

Characterization of the volatile compounds and anatomical features in commercial samples of *Echinodorus* plant species

Caracterização dos compostos voláteis e características anatômicas em amostras comerciais de espécies de plantas *Echinodorus*

Caracterización de compuestos volátiles y características anatómicas en muestras comerciales de especies de plantas *Echinodorus*

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Hebert Vinícius Pereira

ORCID: <https://orcid.org/0000-0002-7893-7333>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: hebert.pereira@yahoo.com.br

Fernando Augusto Gouvêa Reis

ORCID: <https://orcid.org/0000-0002-32740-0850>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: fernandogouveareis@hotmail.com

Ana Maria de Resende Machado*

ORCID: <https://orcid.org/0000-0002-1587-5024>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: anamariaderesendemachado@gmail.com

Andréa Rodrigues Marques

ORCID: <https://orcid.org/0000-0001-5658-3012>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: andreamg@gmail.com

Cleverson Fernando Garcia

ORCID: <https://orcid.org/0000-0001-9354-1401>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: cf.garcia@terra.com.br

Fátima de Cássia Oliveira Gomes

ORCID: <https://orcid.org/0000-0001-7358-7154>

Centro Federal de Educação Tecnológica de Minas Gerais, Brazil

E-mail: fatimaog@cefetmg.br

David Lee Nelson

ORCID: <https://orcid.org/0000-0001-7435-3675>

Universidade Federal dos Vales de Jequitinhonha e Mucuri, Brazil

E-mail: dleenelson@gmail.com

Abstract

Echinodorus grandiflorus and *Echinodorus macrophyllus* are medicinal plants that are widely used in Brazilian folk medicine. The aims of this study were to evaluate the effect of oven drying with ventilation on the chromatographic profile of volatile compounds and to compare the leaf anatomy and volatile compounds of different commercial *E. macrophyllus* (Kunth) Micheli and *E. grandiflorus* Mich. Samples. The components found in fresh and dried samples were extracted by SPME and analysed by GC-MS, and the anatomical features of the leaves were observed microscopically. A total of 46 compounds were identified; five compounds were present in the dried and fresh samples of *E. grandiflorus* and all of the commercial samples. The anatomy analyses confirmed the authenticity of the species. Apparent differences in the volatile composition between the species were observed, allowing the identification of chemical marker. The results showed that commercial establishments do not conform to regulations, and the techniques used were effective for the characterization of volatile compounds and for quality control of medicinal plants.

Keywords: Anatomical features; *Echinodorus*; GC-MS; SPME; Volatile compounds.

Resumo

Echinodorus grandiflorus e *Echinodorus macrophyllus* são plantas medicinais amplamente utilizadas na medicina popular brasileira. Os objetivos deste estudo foram avaliar o efeito da secagem em estufa com ventilação no perfil cromatográfico de compostos voláteis e comparar a anatomia foliar e os compostos voláteis de diferentes amostras comerciais de *E. macrophyllus* (Kunth) Micheli e *E. grandiflorus* Mich. Os componentes encontrados nas amostras frescas e secas foram extraídos por SPME e analisados por CG-EM, sendo as características anatômicas das folhas observadas microscopicamente. Um total de 46 compostos foram identificados; cinco compostos estavam presentes nas amostras secas e frescas de *E. grandiflorus* e em todas as amostras comerciais. As análises de anatomia confirmaram a autenticidade da espécie. Diferenças aparentes na composição de voláteis entre as espécies foram observadas, permitindo a identificação de marcador químico. Os resultados

mostraram que os estabelecimentos comerciais não estão em conformidade com a regulamentação e as técnicas utilizadas foram eficazes para a caracterização de compostos voláteis e para o controle de qualidade de plantas medicinais.

Palavras-chave: Características anatômicas; *Echinodorus*; CG-EM; SPME; Compostos voláteis.

Resumen

Echinodorus grandiflorus e *Echinodorus macrophyllus* son plantas medicinales muy utilizadas en la medicina popular brasileña. Los objetivos de este estudio fueron evaluar el efecto del secado en horno con ventilación sobre el perfil cromatográfico de compuestos volátiles y comparar la anatomía foliar y compuestos volátiles de diferentes muestras comerciales de *E. macrophyllus* (Kunth) Micheli y *E. grandiflorus* Mich. Los componentes encontrados en las muestras frescas y secas fueron extraídos por SPME y analizados por CG-EM, observándose microscópicamente las características anatómicas de las hojas. Se identificaron un total de 46 compuestos; cinco compuestos estuvieron presentes en las muestras secas y frescas de *E. grandiflorus* y en todas las muestras comerciales. Los análisis de anatomía confirmaron la autenticidad de la especie. Se observaron diferencias aparentes en la composición de volátiles entre especies, lo que permitió la identificación de un marcador químico. Los resultados mostraron que los establecimientos comerciales no cumplen con la normativa y las técnicas utilizadas fueron efectivas para la caracterización de compuestos volátiles y para el control de calidad de plantas medicinales.

Palabras clave: Características anatómicas; *Echinodorus*; CG-EM; SPME; Compuestos volátiles.

1. Introduction

There has been a widespread increase in the use of medicinal plants as a result of their potentially beneficial effects on human health (Vida et al., 2010). However, there is little information available on the toxic effects of most of the active principles found in these medicinal plants. In the past, the harvesting of medicinal herbs was primarily performed by traditional healers. However, in recent years, medicinal herbs remain the most important health care source for the vast majority of the world's population. According to the World Health Organization (WHO), it is estimated that 70-95% of the world's population depends on traditional herbal medicine to meet their primary health care needs (Carmona & Pereira,

2013). There are more than 1000 herbal medicines in Brazil were evaluated for their medicinal properties (ANVISA, 2014).

Two species widely used in Brazilian folk medicine are *Echinodorus grandiflorus* and *Echinodorus macrophyllus*, which are known as “chapéu de couro” and have similar botanical characteristics. These plants belong to the monocotyledonous Alismataceae family, which consists of 13 aquatic and semi-aquatic genera, and 75 species comprising 11 genera are found in tropical, subtropical, and subtemperate regions in the Eastern and Western Hemispheres (Crow, 2013). Their leaves, in the form of a decoction, infusion, or bottled solution, are used for the treatment of various diseases due to pharmacological activities described in the literature indicate its effectiveness as anti-inflammatory, antioxidant, antiedematogenic, antiproliferative, diuretic, analgesic, anti-rheumatic, antihypertensive and with cardioprotective effects (Pimenta et al., 2006; Garcia et al., 2010; Prando et al., 2015; Coelho et al., 2017; Marques et al., 2017; Gasparotto et al., 2018, 2019; Gomes et al., 2020).

The chemical profile of the extracts and the essential oil of the leaves of the *Echinodorus* species is basically represented by the presence of tannins, alkaloids, flavonoids, anthraquinones, steroids, triterpenes, saponins, polysaccharides, and coumarins (Pimenta; Figueiredo & Kaplan, 2006; Lima-Dellamora et al., 2014; Garcia et al., 2016; Bonetti et al., 2020).

The quality control and standardization of herbal medicines involve several steps (Brasil, 2016; Calixto, 2000). The use of fresh plants, temperature, light exposure, water availability, nutrients, time and method of collection, drying, packaging, storage and transportation of raw material, age of the plant and the part of the plant collected can affect the quality and, consequently, the therapeutic value of the resulting herbal medicines. Some constituents of the plants are heat-labile and need to be dried at low temperatures. Additionally, other active principles are destroyed by enzymatic processes that continue for long periods of time after plant collection.

According to Souza-Moreira et al. (2010), several Brazilian herb-traders who seek to increase their profits associated with this product do not care about the data collection (locality of the planting and time of year of harvesting) and treatment of the collected material (e.g., drying). In addition, analyses of marketed herbs have shown that it is common to find foreign material contaminants from other plants, exchanges of a species by another species, and the presence of microorganisms. A likely consequence is a lack of confidence in these types of products, which may or may not contain the active compounds in the samples that are sold (Souza-Moreira et al., 2010).

The drying method used has an effect on the compositions of the volatile components, in the amount of water present, and in the structure and integrity of the commercialized plants. There are various methods used for drying, and each method has advantages and disadvantages. The hot-air drying method may cause alterations in the physical and chemical characteristics; freeze-drying can minimize the damage caused by heat drying, but it is very expensive; microwave drying can produce losses in volatile compounds, but it can preserve the colour; and vacuum-microwave is very rapid, but the absence of air might inhibit oxidation and allow a better preservation of the sensory proprieties (Pu; Hui & Raghavan, 2016).

The choice of the drying method must take into account the lower energy expenditure, the maintenance of the integrity of the chemical compounds of the plant and the drying time.

The chemical composition of herbal medicines is complex, and the active components found in small amounts are difficult to identify. Numerous methods have been proposed for the description of the chemical compositions of volatile compounds in plants and to correlate study results with known descriptors of plant activity (Tigrine-Kordjani et al., 2007). For the analysis of these volatile compounds, solid-phase microextraction (SPME) is a simple and rapid modern tool that offers a valid alternative to hydrodistillation, but it has a high cost and excessive preparation time. In SPME, the analytes are adsorbed from a solid sample by headspace extraction using a fibre immersed in the vial, and the extracted sample is immediately analysed, thus providing a “green chemistry” sampling method that requires no solvent (Gama et al., 2019). SPME has been widely used for the rapid extraction of volatile compounds from aromatic and medicinal plants and the extracted compounds are identified by gas chromatography coupled with mass spectrometry (Cui et al., 2020).

We propose that SPME together with GC-MS could be an efficient analytical method for the characterization of volatile compounds, and it ensures the quality control of the *Echinodorus* species. The aims of this study were to evaluate the effect of oven drying with ventilation on the chromatographic profile of volatile compounds and to compare the leaf anatomy and volatile compounds of different commercial *E. macrophyllus* (Kunth) Micheli and *E. grandiflorus* Mich. samples. These evaluations might facilitate the identification of these medicinal herbs, the analysis of the information contained in labels and the determination of the presence of foreign materials, moisture content, and total ash to assess whether such items are concordant with the regulations.

2. Materials and Methods

2.1 Methodology

Two *Echinodorus* species were studied, relating the anatomical nature of the leaves and the chemical composition of volatile compounds for the real knowledge of the species sold.

2.2. Plant material

Leaves of *E. grandiflorus* were collected from the surroundings of Itatiaiuçu, Minas Gerais, Brazil (20°12'S and 44°23'W, 886 asl). Leaves of *E. macrophyllus* were collected from the banks of a lake in the Natural History Museum and Botanical Garden (UFMG), Belo Horizonte, Minas Gerais, Brazil (19° 89'S and 43° 91'W, 705 asl. Dried specimens of *E. grandiflorus* and *E. macrophyllus* were deposited in the herbarium of the Instituto de Ciências Biológicas of the UFMG (BHCB) with the numbers BHCB166577 and BHCB166578, respectively. Just after harvesting, fully developed leaves of these species were separated from the petioles, washed under running water and then with distilled water. Samples of these plants *in natura* were immediately employed in the analyses. The remaining portion of each sample was dehydrated in a forced-air drying oven at 35°C.

2.3. Analysis of commercial plants samples

Commercial herbs of *Echinodorus* were randomly sampled from 10 herb-traders in commercial establishments in Belo Horizonte, MG, Brazil. Three packing units were bought at each commercial market and inspected in terms of their packaging and labelling, the presence of foreign material, and the determination of the moisture content and total ash (ANVISA, 2010).

2.4. Leaf anatomy analysis

Leaf samples from the plants *in natura* were fixed in 70°GL ethanol (Jensen, 1962). Dry leaves of the commercial samples selected from the packaging unit of each commercial market were rehydrated in water that was warmed with 20% glycerin for 60 min. Sections of

the leaf blade (*in natura* and rehydrated leaves) were bleached with 20% sodium hypochlorite solution until depigmentation of the leaves was observed and then washed with water. The sections were stained with astrablau-safranin and then assembled into semi-permanent slides (Jensen, 1962). The histological observations were performed using an Olympus BX53 microscope and micrographs were obtained. The anatomical features of the leaves were analysed as described by Matias (2007) and Leite et al. (2007).

2.5. Chromatographic analysis

Samples from fresh and dried leaves were cut for the SPME analysis, and 0.5 g of the samples was placed in a glass vial containing 10 mL of the 17% NaCl solution. This solution was hermetically closed in a 20-mL vial with a Teflon septum and an aluminium cap. The SPME device was then inserted into the sealed vial by manually penetrating the septum, and the fiber was exposed to the headspace of the plant material during 20 min at 70 °C (Pimenta; Figueiredo & Kaplan, 2006). After extraction, the needle on the SPME manual holder was inserted into the GC injector, and the fiber was directly exposed to the hot injector at 250 °C in splitless mode. A 100 µm polydimethylsiloxane (PDMS) fiber with a manual holder (Supelco, Bellefonte, PA, USA) was used.

The plant samples were analysed on a gas chromatograph (Agilent Technologies 7890A GC System) coupled with a mass spectrometer (Agilent 5975C inert MSD Triple-Axis Detector and quadrupole analyzer) using solid-phase microextraction to extract volatile.

A series of alkanes (C8–C20) were analysed using the same method to identify the compounds through comparison with the literature (NIST 2005 library). The column employed was a HP5-MS column (length of 30 m I.D. of 0.25, and film thickness of 0.25 µm). The oven temperature program commenced at 70 °C, and increased to 230 °C at a rate of 3 °C/min, with a helium flow rate of 1.3 mL/min. The characterization of tentatively identified compounds was achieved by comparing the Kovats Indices and the mass spectra generated in GC-MS.

2.6. Multivariate analysis

The species identification was performed through principal component analysis (PCA) and hierarchical cluster analysis (HCA), and the data was processed using the Minitab 17 software. We used a data matrix with 12 rows (10 commercial samples and two reference

samples (*E. macrophyllus* and *E. grandiflorus*) and 14 columns (area of 14 identified principal volatile compounds). PCA was performed using a covariance matrix, and the scores of the principal components (cumulative eigenvalue greater than 80 %) were used for the construction of the scatter plot to assess the possible groupings of the commercial samples with respect to the reference species. The HCA analysis was conducted using the Euclidean distance measure, unstandardized data, and the method of median linkage, and the respective dendrograms were evaluated for possible groupings of the commercial samples with respect to the reference species.

3. Results and Discussion

3.1. Evaluation of commercial herbs

The analysis showed that the labels on the packages in which herbs of *Echinodorus* species were sold did not contain information on their scientific name (100%), contraindications (100%), instructions (100%) and indications (87%) for use, and the parts of the vegetal materials that were used (100%). However, 90% of the labels on the packages evaluated exhibited an expiration date. This analysis showed that herb-traders do not make an effort to normalize the data of the plants and their therapeutic action. Moreover, it was observed that commercial establishments did not comply with the Brazilian Pharmacopoeia (ANVISA, 2010), which delineates the processing and storage conditions and the labelling and quality of the plant.

The conditions of the material within the packaging were evaluated, and some irregularities were observed (Table 1). Among the packages analysed, all of the samples had masses exceeding that indicated (20 g) on the label. As specified by the pharmacopoeia monograph, the percentage of foreign materials should not exceed 2% (m/m) (ANVISA, 2010). However, it was noted that the amount of foreign matter present in the samples ranged from 0.61% to 16.59%, thereby exceeding the allowed limit, and the foreign matter contained foreign leaves, stems, and seeds of other plants, insect wings, whole insects, spider webs, arthropod nests and eggs, and hair strands (Table 1). In addition, 50% of the samples exhibited a percentage of foreign materials greater than the maximum established limits.

Table 1. Analyses of the weight package, presence and identity of foreign material, moisture content, and total ashes of *Echinodorus* samples marketed in Belo Horizonte-MG.

S	Measured Weight (g)	% of foreign material	Description of foreign material	Moisture content (%)	Total ashes (%)
1	22.57 ± 1.19	1.0 ± 0.05	Ant, soil, seeds	11.33 ± 1.15	10.48 ± 0.22
2	22.63 ± 2.02	2.06 ± 0.22	Seeds, stems, dust	11.01 ± 0.80	10.67 ± 1.5
3	27.71 ± 2.73	4.07 ± 0.77	Insect, strands of hair, spider webs	10.32 ± 0.35	10.21 ± 1.75
4	30.67 ± 2.77	0.61 ± 0.01	Seeds, plastic traces, dust	10.88 ± 0.40	10.25 ± 0.03
5	26.39 ± 3.87	4.09 ± 0.03	Leaves, stems, eggs of insects, dust	10.94±0.01	11.68 ± 2.13
6	36.42 ± 3.09	2.61 ± 0.02	Leaves, dust	11.31±0.25	10.36 ± 1.86
7	21.56 ± 6.24	0.69 ± 0.15	Mould, spider webs, insect wings, hair, soil	11.63±0.33	12.72 ± 1.66
8	28.04 ± 0.44	–	Dust	10.82±0.02	11.18 ± 1.44
9	42.57 ± 4.24	–	–	11.73±0.15	10.42 ± 2.10
10	31.54 ± 4.12	16.59 ± 1.65	Seeds, stems, nests and eggs of insects, spider webs, excreta	11.48±0.60	11.50 ± 1.55

S = samples
 Source: Authors.

The moisture in the samples ranged from 10.32% to 11.73% (Table 1). The maximum moisture content established for this species is 9%; all of the samples exceeded the established limit. Excessive moisture in the samples can be damaging to their quality because it favours enzymatic activity and proliferation of microorganisms that can decompose the active principles of the plant and produce substances that can cause intoxication if ingested (ANVISA, 2010).

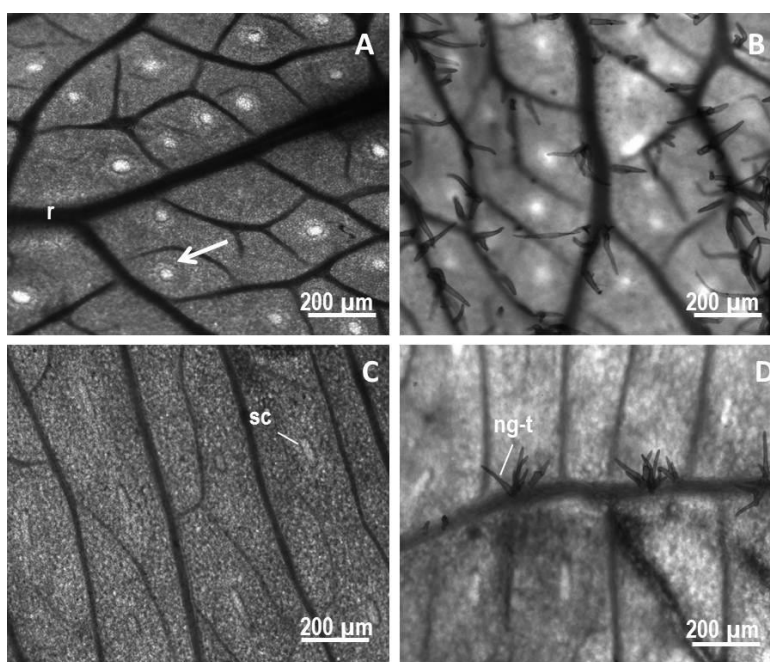
The analysis of the total ash determines the amount of residual non-volatile substances present in the sample after the organic substances are removed through the incineration process. As is shown in Table 1, the total ash contents of the samples varied between 10.21 and 12.72%. The Brazilian Pharmacopoeia admits a maximum value of 11% for the ash content (ANVISA, 2010). The amount of total ash indicates that care must be taken in

preparing the product to ensure that it is not mixed with foreign materials and result in adulteration of the final product (Araújo et al., 2006).

3.2. Anatomical study

Micromorphological examinations revealed that the fresh leaves from *Echinodorus* possess abaxial surface epidermal cells with thin sinuous walls and non-glandular trichomes located in the region of the ribs on both surfaces (Figures 1B and 1D) that are sometimes branched (Figures 2A and 2B). Secretory ducts (laticiferous) were observed microscopically in the surface of leaves (Figures 1A and 1C).

Figure 1. Frontal view of the epidermis in the abaxial surface of *E. grandiflorus* (A and B) and *E. macrophyllus* (C and D).

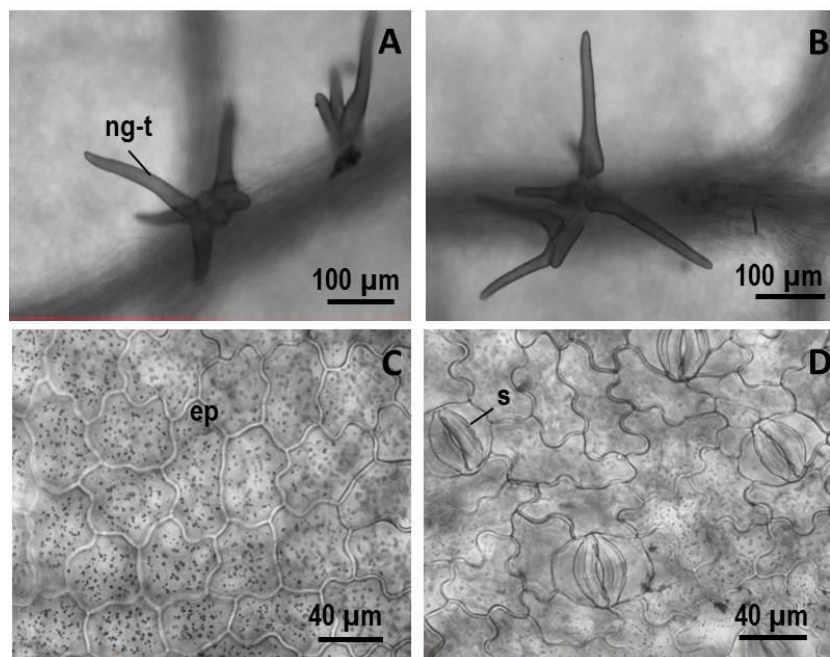


Legend: non-glandular trichomes (ng-t), ribs (r), and secretory ducts (sc). Bars = 200 μ m.
detailed view of translucent secretory ducts (arrow).
Source: Authors.

Haynes and Holm-Nielsen, (1994) considered *E. grandiflorus* to be a single species with two subspecies (ssp. *grandiflorus* and ssp. *aureus*), but Lehtonen (2008) split the species into three taxa, *E. grandiflorus* ssp. *aureus*, *E. grandiflorus* ssp. *grandiflorus* a (*E. Argentinensis* Rataj *sensu* Rataj, 1970), and *E. grandiflorus* ssp. *grandiflorus* b (*E. Longiscapus* Arechavaleta *sensu* Arechavaleta, 1903), which possess pellucid markings as

dots in their leaves (Fig. 1A). Matias (2007) described the secretory ducts of *E. macrophyllus* ssp. *scaber* (Rataj) R. R. Haynes & Holm-Niels as non-translucent, whereas the secretory ducts of *E. grandiflorus* ssp. *aureus* (Fassett) R. R. Haynes & Holm-Nielsen were translucent, conspicuous, and detected as pellucid markings in the form of dots on the leaves [Haynes & Holm-Nielsen, 1994]. These anatomical characteristics confirm the morphological identification of the species. The leaf blade of *E. macrophyllus* is hypostomatic (Figure 2C), as described by Leite et al. (2007), whereas that of *E. grandiflorus* is amphistomatic; both species present paracytic stomata in which the guard cells are accompanied by a pair of subsidiary cells (Figure 2D).

Figure 2. Surface view of the epidermis in the abaxial side showing non-glandular trichomes (ng-t) (A and B) and paracytic stomata (s) (D); surface view of the epidermis in the adaxial side showing the epidermal cell wall (ep) (C).



Source: Authors.

The effect of dehydration and rehydration on the leaf structures was observed under an optical microscope. After a fresh sample of *Echinodorus* was dried, the leaves exhibited significant changes in their botanical structures, such as a severe shrinkage of the cuticle, its associated structures, and the underlying epidermal layers. Internally, the secretory ducts were extremely affected, and many of them disappeared. The non-glandular trichomes were

observed with difficulty. All of these characteristics were observed after rehydration of the commercial leaf samples. The anatomical criteria based on the leaf structures that were used for the identification of the commercial species were the presence of stomata in both surfaces of *E. grandiflorus* leaves and only in the abaxial surface of *E. macrophyllus* leaves because these structures were easily visualized after rehydration, even if damaged. The anatomical results show that all of the commercial species were *E. grandiflorus*.

3.3. Chromatographic analysis

The results of the SPME and GC-MS analysis indicated that the forty-six organic compounds found are distributed into seven classes: terpenes (thirty-one), carotenoide derivatives (seven), aldehydes (three), esters (one), phenylpropanoids (two), alcohol (one), and coumarin (one) (Table 2). The terpenes were the principal compounds found in the samples. Terpenes are a large class of plant substances, and the most common of these compounds are monoterpenes and sesquiterpenes (Simões et al., 2010).

A great compatibility between the reference samples of *E. macrophyllus* before and after drying was observed. A total of 11 compounds were tentatively identified in fresh samples of *E. macrophyllus*, whereas 13 compounds were tentatively identified in the same samples after drying; 9 of the detected samples were identified both before and after drying. The principal compounds found in the dry and fresh samples of *E. macrophyllus* were β -caryophyllene, α -curcumene and β -bisabolene.

The principal compounds found in the dry sample of *E. grandiflorus* were geranyl acetone and β -ionone. Bicas et al. (2011) studied the composition of volatile components of Brazilian exotic fruits and found β -ionone using the same chromatographic technique and SPME extraction employed in this work. This compound was found in all the commercial samples, and it was identified at high percentages in samples 3 and 6 and the reference sample of *E. grandiflorus* after the drying process. Bastos et al. (2006) identified geranyl acetone as a major compound in the essential oil from mate tea. This compound was found at high percentages in all the dried *E. grandiflorus* samples. The analyses of the reference samples of *E. grandiflorus* before and after drying also revealed a large compatibility between the profiles (Table 2).

Table 2. Tentatively identified and quantified compounds in *Echinodorus* samples through SPME extraction, GC-MS

Compounds	KRI*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(E)-6-methyl-3,5-heptadien-2-one ^t	1080 (1074)	-**	-	4.8	2.1	-	-	1.6	2.9	3.3	1.8	3.0	-	-	-
2-ethylidene-6-methyl hepta-3,5-dienal ^t	1187 (1182)	-	1.4	-	-	-	-	1.1	-	1.1	-	-	-	-	-
decanal ^a	1189 (1183)	-	2.5	3.0	-	1.8	2.4	1.5	2.4	2.2	2.5	2.0	-	7.2	-
β -cyclocitral ^t	1203 (1196)	-	-	-	-	-	-	-	-	-	-	3.2	2.9	-	-
4-propenylanisole ^p	1264 (1269)	3.2	-	2.2	-	1.8	-	-	-	-	-	-	-	-	-
dihydroedulan ^{dc}	1284 (1290)	-	-	-	-	-	2.9	3.5	-	-	-	3.9	-	4.7	-
megastigma-4,6,8-triene ^{dc}	1309 (1317)	-	1.2	-	-	-	-	0.8	1.5	2.4	-	-	3.2	-	5.7
p-mentha-[1(7),8]-diene-2-hydro peroxide ^t	1340 (1338)	-	0.8	1.8	1.1	0.7	-	1.9	1.9	1.2	2.3	-	-	-	-
isopulegol acetate ^t	1342 (1335)	-	-	-	-	-	-	-	-	-	-	0.9	-	-	-
β -cubebene ^t	1383 (1384)	1.9	-	-	-	1.6	-	-	-	-	-	1.5	1.0	-	-
ethyl decanoate ^e	1387 (1382)	1.5	1.6	1.5	1.4	0.6	-	1.6	9.4	2.3	0.9	1.5	-	4.7	-
3,7-dimethyloct-2-enyl-2-methyl- propanoate ^t	1398 (1417)	0.7	1.2	-	0.8	0.9	3.2	1.2	1.6	0.9	1.4	1.5	-	-	-
6,10-dimethylundecan -2-one ^t	1397 (1391)	1.5	-	-	-	-	1.8	1.6	3.9	-	2.8	-	-	-	-
2,8,8-trimethyl-4-methylene-2-vinyl-bicyclo[5.2.0]nonane ^t	1406 (1407)	-	-	-	-	-	-	-	-	-	1.2	-	2.7	-	4.2
dehydrodihydroionone ^{dc}	1415 (1424)	-	2.7	-	1.9	-	-	1.5	2.8	2.2	-	-	-	-	-
β -caryophyllene ^t	1419 (1424)	2.6	-	-	1.2	7.9	-	1.5	1.3	-	0.9	-	37.7	7.7	31.1

2H-1-benzopyran-2-one ^e	1420 (1414)	-	11.2	-	3.8	0.7	5.3	6.5	4.8	12.4	4.0	-	-	-	-
trans- α -bergamotene ^t	1433 (1433)	-	-	-	-	-	-	-	-	-	-	-	3.2	-	2.8
geranylacetone ^t	1446 (1453)	11.8	13.2	28.1	27.3	3.5	11.2	18.1	19.6	12.5	25.3	19.4	-	1.8	-
α -caryophyllene ^t	1448 (1456)	3.0	-	-	-	4.8	-	-	-	-	-	-	-	-	-
β -farnesene ^t	1449 (1449)	-	-	-	-	-	-	-	-	-	-	0.9	2.1	-	-
1-dodecanol ^{alc}	1457 (1457)	2.8	11.7	16.0	23.3	11.8	11.4	4.6	5.4	9.6	6.9	5.4	-	20.3	-
dehydro- β -ionone ^{dc}	1466 (1460)	-	3.2	-	2.8	0.5	3.4	3.9	4.1	3.0	2.9	-	-	-	-
β -ionone ^{dc}	1479 (1486)	4.5	6.8	16.8	5.4	6.9	12.1	6.7	4.7	5.0	6.8	21.0	-	7.3	-
α -curcumene ^t	1480 (1472)	3.6	1.8	7.0	2.0	3.5	7.5	0.9	3.7	3.0	0.8	5.7	24.6	2.6	10.1
α -muurolene ^t	1487 (1479)	-	-	-	-	2.8	-	-	-	-	-	-	2.1	-	5.2
2-hydroxy-1,1,10-trimethyl-6,9-epi-dioxydecalin ^{dc}	1491 (1507)	0.6	2.4	-	0.8	0.6	1.0	1.6	1.3	1.5	1.9	1.2	-	2.3	-
α -farnesene ^t	1501 (1499)	-	-	-	-	-	-	-	-	-	-	6.0	-	20.4	3.0
tridecanal ^a	1502 (1495)	-	3.5	2.1	1.1	0.8	2.6	1.2	1.1	3.1	1.4	-	-	-	-
β -bisabolene ^t	1506 (1500)	-	9.9	-	-	-	-	7.6	-	-	-	-	11.9	-	22.1
γ -amorphene ^t	1508 (1505)	1.2	-	-	-	3.6	-	-	-	-	-	-	-	-	-
δ -cadinene ^t	1516 (1514)	-	-	-	-	-	-	-	-	-	-	1.2	-	3.5	-

cubenene ^t	1525 (1515)	-	-	-	-	1.2	-	-	-	-	-	-	2.6	-	-
elemol ^t	1539 (1535)	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-
elemicin ^p	1540 (1531)	46.6	-	-	0.3	0.2	0.2	-	-	-	5.8	-	-	-	-
limonen-6-ol, pivalate ^t	1551 (1560)	-	0.8	0.3	0.3	-	0.2	0.6	0.2	0.3	0.4	-	-	-	-
nerolidol ^t	1552 (1545)	-	0.3	-	-	3.4	-	0.3	-	0.5	-	2.0	-	5.8	-
2,6,10-trimethyltetra decane ^t	1560 (1557)	1.0	-	-	-	-	-	1.1	-	0.9	-	-	0.6	-	1.3
spathulenol ^t	1568 (1569)	1.2	-	-	0.7	3.4	0.6	3.8	-	-	0.7	-	-	-	-
(E,Z)-pseudoionone ^t	1574 (1562)	2.2	1.8	-	4.4	13.8	8.8	3.1	3.3	2.0	5.4	2.8	2.1	-	3.0
caryophyllene oxide ^t	1579 (1576)	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-
humulene epoxide ^t	1599 (1601)	2.0	2.0	3.2	4.0	13.3	8.1	1.7	3.6	5.2	6.1	0.6	-	5.0	-
tetradecanal ^a	1601 (1592)	-	-	-	-	-	-	1.2	-	-	-	-	-	-	1.3
5,6,6-trimethyl-5-(3- oxobut-1-enyl)-1-oxa spiro[2,5]octan-4-one ^{dc}	1739 (1746)	2.2	2.0	9.0	4.6	3.9	6.5	2.6	4.8	4.8	3.5	7.8	-	2.0	-
(4E,8E)-5,9,13-tri methyl-4,8,12-tetra decatrinal ^t	1854 (1855)	-	1.2	-	0.4	-	-	-	-	-	-	-	-	-	-
(R,R)-phytone ^t	1860 (1868)	-	0.6	-	0.9	0.3	0.3	3.3	1.0	0.4	1.0	2.5	-	-	-

^t terpene; ^a aldehyde; ^p phenylpropanoids; ^{dc} derivatives of carotenoids; ^e ester; ^c coumarin; ^{alc} alcohol.

*I – calculated Kovats retention index; the theoretical Kovats retention index is shown in brackets.

** absent compound. 1 to 10 - commercial dried samples; 11 - dried sample of *E. grandiflorus*; 12 - dried sample of *E. macrophyllus*; 13 - fresh sample of *E. grandiflorus*; 14 - fresh sample of *E. macrophyllus*.

Source: Authors.

More compounds were identified in the dry samples than in the fresh samples, and the majority of the identified compounds belong to the class of terpenes. The comparison of the results of both species revealed a decrease in the percentage of compounds and an increase in the number of compounds identified after processing (Table 2). Silva and Casali (2000) demonstrated that drying has the function of reducing the rate of deterioration of the plant material by reducing the water content that interferes with the action of enzymes so that the plants are preserved longer. With the decrease in water content, the concentration of active compounds found in the dried sample increases. This observation explains the larger number of compounds isolated from the dried than from the fresh samples.

The study conducted by Pimenta; Figueiredo & Kaplan (2006), who analysed a hydrodistilled extract of *E. grandiflorus* by GC-MS, identified some compounds that were also found in the present work: dihydroedulan, β -caryophyllene, α -caryophyllene, α -farnesene, δ -cadinene, and caryophyllene oxide. It should be emphasized that the work conducted by Pimenta; Figueiredo & Kaplan (2006) identified phytol as a major component of the extract of *Echinodorus* samples and β -caryophyllene, nerolidol, and α -caryophyllene as significant sesquiterpenes. The variation in the composition of the main compounds is due to the different extraction techniques used. Liquid-liquid extraction allows the isolation of compounds of low volatility, such as cembranos and cleoredanos. In contrast, the highly volatile compounds are more capable of being adsorbed by the fiber in the SPME method; therefore, these compounds cannot be detected by the headspace method because of their high molecular weight. However, a greater number of terpene compounds and derivatives of carotenoids, which are primarily responsible for the formation of the aroma of plants (Uenojo; Marostica & Pastore (2007), can be identified using the SPME technique because of its high sensitivity.

The comparison of the two species of fresh samples revealed that a similar number of compounds were identified in both species (thirteen compounds were found in *E. grandiflorus* and eleven compounds were identified in *E. macrophyllus*). However, only three of these compounds were common to both species. In the dry and fresh reference samples of *E. grandiflorus*, a greater number of compounds belonging to the class of terpenes and other chemical classes were identified, while in the samples of *E. macrophyllus* a smaller number of compounds were identified, which belong almost exclusively to the class terpenes.

The principal compounds obtained from the fresh samples of *E. macrophyllus* were β -caryophyllene, α -curcumene and β -bisabolene. β -caryophyllene was found in samples of *E. grandiflorus* and *E. macrophyllus*, and the highest percentages of this compound were found

in fresh (31.1%) and dried (37.7%) *E. macrophyllus* samples. This compound has been previously identified in leaf samples of medicinal plants (Sá et al., 2012).

The compounds tentatively identified in the fresh sample of *E. grandiflorus* were 1-dodecanol and α -farnesene. These compounds were found in the fresh samples of the two species, and a significant percentage was found in the fresh sample of *E. grandiflorus*. These compounds were also identified in the essential oil from *Myrcia tomentosa* by Sá et al. (2012) using GC-MS.

There was a wide variation in the percentages of the majority of the compounds. Some of the compounds identified in the commercial samples have been described in the literature. elemicin is predominantly found in the essential oil (56%) from tarragon (Zawioelak & Dzida, 2012). This compound was identified in the present study in five of the commercial samples, and it accounted for 47% of the total compounds in commercial sample 1. Mockutė; Bernotienė & Judpėntienė (2005) studied samples of *Leonurus cardiaca* L. and found that the percentages of humulene epoxide were very similar in fresh and stored essential oils. In the present study, this compound was found in all of the commercial samples and in the fresh and dry samples of *E. grandiflorus*. 2H-1-Benzopyran-2-one (coumarin) was present in most of the commercial samples, and a significant percentage was found in samples 2 and 9. A similar amount of this substance was described by Wang; Wang & Yang (2009) in essential oils from cinnamon leaves.

This variability in the volatile components of both species and between samples was expected because the type and concentration of secondary metabolites is strongly influenced by the geographical origin, exposure to sunlight, availability of water, soil type, presence of inflorescences, growing conditions, crop and stage of plant development (Pimenta; Figueiredo & Kaplan, 2006).

The following five compounds were found in the dried and fresh reference samples of *E. grandiflorus* and in all of the commercial samples: geranyl acetone, 1-dodecanol, β -ionone, humulene epoxide, and 5,6,6-trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro [2,5]octan-4-one. The analysis of fresh and dried samples of *E. macrophyllus* revealed only one common compound (trans- α -bergamot), which was not found in any of the commercial samples. This compound can be considered a chemical marker of this species. The identification of this marker is important for monitoring the authenticity of the marketed species, and it would allow greater quality control and the ability to monitor the adulteration and counterfeiting of medicinal plants obtained by consumers.

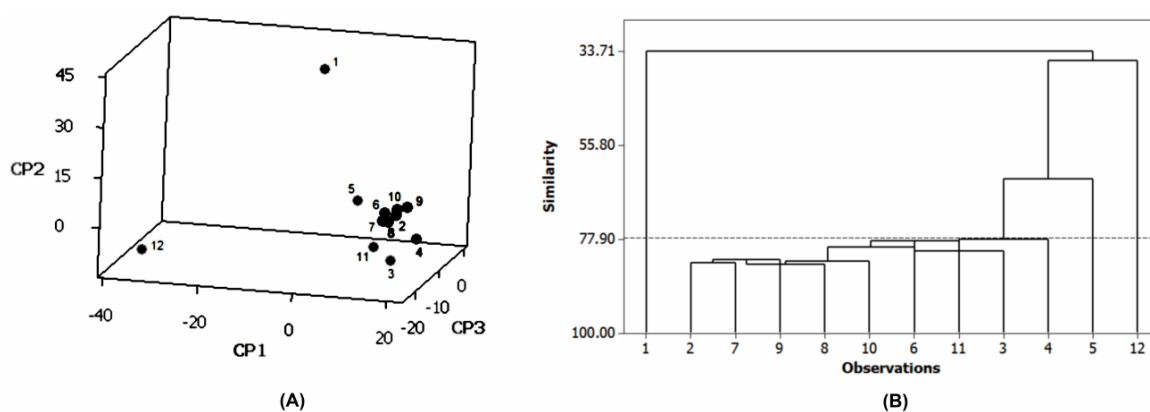
Several major compounds identified in the plant under study, have already had their pharmacological actions scientifically proven, such as spatulenol - antioxidant, antiinflammatory and antimicrobial activity (Nascimento et al., 2018); β -caryophyllene - anti-inflammatory, antioxidant, sedative, anxiolytics, antidepressant, anticonvulsant and antitumor (colon, skin and pancreas)(Francomano et al., 2019); α -curcumene - antimicrobial activity (Silva et al., 2015); geranylacetone - antimicrobial activity (Bonikowski; Świtakowska & Kula, 2015)and β -ionone – antitumor activity (Ansari & Emami, 2016).

According to Dias et al. (2013), the species *E. grandiflorus* and *E. macrophyllus* are used in folk medicine indistinctly for the same medicinal fins in different regions of Brazil. However, data from the literature show differences in chemical composition between two species (Santos et al., 2017), which corroborate with our study.

3.4. Multivariate analysis

The three principal components (PC1, PC2 and PC3) accounted for 81.6% of the total variance and provided discriminatory information for the “chapéu de couro” samples. The results of the matrix made it possible to generate a three-dimensional graph in which each axis represents a set of defined PCs (Figure 3A). To perform the analysis, we selected high values of the relative area (>5%) and included at least one of the reference samples.

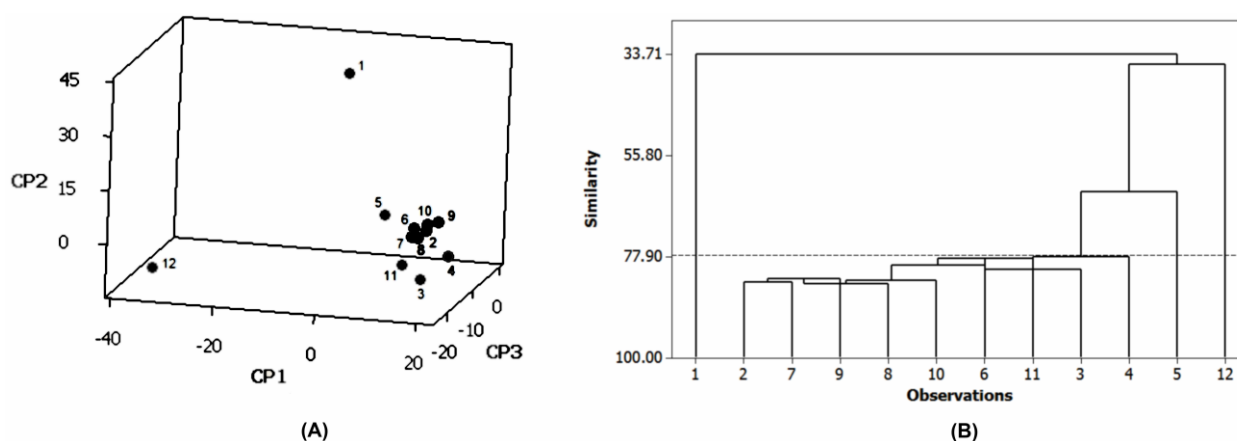
Figure 3. (A) Score plots with three principal components (PC1, PC2, and PC3) from the covariance matrix, which was generated with the CG-MS data for dry references samples of chapéu de couro (*E. macrophyllus* and *E. grandiflorus*). (B) Dendrogram built from the CG-MS data for dry samples of chapéu de couro using the method of median linkage. 1 to 10 - commercial dried samples; 11 - dried sample of *Echinodoros grandiflorus*; 12 - dried sample of *Echinodoros macrophyllus*



Source: Authors.

The reference samples are located at distant positions in the graph, confirming the difference in the chemical composition profiles between the different species. The commercial samples (with the exception of sample 1) are distributed as a single group surrounding the reference sample of *E. grandiflorus* (sample 11). This separation of the samples confirms the results obtained from the histological study and asserts that these species belong to *E. grandiflorus*. One hypothesis for the separation of sample 1 would be a possible adulteration or an inadequate processing stage, such as the cultivation and collection period (lack of water and nutrients, excessive sunlight, season and stage of the maturation of the plant), excessive time between collection and drying, drying at high temperatures, and prolonged storage time, which can lead to the loss of volatiles from the samples.

Figure 3. (A) Score plots with three principal components (PC1, PC2, and PC3) from the covariance matrix, which was generated with the CG-MS data for dry references samples of chapéu de couro (*E. macrophyllus* and *E. grandiflorus*). (B) Dendrogram built from the CG-MS data for dry samples of chapéu de couro using the method of median linkage. 1 to 10 - commercial dried samples; 11 - dried sample of *Echinodorus grandiflorus*; 12 - dried sample of *Echinodorus macrophyllus*.



Source: Authors.

The drying of vegetable products is an effective method that increases the shelf life of the final product by slowing the growth of microorganisms and preventing certain biochemical reactions that might alter the organoleptic characteristics. However, drying cause changes in the cell structure that is often associated with the release or retention of volatile compounds (Wojdyło et al., 2016). The division into groups promoted by the PCA were confirmed by HCA (Figure 3B), which indicates that the identity of the two species and of most of the commercial species is *E. grandifloras*.

4. Conclusions

In this work, the drying in a forced-air oven was found to lead to decreases in the concentrations of most of the volatile compounds compared with the levels found in the fresh samples, although certain compounds were observed to increase after drying. The anatomical results corroborate the findings obtained from the chemical tests, which is fundamental for the confirmation of the authenticity of the species. SPME coupled with GC-MS is a rapid and effective method for the characterization and identification of volatile components and for the quality control of commercial samples of medicinal plants. The analysis indicated which apparent differences in the volatile compounds between the species are reflected by

differences in the principal compounds. Moreover, the identification of a compound that can be considered to be chemical marker of one of the species was achieved with this technique. The results showed that commercial establishments have no knowledge of the information that should be included on labels, and they perform the drying process without any criterion of standardization and without concern for the quality of the final product drying.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Percentage of contribution of each author in the manuscript

Hebert Vinícius Pereira - 30%

Fernando Augusto Gouvêa Reis - 15%

Ana Maria de Resende Machado - 15%

Andrea Rodrigues Marques - 10%

Cleverson Fernando Garcia - 10%

Fátima de Cássia de Oliveira Gomes - 15%

David Lee Nelson - 5%