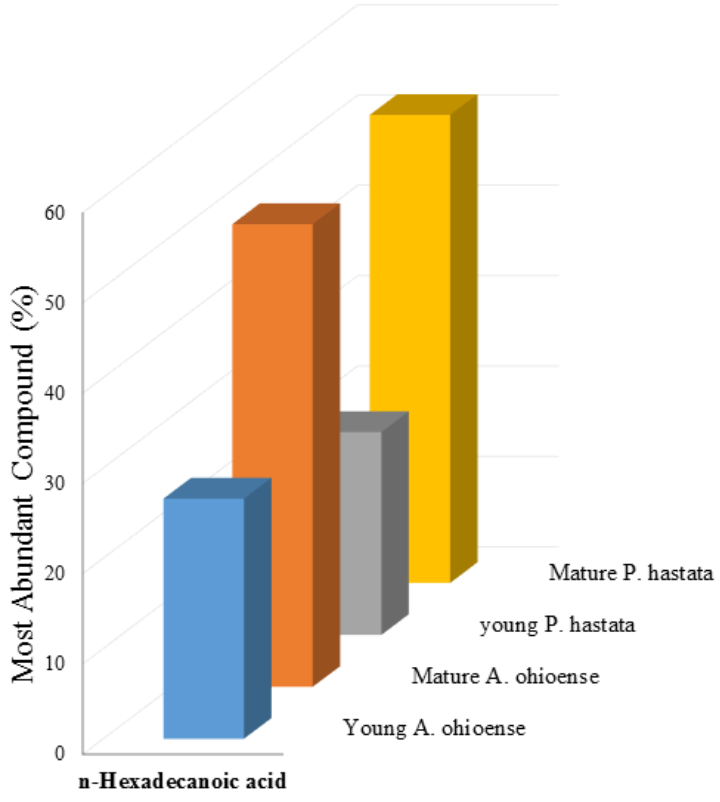


Figure 8: Highlight on the Proportion of n-Hexadecanoic acid (the most abundant compound) in both the Young and Mature *A. ohioense* and *P. hastata* Methanol Extracts

DISCUSSION

The use of Gas Chromatography-Mass Spectrometry (GC-MS) as an established technique for reliable identification of some bioactive compounds existing in medicinal plants including volatile matter, long chain and branched chain hydrocarbons, alcohols, acids and esters has been reported (Kumar *et al.*, 2014; Godwin *et al.*, 2015; Dienish *et al.*, 2018). GC-MS analyses carried out in this study showed remarkable differences between the bioactive compounds identified from the young and mature moss species samples. The identification of 20 compounds each from the young moss extracts of both *A. ohioense* and *P. hastata* and only 13 and 9 compounds respectively from their mature stage samples could be attributed to the differences in developmental stages of the analysed moss samples (Majd *et al.*, 2019). The observations on the young stage moss samples showing more bioactive compounds than the mature ones was in line with the findings of Muhammad *et al.* (2013) who reported higher amounts of volatiles production in young leaves than mature leaves of different *Citrus* types.

According to Gobbo-Neto and Lopes (2007), the levels of growth among other factors affect plant secondary metabolites production. The influence of age and plant development on secondary metabolite contents and the relative proportions of these chemical components as demonstrated by several authors for different plant species showed that young tissues generally have higher rates of metabolites' biosynthesis (Carlos *et al.*, 2015). Plants under heterogenic environments may also accumulate secondary metabolites that have different features (Huang and Guo, 2007). Some of the identified bioactive compounds by the GC-MS analyses such as octanoic acid, n-hexadecanoic acid, phytol, methyl palmitate and hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)-ethyl ester were identified in both the young and mature stage samples of *A. ohioense.* Similarly, both the young and mature stage samples of *P. hastata* revealed the presence of 2,6,6-trimethyl-bicyclo[3.1.1]heptane, 2-pentadecanone 6,10,14-trimethyl, n-hexadecanoic acid, methyl palmitate, isophytol, hexadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester and bis(2-ethylhexyl) phthalate. However, the concentration of each bioactive compound varied from one maturity stage to another with some having higher concentrations in the mature samples and lower concentration in the young stage samples. It is evident from this study that developmental phases have an impact on the studied mosses' bioactive compounds production; some compounds' concentrations were increased during the developmental transition from young to mature stage. n-Hexadecanoic acid was found to have higher concentration in the mature stage samples of the studied mosses compared with the young ones, as it was reported for leaf volatiles in *Citrus* cultivars (Flamini and Cioni, 2010; Lin *et al.*, 2010; Muhammad *et al.*, 2013).

Some compounds were also noted to be present only at a stage and absent at the other stage of maturity. [1R-(1.alpha.,2.beta.,5.alpha.)]-2,6,6-trimethyl-bicyclo [3.1.1] heptane, which formed 11.59% of the total identified compounds in the young *A. ohioense* extract was not revealed in the mature sample extract. Conversely, n-propyl-9-octadecenoate (7.47%) was present in the mature *A. ohioense* extract but it was not detected in the young stage sample. Similarly, in *P. hastata*, phenol (2.01%) was identified only in the young stage sample and decan-2-yl (E)-2-methylbut-2-enoate (2%) was identified in the mature stage sample and not in the young stage sample. The variations observed in both the concentrations and the amount of bioactive compounds of the mosses at the different developmental stages could also be a means by which the mosses adapted to resist biological, physical or some chemical environmental stresses along the developmental stages (Wink and Mohamed, 2003; Guo *et al.*, 2013; Chukwunonso and Macmanus, 2017). Secondary metabolites, particularly low molecular weight phenolic compounds, have been reported to be at the highest levels in the early stage of growth or only synthesized at young plant vegetative stages when leaves are most in need of defense from herbivores (Parr and Bolwell, 2000). Changes in bioactive compound composition between the young and mature mosses suggest some biological roles for these biological compounds, for example, in the protection of reproductive organs and defense against pathogens and/or herbivory (Muhammad *et al.*, 2013).

Most of the identified compounds from the moss extracts have been reported to be bioactive compounds of varying pharmacological relevance. 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, cancer preventive, immunostimulant, chemopreventive, haemolytic-5-α reductase inhibition, lipo-oxygenase inhibition properties (Lalitha *et al*., 2015; Adeoye-Isijola *et al*., 2018). 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 (Lalitha *et al*., 2015). Phytol, a diterpene is used in artificial synthesis of vitamin E and vitamin K (Daines *et al*. 2003). The roles of phytol as an antimicrobial, anti-inflammatory, diuretic, antimalarial, anti-cancer and antibacterial against *Staphylococcus typhi* have also been reported (Tulika and Mala, 2017). Furthermore, the applications of phytol in cosmetics and fragrance industry (McGinty *et al*., 2010; Chavan and Gaikwad 2017) suggest the application of these plants in cosmetics, and which may prove as a cost effective alternative to synthetic chemicals used in current cosmetic industry.

The antibacterial activity of bis (2-ethylhexyl) phthalate against *Staphylococcus* has been reported (Habib and Karim, 2009). The compound bis (2-ethylhexyl) phthalate was also reported to be synthesized by some bacteria as well as fungi (Aurelio and Estibaliz, 2018), and some industries use it as plasticizers to improve the plasticity and the flexibility of some materials like food packages, toys, medical devices such as blood storage bags, fluid bags, e.t.c. (Aurelio and Estibaliz, 2018). Octanoic acid (caprylic acid), an 8-carbon, short-chain fatty acid and its monoglyceride, monocaprylin, were reported to be effective in inactivating infant pathogens such as herpes simplex virus, respiratory syncytial virus, *Haemophilus influenzae*, and Group B streptococci (Isaac *et al*., 1995). Methyl palmitate is used in the preparation of detergents, emulsifiers, wetting agents, stabilizers, resins, lubricants, plasticizers and animal feeds (Larranaga *et al*., 2016). It exhibits anti-inflammatory and anti-fibrotic agent and prevents bleomycin-induced lung inflammation and fibrosis in rats as well as preventing carbon tetrachloride-induced liver fibrosis. It is also linked to transforming growth factor beta reduction, which is a secreted protein that controls proliferation, cellular differentiation and other functions in most cells (Mantawy *et al*., 2011; Roopa *et al*., 2020).

CONCLUSION

The findings of this study suggest that, maturity stages of mosses affect their bioactive compounds composition as observed in the most abundant component, n-hexadecanoic acid, with higher concentration in the mature moss samples and methyl palmitate in the young leaves. Also, the young stage samples of the mosses revealed the presence of more bioactive compounds in their crude methanol extracts than the mature ones. Further investigation on the identified bioactive compounds from the studied mosses will be beneficial to formulate novel drugs for the management and treatment of diseases.

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