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Analysis of volatile organic compounds from the the moss *Calymperes palisotii* Schwaegr.

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A B S T R A C T

Bryophytes are natural sources of volatile organic compounds (VOCs) with bioactive properties whese can be used for bioprospecting and biotechnological applications. This study determines the VOC composition from the moss Calymperes palisotii Schwägr. The volatile concentrate of the species was obtained by simultaneous micro hydrodistillation-extraction (SDE), and analyzed by gas chromatography coupled to mass spectrometry (GC/MS). Among 13 compounds, hexanal, 3-methyl-2-pentanone, phenylacetaldehyde, and E-nerolidol were major compounds,. This was first record of the volatile chemical composition of C. palisotii, with some substances was unprecedented in moss species.

1. Introduction

Bryophytes comprise more than 24 000 species of liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Anthocerotophyta), which is taxonomically placed between algae and pteridophytes, and distributed in several ecosystems (Asakawa and Ludwiczuk, 2018; Asakawa et al., 2020). In evolution, these plants had the ability to develop cormophytic structures - some species contained stomata, conductive tissues and cuticle but not functional while alternative life strategy of poikilohydry succeeded and due to lack of protection mechanics, these plants use ‘chemical weapons’ for defense against biotic factors (Frahm et al., 2003; Frahm, 2004). For this, bryophytes are considered a rich source of bioactive secondary metabolites, which can be easily bioprospected for developing bioproducts in the health and agriculture sectors (Santra and Banerjee, 2020).

The Calymperaceae Kindb. is a diverse group of bryoflora that comprises acrocarpic mosses distributed mainly in the tropics and subtropics, but with some species in temperate latitudes above and below the equator (Reese, 1993). Calymperaceae is composed of eight genera: *Arthrocormus* Dozy & Molk., *Exostratum* L. T. Ellis, *Mitthyridium* H. Rob., *Exodictyon* Cardot, *Leucophanes* Brid., *Calymperes* Sw. ex Weber., *Syrrhopodon* Schwägr., and *Octoblepharum* Hedw.(Shaw and Goffinet, 2000). The last four occur in the Neotropics. In particular, *Calymperes* Sw. is a pantropical genus with about 50 species, 15 of which occur in Brazil, from the north to the south and in all phytogeographic domains of the country except for the pampas (Gradstein et al., 2001; Costa and Peralta, 2015). However, literature on this taxon is meagre, in terms of phytochemistry and biological activities.

The species *Calymperes palisotii* Schwägr. posesess consistent characteristics such as oblong-ligulate leaves with broad and obtuse apices, base without shoulders, rectangular cancellinae forming broad obtuse angles, intramarginal teniolae, cells of upper lamina clearly papillose, broad, distally truncate, and frequent presence of gemmae in tufts at leaf apices (Moraes and Lisboa, 2009). The species occurs throughout Brazil, in the Amazon, Caatinga, Cerrado, and Atlantic Forest biomes on tree trunks, fences, walls, rocks, and rocky outcrops (Costa and Peralta, 2015). *C. palissotii* are found in tufts, usually intermingled with other bryophytes and provide microhabitats for hymenoptera, annelids, diplopods (Parker et al., 2007). In this perspective, the present study deals with the analysis of VOCs from *C. palisotii.*

2. Methodology

2.1. Botanical material and extraction procedure

Tufts of *C. palisotii* were collected from trunks of living trees in domestic backyards in the municipality of Marapanim, state of Pará,

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(0° 43’52” S e 47° 41’54” W) by the protocol of Yano (1984), and collected samples were kept in zip-lock plastic bags. These samples were identified by Professor Ana Cláudia Caldeira Tavares-Martins, Environmental Monitoring and Conservation Laboratory, University of the State of Pará, Brazil − LCMA/UEPA. In the laboratory, the specimens were screened and separated from other intertwined bryophyte species using a magnifying glass and tweezers. A fresh sample of all parts of *C. palisotii* (8 g) was subjected to simultaneous micro hydrodistillation-extraction (SDE) using a Nickerson & Likens extraction apparatus from Chrompack and *n*-pentane (3 mL) as solvent, coupled to a refrigeration system to maintain the temperature of the condenser between 5 and 10 °C for 2 h.

2.2. Chemical analysis

The volatile concentrate was analyzed by gas chromatography coupled to mass spectrometry (GC/MS) using a Shimadzu QP-2010 Plus system equipped with a Rtx-5MS capillary column (30 m x 0.25 mm, 0.25 mm film thickness) under the following operating conditions: carrier gas: helium, with a linear velocity of 36.5 cm/s, injection type: splitless (2 μL), injector and detector temperature: 250°C, oven temperature program: 40 and 60°C (2°C/min), 60 and 250 °C (3° C/min), MS: electron impact, 70 eV, temperature of ion source and connecting parts: 220°C. The individual identification of compounds was performed by comparing the mass spectra and retention indices (RI) with those of substances in the libraries of the system (NIST) and literature data (Adams, 2007). The RI were obtained using the homologous series of *n*-alkanes (C8-C40) (Sigma-Aldrich, Milwaukee, WI, USA). The components were quantified by means of GC in a Shimadzu QP-2010 Plus instrument equipped with a flame ionization detector (FID) under the same operating conditions above mentioned except for the carrier gas, which was hydrogen.

3. Results

Nineteen compounds were detected in the volatile concentrate of *C. palisotii*, and 13 (89.76%) were identified. The major compounds identified were hexanal (22.43%), 3-methyl-2-pentanone (20.53%), E-nerolidol (15.71%), and phenylacetaldehyde (15.01%). The other compounds had concentrations below 4.74% (undecanal) or were not recognized (six substances, corresponding to 10.24% of the volatile concentrate) based on spectral data from the libraries and the literature used (Table 1, Fig. 1). The classes of compounds present in the aroma of *C. palisotii* were aldehydes (31.6%), sesquiterpenes and hydrocarbons (10.5% each), ketone, phenylpropanoid and furan (5.3% each), and unidentified compounds (31.6% in total).

Table 1
Volatile organic compounds (%) detected in the pentane extract of *Calymperes palisotii* Schwägr.

Constituents	RI_C	RI_L	%
3-methyl-2-pentanone	*745*	*745*	*20.53*
Hexanal	*794*	*801*	*22.43*
Furfural	820	828	1.54
2-Pentylfuran	988	984	1.37
NI	998	-	0.35
Phenylacetaldehyde	*1038*	*1036*	*15.01*
Nonanal	1102	l100	3.03
Naphthalene	1175	1178	0.33
Safrole	1284	1285	0.72
Undecanal	1305	1305	4.74
(2E,4E)-Decadienal	1314	1315	0.62
NI	1334	-	0.34
NI	1390	-	0.14
NI	1404	-	0.27
NI	1484	-	0.35
β-Bazzanene	1518	1519	1.17
E-nerolidol	*1563*	*1561*	*15.71*
n-Heptadecane	1699	1700	2.56
(NI)-MM251	2258	-	8.79
Total	-	-	100.0

RI_C: Calculated retention index. RI_L- Retention index from literature.
Main constituents in italics.

4. Discussion

In the current study, hexanal (aldehyde) was the most abundant (22.43%) in the VOCs of *C. palisotii*, which is also predominant in VOCs of moss *Rhodobryum giganteum* (Hook.) Par. with concentrations of 8.55 and 19.67% (Li and Zhao, 2009). This constituent has been identified at various concentrations in other moss species from different families, such as *Fontinalis antipyretica* Hedw., *Brachymenium capitulatum* (Mitt.) Paris, *Hydrogonium consanguineum* (Thwaites & Mitt.) Hilp., *Barbula hastata* Mitt., *Octoblepharum albidum* Hedw., and *Neckeropsis undulata* (Hedw.) Reichardt.

Hexanal belongs to the oxylipin family of smaller oxygen compounds, generated from long-chain polyunsaturated fatty acids, which play a key role in the chemical defense of mosses (Mitra et al., 2017; Miranda et al., 2021). The hexanal found in *C. palisotii* may have been generated from arachidonic acid or linolenic acid by the action of the enzyme lipoxygenase (LOX), as previously reported in *Marchantia polymorpha* L. and *N. undulata*, as short-chain aldehydes are generated from these two acids (Boonprab et al., 2019; Miranda et al., 2021; Tawfik et al., 2017).

3-methyl-2-pentanone is the concentration second major VOC of *C. palisotii* (20.53%) and has already been reported in angiosperms, such as hazelnut (*Corylus avellana* L. var. Katalonski) (Marzocchi et al., 2017), but no records existed in bryophytes until now. This chemical constituent has been reported in endophytic bacteria which releases antifungal VOCs and exhibits favorable effects on plant growth of *Physalis ixocarpa* Brot. ex Hornem., corroborating the importance of the microbiota for plants (Rojas-Solís et al., 2018). Mosses living in tufts and in humid environments are likely to host microbes and for this reason, they can produce secondary metabolites with antimicrobial action (Nugraha et al., 2019).

E-nerolidol found in the VOCs of *C. palisotii* (15.71%) had already been reported in some species of anthoceros and liverworts (Asakawa et al., 2013; Jia et al., 2016; Salazar-Allen et al., 2017). There is no report in the literature of E-nerolidol in mosses, and thus this is the first record. In the last 40 years, there has been greater phytochemical prospection of liverworts due to the presence of oil bodies, resulting in a deeper knowledge of the chemical composition of this phylum (Asakawa et al., 2013; Ludwiczuk and Asakawa, 2020; Commisso et al., 2021). Another important aspect is that, phylogenetically, mosses are placed between liverworts and hornworts (Qiu et al., 2006) and, in these two phyla, E-nerolidol was found as a secondary metabolite, which does not rule out the possibility of this compound occurring in *C. palisotii*.

Phenylacetaldehyde found in the VOCs (15.01%) of *C. palisotii*) is also in VOCs of the livertwort *Marsupella emarginata* (Ehrh.) Dumort. and the mosses *Homalia trichomanoides* (Hedw.) Brid. and *Mnium stellare* Hedw. (Saritas et al., 2001; Adio et al., 2002). This constituent can be considered a phenolic precursor and, as enzymes involved in the secondary metabolism of plants, phenylacetaldehyde reductase produces the volatile compound 2-phenylethanol (Moummou et al., 2012; Yonekura-Sakakibara et al., 2019).

The aldehydes undecanal (4.74%) and nonanal (3.03%) identified in VOCs of *C. palisotii* had already been widely reported among moss species of different families (Tosun et al., 2014, 2015; Valarezo et al., 2018; Carranza et al., 2019; Yucel, 2021, 2020). Many fragrances in nature belong to the group of aldehydes, which have different properties, acting for example as animal attractant (Bojke et al., 2020).

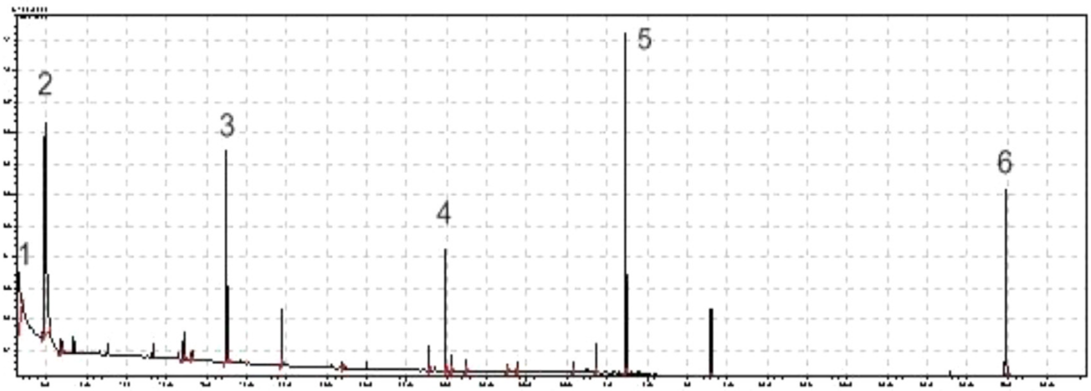

Fig. 1. Ion chromatogram of the volatile concentrate of *Calymperes palisotii* Schwägr. Major Compounds: 1. 3-Methyl-2-pentanone, 2. Hexanal, 3. Phenylacetaldehyde, 4. Undecanal, 5. E-nerolidol, 6. Unidentified.

Aggregation chemicals, including aldehydes, can be used as attractive semiochemical lures in traps for the management and control of agricultural pests (Weeks et al., 2020).

The hydrocarbon n-heptadecane found in VOCs (2.56%) of *C. palisotii* as well as other n-alkanes, are widely found in mosses (Cuvertino-Santoni et al., 2017; Salazar-Allen et al., 2017). Fractions of the extract of the angiosperm *Chenopodium ambrosioides* L., which mainly contained long-chain hydrocarbons and fatty acid derivatives (heptadecane, ethyl hexadecanoate and arachidonic acid), were active against stored grain pests (Peterson et al., 1989), suggesting a synergistic action.

Furfural found in VOCs (1.54%) of *C. palisotii* had not been recorded in bryophytes until now. In turn, 2-pentylfuran found in VOCs (1.6%) of *C. palisotii*, had already been reported for the moss *R. giganteum* and *N. undulata* (Li and Zhao, 2009; Miranda et al., 2021). In angiosperms, 2-pentylfuran was derived from lipids and a product of the degradation of fatty acids in transformed roots of *Codonopsis pilosula* (Franch.) Nannf. (Makowczyńska et al., 2021).

β-Bazzanene identified in VOCs (1.17%) of *C. palisotii* has been widely recorded in liverwort species, but less frequently in mosses and hornworts (Cuvertino-Santoni et al., 2017; Mitra et al., 2017; Pannequin et al., 2017; Asakawa et al., 2018). In turn, the aldehyde (2E,4E)-decadienal, which made up 62% of VOCs of *C. palisotii*, have the biosynthesis, as well as of other short-chain aldehydes, through the conversion of arachidonic acid and linoleic acid (Boonprab et al., 2019), whose oxylipin (2E,4E-decadienal) may participate somehow in the wound-induced chemical defense mechanism of mosses, as proposed for hexanal.

The phenylpropanoid safrole (0.72%) and the aromatic hydrocarbon naphthalene (0.33%) had the lowest concentrations in VOCs of *C. palisotii*. Safrole had already been reported in African liverworts and the naphthalene was the major compound in the Amazonian mosses *Sematophyllum subsimplex* (Hedw.) Mitt., *L. martianum* and *N. undulata* (Linde et al., 2016; Miranda et al., 2021; Moraes et al., unpublished data).

Eight VOCs of *C. palisotii* were not identified, and (NI)-MM251 was one of the most abundant (8.79%). In the phytochemical and pharmacological context, non-vascular plants are the least studied in the entire plant kingdom (Vollár et al., 2018), making the discovery of many compounds unfeasible due to the limited bank of mass spectra of substances. Many authors have stated that this group of plants produces some unique phytochemicals, with unprecedented structures, or phytochemicals produced by specific species, such as (bis)bibenzyls which are produced solely by liverworts, and auronidins, a unique class of phenylpropanoids recently discovered in the liverwort *M. polymorpha* (Asakawa et al., 2013; Sabovljević et al., 2016; Greeshma et al., 2017; Berland et al., 2019).

5. Conclusion

In this first record of the volatile chemical composition of *C. palisotii*, the presence of several groups of compounds (aldehydes, terpenes, hydrocarbons, phenylpropanoids, furans, and ketones) was observed and the finding of the substances 3-methyl-2-pentanone, E-nerolidol and furfural was unprecedented in moss species, although they occur in other bryophyte phyla and/or groups of plants. The supposition of the coexistence of endophytic microorganisms, which also act as producers of these bioactive compounds, is not ruled out. Studies already conducted with these metabolites from oils, extracts or isolates have shown that they have promising properties for bioprospecting and biotechnology.

Techniques for the cultivation of bryophyte cells and tissues with the objective of *in vitro* production of compounds - and on a large scale through bioreactors - can be used to induce or increase the production of metabolites of the moss *C. palisotii*. Also, chemical studies are necessary for the isolation and characterization of substances not identified in this investigation, as many bryophyte compounds are rare, unique, and still unknown in this taxon. *In vitro* and *in vivo* research on the biological activities of these metabolites must be developed to contribute to the production processes of phytopharmaceuticals and agricultural drugs and to add economic value to non-vascular plants.

Declaration of Competing Interest

There are no conflicts of interest.

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