Volatile chemical constituents of two species of bryophytes (Bryophyta) occurring in the Brazilian Amazon

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Short Report

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Abstract

Bryophytes are a group of plants that present a variety of chemical compounds, but information on the phytochemistry of the species, especially those in the Brazilian Amazon, is still scant. Thus, the objective of this work was to determine the volatile chemical constituents of the Amazonian moss species *Sematophyllum subsimplex* (Hedw). Mitt. and *Leucobryum martianum* (Hornsch.) Hampe ex Mull Hal. Samples were collected at the Gunma Ecological Park, in the municipality of Santa Bárbara, Pará, and subsequently dried, identified, screened, kept in a refrigerated place, and subjected to extraction by means of simultaneous distillation-extraction (SDE). Volatile concentrates were analyzed by gas chromatography/mass spectrometry (GC-MS). Forty-two chemical constituents were identified in the pentane extracts of the two species studied, mainly belonging to the group of aldehydes, terpenes, and carboxylic acids. Naphthalene was predominant in both species, mainly in *L. martianum*. Safrole, found in *L. martianum*, is being recorded for the first time in a moss. This study represents the first report of the chemical composition of the two selected taxa and the results indicate that these mosses are natural sources of compounds of interest for bioprospecting.

Highlights

- Forty-two chemical constituents were identified in the pentane extracts of the two species studied
- The species are rich in composts belonging to the group of aldehydes, terpenes, and carboxylic acids.
- Naphthalene found in *L. martianum*, is being recorded for the first time in a moss.

1. Introduction

Bryophytes are represented by three distinct lineages of terrestrial cryptogamic plants, liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Antocerotophyta), and can be found on all continents, including Antarctica, except for the marine environment (Asakawa and Ludwiczuk 2018).

The number of bryophyte species known globally is approximately 20,300, but as they are small, difficult to collect, and have no nutritional value for people, or in better words, there is no record of the use of bryophytes as human food so far, their chemistry was neglected for a long time (Asakawa et al. 2021).

Over the last 40 years, more than 3,000 compounds have been found in this group of plants, many of which are characterized by unprecedented structures, indicating that many constituents can still be discovered through phytochemical studies (Ludwiczuk and Asakawa 2020).

The group of mosses is of biochemical and biotechnological interest because it has an abundance of aldehydes and terpenes (mainly mono-, sesqui- and diterpenes) in addition to aromatic compounds; flavonoids; bioflavonoids; saturated, mono- and di-unsaturated hydrocarbons, among others (Cansu et al. 2014).

A total of 1,527 species of bryophytes have been recorded in Brazil, of which 571 occur in the Brazilian Amazon and 331 in the state of Pará (Costa and Peralta). In these territories, these plants are well studied as to their floristics, taxonomy and ecology, but the first chemical researches were carried out by Pinheiro, Brazão and Lisboa (1989), in Pará, who confirmed the bactericidal potential of some bryophyte species, and Vidal et al. (2013), in Ceará, who conducted a chemical analysis of the extract of the moss *Octoblepharum albidum* Hedw., with a positive result for antibacterial activity.

The volatile profile of the moss *Neckeropsis undulata* (Hedw.) Reichardt, that occurs in the Amazon, was recently published, listing 13 constituents, and contributing to the chemotaxonomic knowledge of the species (Miranda et al. 2021). The ethanol extract of *Sematophyllum subsimplex* (Hedw). Mitt. showed antifeedant and insecticidal effect on second instar larvae of *Spodoptera frugiperda* (JE Smith, 1797) in maize (*Zea mays* L.) leaves (Rezende-Moraes et al. 2021).

The moss species *S. subsimplex* and *Leucobryum martianum* (Hornsch.) Hampe ex Mull Hal., object of the present study, belong to the families Sematophyllaceae Broth. and Leucobryaceae Schimp., respectively, which are common throughout the Amazon region and well-explored in botanical studies (Santos and Lisboa 2003; Ilkiu-Borges and Lisboa 2004; Tavares-Martins et al. 2014).

According to the consulted literature, there are no studies on the chemical composition of these two species. Thus, the objective of this work was to know the volatile constituents of the mosses *Sematophyllum subsimplex* Hedw. Mitt. and *Leucobryum martianum (Hornsch.) Hampe ex Müll Hal.*, which is of great importance for Science for being a fundamental step for future research.

2. Experimental

2.1 Study area and selection of the moss species

The Gunma Ecological Park is located in the municipality of Santa Bárbara, Northeast of the state of Pará, Eastern Brazilian Amazon (01º13’00.86” S and 48º17’41.18” W). The park has an area of 400 ha of native humid tropical forest and 140 ha of multiple-use open area which has undergone several changes as a result of urban growth. The park is irrigated by the Tracuateua and Tauariê inflowing streams (“igarapés”).

The species *S. subsimplex* and *L. martianum* were selected for this study because they are widely distributed in tropical forests, including in the Amazon (Sierra et al. 2018).

These species are known, well described and illustrated in the literature and easy to identify in the field and laboratory (Costa 1986; Visnadi 2006). The selection of the species was also based on phytochemical studies already conducted with these species or with the genera to which they belong (Pinheiro et al. 1989; Veljic et al. 2008; Haines and Renwick 2009; Olofin 2013).

2.2 Collection and identification of botanical material

The samples were collected in a secondary non-flooded ("terra firme") forest ecosystem, close to the "igarapés" and to the plots established at the entrance of the flooded area ("igapó") of the forest. The species *S. subsimplex* was collected on living bark and *L. martianum* on the ground, under medium sunlight intensity, following the collection techniques of Yano (1984).

The samples were identified at the Bryology Laboratory of the Emílio Goeldi Museum of Pará (MPEG), Belém, Pará, Brazil. The botanical material was washed, sun dried for three days, kept in a refrigerated room. As bryophytes often grown in mats consisting of aggregates of several species (He et al. 2016), the samples were screened using tweezers (Flume 5) under a stereomicroscope (Leica, Wild M3Z) for the separation of specimens of the selected mosses. Only gametophytes (vegetative part) were separated, as sporophytes were absent at the time of collection.

The taxonomic classification was made according to Goffinet (Goffinet 2009). Witness material was incorporated into the João Murça Pires Herbarium (MG) of the MPEG.

2.3 Obtaining volatile constituents

After drying at room temperature, the biomass of the screened material of each moss species was weighed on a precision scale (2.6 g was enough for each species), and they were submitted to simultaneous micro hydrodistillation-extraction (SDE). A Nickerson & Likens extractor from Chrompack was used for the procedure and *n*-pentane (2 mL) was used as solvent, coupled to a refrigeration system to maintain the condensing water temperature between 5–10 ºC for 2 hours. The extraction was carried out at the Laboratory of Engineering of Natural Products (LEPRON) at the Federal University of Pará (UFPA) and all extractions were carried out in duplicate.

2.4 GC-MS analysis of the volatile concentrate

Volatile concentrates were analyzed by gas chromatography/mass spectrometry (GC-MS) in a Thermo system, model DSQII, equipped with a DB-5MS fused silica capillary column (30m x 0.25 mm x 0.25 μm film thickness); helium carrier gas adjusted to provide a linear velocity of 32 cm/sec (measured at 100° C); injector temperature, 240°C; injection type: without flow splitting. 0.1 μL of the pentane solution was injected; the oven temperature was programmed for 60–240 ºC (3 ºC/min); ionization was achieved by using an electronic impact technique at 70 eV; temperature of ion source and connecting parts, 200 ºC; mass range, 39 to 450 daltons.

2.5 Identification of chemical constituents

Each constituent was identified by comparing its mass spectrum and retention indices (RI) with those of standard substances in the libraries of the system (Adams 2007; Technology 2014).

The RI were obtained using the homologous series of n-alkanes (C8-C26) under the same chromatographic conditions and calculated by Vanden Dool–Kratz's equation. The quantification of the constituents was obtained by means of GC, on a Thermo/Focus equipment with a flame ionization detector (FID), under the same operating conditions as the GC-MS, except for the carrier gas, which was hydrogen.

3. Results And Discussion

In the chromatographic analysis, 39 chemical constituents were detected in *S. subsimplex*, of which 28 (63.88%) were identified, and 20 were detected in *L. martianum*, with identification of 14 (95.44%) constituents of its volatile concentrate.

The volatile chemical composition of *S. subsimplex* consisted predominantly of aldehydes (1, 3, 5, 6, 8, 9, 10, 11, 12 and 14), terpenes (sesquiterpenes) (2, 15 and 19), carboxylic acids (22, 24, 25 and 27), phenolic compounds (17 and 20), esters (13 and 26), ethers (21 and 28), an aromatic hydrocarbon (7), a ketone (16), and an alcohol (4) (Table 1). The major components were naphthalene (26.62%), phthalic acid derivative (13.98%), curcuphenol (7.74%), hexadecanoic acid (7.51%), and *n*-nonanal (5.41%) (Table 1).

Tabela 1: Volatile chemical constituents identified in the pentane extract of *S. subsimplex.*			
Number	Constituents	RIL	(%)
1	(2E) - heptenal	947	4.61
2	2-pentylfuran	984	1.52
3	(2E) - octenal	1049	1.33
4	(2E) - octenol	1060	2.13
5	n-nonanal	1100	5.41
6	(2E) - nonenal	1157	0.58
7	Naphthalene	1178	26. 62
8	Decanal	1201	1.15
9	(2E, 4E) - nonadienal	1210	1.39
10	(2E) - decenal	1260	2.08
11	(2E, 4Z) - decadienal	1292	1.16
12	(2E, 4E) - decadienal	1315	1.36
13	1-methoxy-4-methyl-bicyclo[2.2.2]octane	1341	1.13
14	(2E)- undecenal	1357	1.30
15	(E)-β-ionone	1487	0.43
16	(E)-5,6-epoxy-β-ionone	1467	0.30
17	Benzophenone	1626	0.71
18	Methyl cis-dihydrojasmonate	1092	0.44
19	Curcuphenol	1717	7.74
20	2-ethylhexyl salicylate	1807	0.50
21	Galaxolide	1913	0.66
22	Phthalic acid derivative	2171	13.98
23	Methyl hexadecanoate	1921	0.65
24	Hexadecanoic acid	1959	7.51
25	Phthalic acid derivative	2470	0.65
26	Methyl linoleate	2095	2.07
27	Methyl 10-octadecenoate	2085	1.92

Tabela 1: Volatile chemical constituents identified in the pentane extract of *S. subsimplex.*			
28	2-ethylhexyl p-methoxycinnamate	2088	1.17

Regarding the volatile chemical composition of *L. martianum*, there was a group of carboxylic acids (11, 13 and 14), phenolic compounds (2 and 9), an aromatic hydrocarbon (1), a sesquiterpene (5), an alcohol (12), an aldehyde (2) and an ether (9) (Table 2). The major components were naphthalene (72.51%), phthalic acid derivative (7.09%), and hexadecanoic acid (5.08%) (Table 2).

Table 2
Volatile chemical constituents identified in the pentane extract of L. martianum.

Number	Constituents	RI_L	(%)
1	*Naphthalene*	*1178*	*72.51*
2	Safrole	1285	0.43
3	(2E, 4E)-decadienal	1315	0.38
4	2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl 2-methylpropanoate	1347	0.34
5	Maaliol	1566	0.61
6	Diethyl phthalate	1590	0.49
7	Methyl *cis*-dihydrojasmonate	1654	0.73
8	2-ethylhexyl salicylate	1807	1.20
9	Galaxolide	1913	1.15
10	6,10,14-trimethyl-2-pentadecanone	1754	0.87
11	*Phthalic acid derivative*	*2008*	*7.09*
12	Hexadecanol	1874	4.00
13	Phthalic acid derivative	2534	0.56
14	*Hexadecanoic acid*	*1959*	*5.08*

IR_L – Retention index according to the literature.

Major constituents in italics, considering ≥ 5.0%.

A high content of the aromatic hydrocarbon naphthalene was identified in the two mosses, corresponding to 26.62% in *S. subsimplex* and 72.51% in *L. martianum*. In the work by Miranda et al.(Miranda et al. 2021), this compound was also reported in the volatile concentrate of *N. undulata*, but at lower concentrations (11.30%). Carranza et al. (Carranza et al. 2019), analyzed the phytochemistry of seven species of mosses from the Philippines and identified naphthalene in *Gymnostomum recurvirostum* Hedw., *Pelekium boniamum* (Besch), and *Hypnum plumiforme* Wilson with contents of 5.88%, 4.97% and 3.57%, respectively.

Naphthalene is frequently found in soil and aquatic environments (Zeng et al. 2021), having usually high bioavailability in the environment (Gesto et al. 2009). It is also known that bryophyte species naturally synthesize naphthalene (Wilson et al. 1989; Asakawa 1995). However, it is assumed that, when this compound occurs at high percentages, part of it may come from persistent organic pollutants (POPs), because bryophytes easily retain and absorb residues of environmental impurities (Harmens et al. 2013).

The area where the two species selected for this study were collected was an environment under human influence. The mosses were collected on trunks of living trees close to the ground and on the ground, adjacent to the “igarapé”, often visited by people who may leave industrial and domestic waste. The area is also close to a road and the vegetation is exposed to motor vehicle engine exhausts.

Phthalic acid derivative was the second major compound in both species, corresponding to 13.96% in *S. subsimplex* and 7.09% in *L. martianum*. This constituent has a repellent effect on insects and acaricide activity, but it can be a contaminant when inappropriately used as a plasticizer, as it is a typical precursor of phthalates, which are widely used in the manufacturing of plastic products to increase their flexibility (Liang et al. 2013).

Hexadecanoic acid was representative in *S. subsimplex* (7.51%) and in *L. martianum* (5.08%). This compound has already been reported in liverworts of the genera *Conocephalum* Hill. and *Marchantia* L. (Asakawa 1995; Deora and Deora 2021) and in the moss *Tortula muralis* Hedw. (Üçüncü et al. 2010).

In this work, there was a predominance of fatty acids in the studied moss species, similarly to other chemical studies with liverworts and mosses. For Lu et al. (Lu et al. 2019), bryophytes are receiving great attention because they are excellent producers of polyunsaturated fatty acids that are important for human and animal health.

The sesquiterpene curcuphenol was recorded in the species *Leptodontium viticulosoides* (P. Beauv.) Wijk & Margad. and *Rhacocarpus purpurascens* (Brid.) Paris. (Valarezo et al. 2018) at much lower levels than those found in *S. subsimplex* (7.74%). Although with lower concentrations, other sesquiterpenes were found in the volatile concentrate of *S. subsimplex*: 2-pentylfuran and (E)-β-ionone. The sesquiterpene maaliol (0.61%), already found in liverworts and hornworts (Sonwa and König 2003; Adio and König 2005; Cuvertino-Santoni et al. 2017), was found in *L. martianum* in the present study, and thus this is the first time that this compound is being reported in mosses.

Mono- and sesquiterpenes are more abundant in liverworts and rare in mosses (Ludwiczuk and Asakawa 2019; Peters et al. 2019). Sesquiterpenes were found in the present study, suggesting that, despite morphologically different from liverworts, some mosses may be evolutionarily related to this phylum, as for example the species *Plagiomnium acutum* (Lindb.) TJ Kop. that has the sesquiterpenes *ent*-β-cedrene, α-cedrene, α-acoradiene, and the diterpene (+)-dolabella-3,7-dien-18-ol in its chemical composition (Toyota et al. 1998; Asakawa 2001).

The aldehyde *n*-nonanal was representative in *S. subsimplex* (5.41%). This constituent has registered in *Homalothecium lutescens* (Hedw.) H. Rob., *Hypnum cupressiforme* Hedw, *Pohlia nutans* (Hedw.) Lindb., and *Rhodobryum giganteum* (Schwaegr.) Par. (Li and Zhao 2009; Üçüncü et al. 2010). Other aldehydes were identified in *S. subsimplex* with low contents, namely, (2*E*)-heptenal, (2*E*)-decenal, (2*E*,4Z)-decadienal, and (2*E*,4*E*)-decadienal. The latter was also found in *L. martianum* (0.38%). Recent works have demonstrated the importance of aldehydes in the composition of cuticles in bryophyte, providing them with protection against water loss (Li and Chang 2021; Matos et al. 2021).

The detection of safrole in the volatile concentrate of *L. martianum* (0.43%) is noteworthy since it is the first record of this constituent in mosses. This compound has been reported in liverworts such as *Fossombronia swaziensis* Perold, *Pallavicinia lyellii* (Hook.) Carruth.), *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph., and *Riccia albolimbata* S.W. Arnell. (Linde et al. 2016).

- This study represents the first record of the volatile chemical composition of the Amazonian mosses *S. subsimplex* and *L. martianum* belonging to the families Sematophyllaceae and Leucobryaceae, respectively. The results indicated that these species are natural sources of aldehydes, terpenes, carboxylic acids, esters and others. The study contributes to the knowledge of the chemistry of bryophytes in the Amazon and its bioprospecting.

Declarations

Conflict of Interest

Not Applicable

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