Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.34122

Chemical profile of the volatile fraction of *Bauhinia forficata* leaves: an evaluation of commercial and *in natura* samples

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Abstract

Bauhinia forficata Link is widely used in Brazilian folk medicine to treat several pathologies. Commercial and botanically identified samples were evaluated via a gas chromatography equipped with a flame ionization detector and a gas chromatography-mass spectrometer. This procedure allowed the identification of 116 compounds, representing 72% to 96% of the total content of the investigated essential oils. The five samples analyzed showed yields of essential oil ranged from 0.03 to 0.10%, being sesquiterpenes and oxygenated sesquiterpenes the major components. Hierarchical Cluster Analysis and Principal Component Analysis were used in order to demonstrate variations in the essential oils' composition of *B. forficata* and were able to clusterize these samples in three groups based on relationships and chemical patterns in essential oils. *In natura* samples showed to be different from commercial samples and CS3 group was the most distinct group of the commercial samples. In spite of differences among samples, it is concluded that essential oils of *B. forficata* presented a rich composition, presenting 11 compounds in common between them, which could be possible to establish a set of compounds as chemical markers for the species, still non-existent in literature.

Keywords: *B. forficata*; pata-de-vaca; essential oils; GC-MS; sesquiterpenes.

Practical Application: This research investigated the volatile composition of essential oils of different samples of *B. forficata*. Findings of the present work show that plant, mainly used to prepare infusions in Brazil, had a volatile profile rich in terpenes, which are known for their positive effects on human health. Thus, our results contribute to increase literature data about *B. forficata* oils composition, mainly the commercial samples, little explored so far.

1 Introduction

The genus Bauhinia, popularly known as "pata-de-vaca", among other denominations, belongs to the Fabaceae family, and in Brazil, 300 native species have already been cataloged. Infusions of leaves of *Bauhinia forficata* Link, also known as "*Brazilian Orchid-tree*" species are used in Brazilian folk medicine as a diuretic, hypoglycemic, tonic, depurative agent, in the fight against lymphatic filariasis (elephantiasis), and for the reduction of glycosuria. Its beneficial effects are generally associated with the presence of phenolic compounds, which are known to have antioxidant properties (Salgueiro et al., 2016; Franco et al., 2018; Tonelli et al., 2022).

In 2009, the Ministry of Health of Brazil published a list of Medicinal Plants of Interest to SUS (Sistema Único de Saúde) or Unified Health System, the Brazilian national healthcare system. This list known in Brazil as RENISUS aims to guide and strengthen research on the species included in the list, especially native ones. The list describes 71 species, and among them is *Bauhinia forficata* Link, highlighting the importance of advancing research that corroborates its use in folk medicine (Agência Nacional de Vigilância Sanitária, 2010).

The main form of commercialization of this herb is dried leaves in plastic bags for preparations of homemade infusions. Thus, the product is treated as a food and under Brazilian law it is not required to indicate the content of bioactive or toxic compounds, as is done in a limited way in herbal products. In this way, the control and regulation of this type of product is practically non-existent, facilitating the possibility of fraud through the inclusion of herbs other than that determined on the label.

The literature is rich when it comes to the composition of leaves extracts (aqueous or hydroalcoholic) of *B. forficata*. Free and glycosylated flavonoids, especially canferolic and quercetinic glycosides, represent important chemical groups typical of the genus, and are the main constituents of *B. forficata* extracts (Ferreres et al., 2012; Jung et al., 2021).

Regarding the composition of the essential oils of this species, only two papers were reported in the literature and presented controversial results. Duarte-Almeida et al. (2004) reported for the first time the occurrence and chemical composition of

Received 01 Apr., 2022
Accepted 11 Aug., 2022
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volatile oils in some species of Bauhinia, the major constituents being sesquiterpenes, namely β–caryophyllene (18.5%) and a copaene isomer (28.8%) found as major components in *B. forficata*. Controversely, Sartorilli & Correa (2007) did not find those compounds in the studied oil of the same species. Instead, they identified γ-elemene (38.4%) and α-bulnesene (17.3%) as major components. These are the only records of the composition of essential oils of *B. forficata*, showing how scarce and controversial the information about the composition of this fraction of the plant actually is. When we expanded the scope of the research, considering only the genus *Bauhinia*, there are only fourteen articles that address the chemical composition of essential oils, comprising a total of seventeen species. With exception of the oils analyzed by Vasudevan et al. (2013, 2014) and Almeida et al. (2015) which presented the diterpene phytol as a major constituent of the species *Bauhinia acuminata* (65.9%), *Bauhinia scandens* (88.32%), *Bauhinia purpurea* (90.38%) and *Bauhinia malabarica* (62.17%); all other samples are characterized by a major composition of sesquiterpenes.

A broader knowledge about the chemical composition of the essential oil of *B. forficata* can contribute to the elucidation of the mechanisms that involve its known pharmacological actions, since part of the volatile terpenic composition can be transferred when preparing the infusions, a form that is normally consumed. Volatile compounds also play a significant role in plant essential oil and infusion aroma, and they are influential in consumer choice (Arsenijević et al., 2016). Furthermore, a detailed knowledge of a representative number of samples can contribute to the determination of a chemical marker of the species, not yet established, helping to standardize these oils, as a way of monitoring fraud (Aquino et al., 2022).

In this way, the association of chemical data with multivariate tools to study plants allow the comparison among samples based on chemometric methods, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Sarabi & Ghashghaie, 2022; Pandeirada et al., 2022).

In this way, the present work aimed to characterize essential oils extracted from leaves of *B. forficata*, comparing a botanically identified sample and four commercial samples labeled as "pata-de-vaca" (*Bauhinia forficata* Link). A chemometric evaluation was carried out to verify the similarities between the botanically identified samples essential oil and the commercial ones.

2 Material and methods

2.1 Plant material

Leaves of *Bauhinia forficata* Link, herein named as *in natura* sample (IN), were collected on three different dates in Petropolis city, Rio de Janeiro State (22° 30' 04.63"S, 43° 07' 58.20"W; Altitude: 958 m), and voucher specimens were deposited at the Herbarium of the Department of Botany of the Federal University of Rio de Janeiro under the registration number RFA 40.615. Four brands of commercial samples were purchased from local markets in Rio de Janeiro city. For three of them, it was possible to acquire three different lots and for one brand, it was possible to acquire only two different lots. The samples were labeled as "pata-de-vaca", and were coded as CS1, CS2, CS3 and CS4. The reference samples (IN) were dried in an air circulation oven at 45 °C. A residual moisture level of 12% (w/w) was attained and, then it was powdered (Agência Nacional de Vigilância Sanitária, 2010; Jung et al., 2021). The Brazilian pharmacopoeia recommends that this type of product present up to 12% of moisture. In order to be in according to commercial samples, this processing was required. The commercial samples were also powdered before hydrodistillation. It is worth highlighting all content of their package was processed since the consumers wholly use this product in infusion preparations.

2.2 Essential oil extraction from the reference and commercial B. forficata samples

The essential oils were extracted by hydrodistillation (Clevenger apparatus) using a 2000 mL flask containing 70 g of plant and 1000 mL of distilled water. The isolation process was carried out during four hours at 100 °C. The essential oil was collected with ethyl acetate, with posterior solvent evaporation under an inert atmosphere of nitrogen gas and the final product was stored in a freezer at -18 °C until the chromatographic analysis.

2.3 Chromatographic analysis

Gas Chromatography (GC) analysis

Oils obtained of *B. forficata* and the commercial samples were analyzed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with a flame ionization detector (FID). Oven temperature was maintained at 50 °C for 2 min initially, and then raised at the rate of 5 °C/min to 240 °C, staying at this temperature for 10 min. Injector and detector temperatures were set at 250 °C and 260 °C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min, and 1 μL of diluted samples (0.01 g/mL) were injected in the splitless mode. Normalization technique was used for obtaining quantitative data.

Gas Chromatography/Mass Spectrometry (GC/MS) analysis

GC/MS analysis of the oils was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components using a homologous series of n-alkanes injected in the same conditions of the samples. Identification of components of essential oils was based on linear retention indices (LRI) relative to n-alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature (Adams, 2001).

2.4 Chemometric analysis

The chemometric methods used for data analysis were hierarchical cluster analysis (HCA) and principal component analysis (PCA). HCA comprises an unsupervised classification procedure that involves measuring either the distance or the similarity between the objects to be clustered. The samples with close similarities are sorted into the same cluster. PCA is widely used for reducing the dimensions of original data set by explaining the correlation among a large number of variables in terms of a smaller number of underlying factors (principal components, PCs) without losing much information (Hu et al., 2014). One data matrix (14 × 116) was constructed in such a way that each row corresponded to a sample and each column corresponded to a compound identified by GC-MS analysis. The HCA and PCA analyses were performed using the Ward method and singular value decomposition (SVD) algorithm, respectively.

3 Results and discussion

3.1 Essential oil yield and composition

The essential oils were obtained from the dried leaves of the *in natura B. forficata* (IN) and the commercial samples (CS1, CS2, CS3 and CS4) by hydrodistillation in yields of 0.08±0.005% (IN), 0.03 ± 0.01% (CS1), 0.05 ± 0.01% (CS2), 0.10 ± 0.04% (CS3), and 0.13 ± 0.05% (CS4), calculated from the average of different lots. For IN, our result was four times higher than that found by (Sartorilli & Correa, 2007) who obtained 0.02% yield from a botanic identified sample from São Paulo. All commercial samples showed higher yields as well. There are no records in the literature for commercial samples, but the genus is known for its low yield of essential oil (Sartorilli & Correa, 2007; Vasudevan et al., 2014; Silva et al., 2020b).

GC-FID and GC-MS analyses were performed and the identities of the compounds, their RI (calculated and literature) and their relative peak area percentages (average of different lots) are listed in Table 1. The chemical composition of the samples proved quite different, with only 11 compouds in common between them, namely, α-copaene, β-cubebene, β-caryophyllene, α-humulene, germacrene-D, δ-cadinene, spathulenol, caryophyllene oxide, humulene epoxide II, isophytol and hexadecanoic acid (Figure 1).

Regarding chemical classes, all samples had a chemical profile major composed by terpene compounds, being sesquiterpenes and their oxygenated derivatives, the predominant classes.

Table 1. Composition of essential oils from *in natura* (IN) and commercial samples.

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$%	CS2 $Area_{(Avg)}$%	CS3 $Area_{(Avg)}$%	CS4 $Area_{(Avg)}$%
α-pinene$^{(M)}$	930	930*	-	-	-	0.25	0.18
o-cymene$^{(M)}$	1023	1022*	-	-	-	0.14	0.09
limonene$^{(M)}$	1028	1028*	-	0.13	-	-	0.05
eucaliptol$^{(OM)}$	1032	1032*	0.06	0.03	0.04	-	-
γ-terpinene$^{(M)}$	1059	1059*	-	-	-	-	0.02
cis linalool oxide$^{(OM)}$	1072	1072#	-	-	0.05	-	-
trans linalool oxide$^{(OM)}$	1088	1088*	-	-	0.03	-	-
linalool$^{(OM)}$	1101	1103*	-	0.13	0.15	-	-
α-tujone$^{(M)}$	1107	1106*	0.09	-	0.08	-	0.04
trans-pinocarveol$^{(OM)}$	1137	1138 *	-	0.18	0.13	-	0.01
verbenol$^{(OM)}$	1143	1143§	-	0.14	-	0.10	0.07
camphor$^{(OM)}$	1145	1145§	0.04	-	0.02	-	0.25
α-phellandren-8-ol$^{(OM)}$	1151	1159§	-	-	-	-	0.03
pinocarvone$^{(OM)}$	1164	1164§	-	0.10	-	0.07	0.09
methyl salicilate$^{(E)}$	1170	1170§		0.20	0.11	-	0.07
4-terpineol$^{(OM)}$	1176	1177*	-	-	-	0.04	0.08
α-terpineol$^{(OM)}$	1193	1194*	0.04	0.08	-	-	-
myrtenal$^{(OM)}$	1198	1198§	-	0.27	-	0.10	0.22
verbenone$^{(OM)}$	1212	1218#	-	0.11	0.09	0.10	0.25
β-cyclocitral$^{(OM)}$	1223	1223§	-	0.154	-	-	0.07
pulegone$^{(OM)}$	1242	1243*	-	0.1	-	-	-
carvone$^{(OM)}$	1247	1248*	-	0.106	-	-	-
geraniol$^{(OM)}$	1260	1260§	-	0.03	-	-	-
anethol$^{(OM)}$	1286	1285§	-	0.21	0.04	0.04	0.07
4-vinyl guaiacol$^{(P)}$	1317	1317§	-	-	0.05	-	-
δ- elemene$^{(S)}$	1337	1337#	0.84	0.35	0.15	-	0.21
α-cubebene$^{(S)}$	1348	1349*	-	0.93	1.10	0.45	0.90

NI: Not identified; LRI_{exp}: Linear retention index calculated for all components using a homologous series of n-alkanes analyzed in the same conditions of the sample; LRI_{lit}: Linear retention index from literature. Area (Avg)%: Percentage area of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value. *National Institute of Standards and Technology (2019). #Pherobase (2019). §Pubchem (2019). **Jordan et al. (2002). ΦMedeiros et al. (2012). (M): Monoterpene. (OM): Oxygenated monoterpene. (S): Sesquiterpene. (OS): Oxygenated sesquiterpene. (P): Phenol. (PP): Phenilpropanoid. (N): Norisoprenoid. (CA): Carboxylic acid. (A): Alcohol. (AL): Aldehyde. (SS): Sulfurated sesquiterpene. (HC): Hydrocarbon. (E): Ester. (K): Ketone. (D): Diterpene. (OD): Oxygenated diterpene.

Table 1. Continued...

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$%	CS2 $Area_{(Avg)}$%	CS3 $Area_{(Avg)}$%	CS4 $Area_{(Avg)}$%
eugenol$^{(PP)}$	1360	1360*	-	-	0.10	-	0.11
ylangene$^{(S)}$	1371	1372*	-	0.12	-	-	0.19
α-copaene$^{(S)}$	1376	1376*	1.07	1.16	1.46	1.49	2.90
β-bourbonene$^{(S)}$	1384	1384*	0.76	0.62	-	0.37	0.78
β-cubebene$^{(S)}$	1389	1389#	1.19	0.18	1.22	0.51	0.51
β-elemene$^{(S)}$	1391	1391#	-	2.1	0.79	0.44	3.92
cyperene$^{(S)}$	1398	1398§	0.13	-	-	0.42	0.77
NI	1406		-	-	-	0.60	-
methyl eugenol$^{(PP)}$	1412	1412§	-	-	-	-	0.95
β-caryophyllene$^{(S)}$	1419	1419§	7.11	3.0	4.36	3.01	3.89
α-ionone$^{(N)}$	1430	1430*	-	0.27	-	-	0.73
γ-elemene$^{(S)}$	1435	1434*	0.77	-	-	0.34	-
α-guaiene$^{(S)}$	1439	1439*	0.30	0.14	-	-	-
aromadendrene$^{(S)}$	1447	1440#	-	0.67	-	0.71	2.09
α-humulene$^{(S)}$	1452	1452*	5.26	1.28	1.12	0.85	1.41
geranyl acetone$^{(N)}$	1456	1456§	-	-	0.15	-	0.24
alloaromadendrene$^{(S)}$	1458	1458*	1.41	-	0.61	1.02	0.78
aristolene$^{(S)}$	1461	1472§	-	0.33	-	-	-
γ-muurolene$^{(S)}$	1473	1475*	-	0.92	-	1.54	-
germacrene D$^{(S)}$	1483	1483*	19.68	2.59	4.52	0.71	5.75
α-curcumene$^{(S)}$	1486	1487§	-	0.12	-	-	-
β-selinene$^{(S)}$	1487	1488§	-	0.85	0.84	1.99	2.70
β-ionone$^{(N)}$	1490	1489§	-	0.17	0.54	-	0.54
α-selinene$^{(S)}$	1493	1494#	-	-	-	1.53	1.23
epi-bicyclosesquifellandrene$^{(S)}$	1493	1491§	-	0.34	-	-	-
bicyclogermacrene$^{(S)}$	1496	1498§	2.37	2.14	2.17	-	-
α-muurolene$^{(S)}$	1501	1499#	0.53	0.28	-	0.34	0.36
bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-$^{(S)}$	1502	1503*	-	-	0.50	-	-
β-bisabolene$^{(S)}$	1504	1505*	-	-	-	1.07	0.62
α-farnesene$^{(S)}$	1507	1507§	-	-	0.54	-	-
γ-cadinene$^{(S)}$	1513	1513#	0.56	-	0.36	0.40	
α-amorphene$^{(S)}$	1516	1514§	-	0.39	-	0.86	1.25
δ-cadinene$^{(S)}$	1516	1516§	2.59	3.39	1.87	0.596	3.99
γ-selinene$^{(S)}$	1537	1544*	-	-	0.87	-	0.8
α-calacorene$^{(S)}$	1543	1543**	-	-	-	-	3.82
eudesma-3,7(11)-diene$^{(S)}$	1545	1545*	-	0.15	1.09	-	-
cadala-1(10) 3,8 triene$^{(S)}$	1546	1548§	-	0.40	-	-	-
NI	1555	NI	-	-	0.43	0.63	-
germacrene B$^{(S)}$	1558	1558§	2.50	-	-	0.56	-
trans nerolidol$^{(OS)}$	1568	1569*	0.51		0.41	-	1.10
dodecanoic acid$^{(CA)}$	1573	1562*	-	0.10	-	-	-
spathulenol$^{(OS)}$	1582	1581.8*	2.46	13.99	2.94	5.64	14.48
caryophyllene oxide$^{(OS)}$	1585	1583*	5.42	3.07	1.52	15.25	5.68
ledol ou viridiflorol$^{(OS)}$	1598	1590#	-	1.28	0.20	-	0.42
Globulol$^{(OS)}$	1603	1604#	-	-	0.32	-	-
humulene epoxide II$^{(OS)}$	1607	1607§	1.48	1.56	0.42	3.43	1.51
aloaromadendrene oxide$^{(OS)}$	1613	1625*	-	-	0.36	-	-
NI	1618		-	1.50	-	-	-
NI	1629		-	-	0.86	-	-

NI: Not identified; LRI_{exp}: Linear retention index calculated for all components using a homologous series of n-alkanes analyzed in the same conditions of the sample; LRI_{lit}: Linear retention index from literature. Area (Avg)%: Percentage area of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value. *National Institute of Standards and Technology (2019). #Pherobase (2019). §Pubchem (2019). **Jordan et al. (2002). ΦMedeiros et al. (2012). (M): Monoterpene. (OM): Oxygenated monoterpene. (S): Sesquiterpene. (OS): Oxygenated sesquiterpene. (P): Phenol. (PP): Phenilpropanoid. (N): Norisoprenoid. (CA): Carboxylic acid. (A): Alcohol. (AL): Aldehyde. (SS): Sulfurated sesquiterpene. (HC): Hydrocarbon. (E): Ester. (K): Ketone. (D): Diterpene. (OD): Oxygenated diterpene.

Table 1. Continued...

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$%	CS2 $Area_{(Avg)}$%	CS3 $Area_{(Avg)}$%	CS4 $Area_{(Avg)}$%
τ-cadinol(OS)	1643	1644*	-	-	0.61	-	-
τ-muurolol(OS)	1647	1647§	-	1.54	-	-	-
selina-6-en-4-ol(OS)	1656	1636*	-	-	0.95	-	-
NI	1658		-	-	-	-	4.97
α-cadinol(OS)	1659	1658*	8.43	7.61	-	2.90	1.37
NI	1662		-	-	-	2.42	-
tumerone(OS)	1671	1680*	-	-	-	-	0.84
eudesma 4(14)-1-diene(S)	1673		-	-	0.23	-	-
NI	1675		-	-	-	3.95	-
cadalene(S)	1676	1676§	-	-	0.41	3.45	4.55
NI	1679		-	-	-	2.82	-
NI	1680		-	-	0.10	-	-
NI	1687		-	-	0.33	-	-
α-bisabolol(OS)	1688	1682*	-	-	-	0.69	-
cedren-8-13-ol(OS)	1690	1690§	-	-	-	0.28	-
NI	1691		1.08	-	-	-	-
eudesma-4,11-dien-2-ol(OS)	1693	1691*	-	1.29	-	-	-
NI	1698		-	-	-	0.35	-
NI	1707		-	-	-	-	0.22
NI	1708			-	-	1.33	-
2-tetradecen-1-ol(A)	1714	1713*	-	-	0.20	-	-
pentadecanal(AL)	1718	1715*	0.70	0.47	-	-	-
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol(OS)	1719	1714*	-	-	0.08	0.42	0.15
NI	1722		-	-	-	0.58	-
mint sulfide(SS)	1739	1740*	-	-	-	-	0.57
NI	1740		-	0.58	-	-	-
NI	1745		-	-	0.11	-	-
aristolone(OS)	1751	1756#	-	-	0.06	-	-
α-cyperone(OS)	1755	1755*	-	-	-	0.66	-
Anthracene(HC)	1776	1770§	-	-	-	-	0.28
myristic acid (tetradecanoic acid)(CA)	1777	1777§	-	2.15	1.48	-	0.39
6,10,14-trimethylpentadecan-2-one(N)	1851	1849§	-	3.88	1.06	0.49	1.28
pentadecanoic acid(CA)	1871	1878#	-	0.24	0.25	-	-
1-hexadecanol(A)	1879	1879*	-	-	0.20	-	-
NI	1882		-	-	-	-	0.25
7,10 hexadecadienal(AL)	1891	1816*	0.48	-	0.06	-	-
7,10,13 hexadecatrienal(AL)	1897	1824*	0.70	-	0.12	-	-
2-heptadecanone(K)	1905	1901*	-	-	0.06	-	-
4(H)cyclopentaphenantrene(HC)	1912	1936§	-	0.20	-	-	-
NI	1918		-	0.08	-	-	-
farnesyl acetone(N)	1923	1924§	0.28	-	0.79	0.17	0.53
methyl palmitate(E)	1930	1930§	-	0.35	0.32	-	0.43
Isophytol(OD)	1950	1950*	0.36	0.47	0.40	0.09	0.20
NI	1958		-	-	0.05	-	-
hexadecanoic (palmitic) acid (CA)	1972	1972*	2.05	19.90	32.13	10.96	5.15
ethyl palmitate(E)	1998	1997§	0.21	-	-	-	0.08
NI	2024		-	0.36	-	-	-
NI	2030		-	-	0.34	-	-

NI: Not identified; LRI_{exp}: Linear retention index calculated for all components using a homologous series of n-alkanes analyzed in the same conditions of the sample; LRI_{lit}: Linear retention index from literature. Area (Avg)%: Percentage area of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value. *National Institute of Standards and Technology (2019). #Pherobase (2019). §Pubchem (2019). **Jordan et al. (2002). ΦMedeiros et al. (2012). (M): Monoterpene. (OM): Oxygenated monoterpene. (S): Sesquiterpene. (OS): Oxygenated sesquiterpene. (P): Phenol. (PP): Phenilpropanoid. (N): Norisoprenoid. (CA): Carboxylic acid. (A): Alcohol. (AL): Aldehyde. (SS): Sulfurated sesquiterpene. (HC): Hydrocarbon. (E): Ester. (K): Ketone. (D): Diterpene. (OD): Oxygenated diterpene.

Table 1. Continued...

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$%	CS2 $Area_{(Avg)}$%	CS3 $Area_{(Avg)}$%	CS4 $Area_{(Avg)}$%
NI	2033		-	-	-	-	0.10
geranyl linalool(OD)	2033	2034*	0.22	-	-	-	-
Fluoranthene(HC)	2055	2057§	-	0.50	-	-	-
heptadecanoic acid(CA)	2073	2071§	-	-	0.30	-	-
9,12-octadecadienoic acid, methyl Ester(E)	2098	2094*	-	-	0.27	-	0.19
9,12,15-octadecatrienoic acid, methyl ester(E)	2105	2105*	-	-	0.66	-	0.23
phytol(OD)	2123	2122*	20.09	-	10.16	3.11	10.64
9,12-octadienal(A)	2146	2150§		-	-	2.64	-
NI	2147		0.12	-	-	-	-
9,12 octadecadienoic acid-linoleic acid(CA)	2153	2154§	-	-	8.30	1.46	1.91
linolenic acid(CA)	2158	2158§	-	-	7.94	-	-
ethyl linoleate(E)	2166	2166§	0.28	-	-	-	-
ethyl linolenate(E)	2173	2173§	0.47	-	-	-	
stearic acid(CA)	2173	2172*	-	-	0.91	0.79	0.21
NI	2188		0.15	-	-	-	-
neophytadiene(D)	2224	2223Φ	0.48	-	-	-	-
eicosane(HC)	2297		-	-	0.04	-	-

NI: Not identified; LRI_{exp}: Linear retention index calculated for all components using a homologous series of n-alkanes analyzed in the same conditions of the sample; LRI_{lit}: Linear retention index from literature. Area (Avg)%: Percentage area of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value. *National Institute of Standards and Technology (2019). #Pherobase (2019). §Pubchem (2019). **Jordan et al. (2002). ΦMedeiros et al. (2012). (M): Monoterpene. (OM): Oxygenated monoterpene. (S): Sesquiterpene. (OS): Oxygenated sesquiterpene. (P): Phenol. (PP): Phenilpropanoid. (N): Norisoprenoid. (CA): Carboxylic acid. (A): Alcohol. (AL): Aldehyde. (SS): Sulfurated sesquiterpene. (HC): Hydrocarbon. (E): Ester. (K): Ketone. (D): Diterpene. (OD): Oxygenated diterpene.

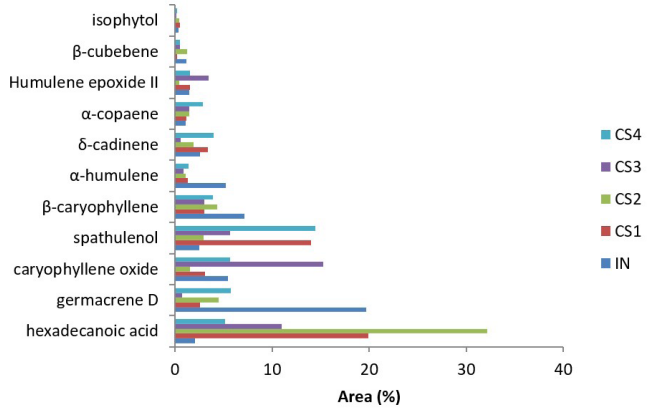

Figure 1. Percentage composition of compounds identified in all essential oils.

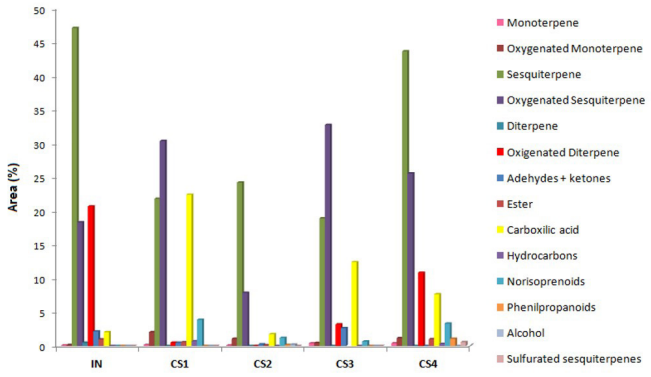

Figure 2. Percentage composition based on chemical classes.

The compounds of these classes represented 65%, 52%, 32%, 52% and 69% of the composition in IN, CS1, CS2, CS3 and CS4 samples, respectively (Figure 2).

For the *in natura* sample (IN), 38 components were identified and represent approximately 91% of the total oil, being phytol (20%), germacrene D (19%), α-cadinol (8%) and β-caryophyllene (7%) the major compounds. Fifty seven compounds were identified in CS1, representing 83% of the total oil. Hexadecanoic acid (19.9%), spathulenol (14%) and α-cadinol (7.6%) were the major compounds. Once again, hexadecanoic acid (32.1%) appeared as the major constituent of the essential oil of CS2 sample, followed by phytol (10.2%) and linolenic acid (8.3%). In this sample, 64 compounds were identified, representing 96% of the total oil. For the CS3 sample, 46 compounds of the oil were identified, representing 72.44% of its total content and the major compounds were caryophyllene oxide (15.2%), hexadecanoic acid (10.9%) and spathulenol (5.7%). CS4 had 94.9% of its oil composition identified, with a total amount of 65 compounds. Spathulenol (14.48%), phytol (10.64%) and germacrene D (5.74%) being the major constituents.

High sesquiterpene content in essential oils from leaves of *Bauhinia* species were previously reported by Gramosa et al. (2009), which showed that *B. ungulata*'s essential oil was

composed exclusively of sesquiterpenes (21.6%) and their oxygenated derivatives (74.3%). Silva et al. (2020a) reported the composition of essential oil from *Bauhinia chileantha* and showed that the sesquiterpenoid compounds represent 78.6% of the total content of the oil. Sartorilli & Correa (2007) also identified 15 compounds in a single sample of *B. forficata*, 14 of them being sesquiterpenes.

It is inferred that the species demonstrates a preference for the metabolic pathways of mevalonic acid, which starts from acetyl CoA and gives rise to sesquiterpenes (C_{15}) and MEP (Metileritritol 4-P) pathway, starting with the condensation of pyruvate and D-glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose 5-phosphate, producing precursors for hemiterpenes (C_5), monoterpenes (C_{10}), and diterpenes (C_{20}), since the composition of its essential oils is mostly terpenic (Aragüez &Valpuesta, 2013).

Many sesquiterpenes, and their alcohol, aldehyde, and ketone derivatives are biologically active or precursors of metabolites with biological functions, while others have desirable fragrance and flavoring properties. Several sesquiterpenes are recognized for their potential as aroma compounds with pleasant and commercial characteristics and have also been studied in the last few years regarding their biological potentials (Butnariu, 2021).

Observing the 11 compounds that were characterized in all essential oils, it can be concluded that some of them are responsible for the characteristic and very similar aroma among the samples. Germacrene D was identified in concentrations varying from 0.70 to 19.68% and for this compound an odor characteristic of woody and greasy cooked flour is attributed (Ajarayasiri & Chaiseri, 2008). Nonetheless, there is no information available on the odor threshold of Germacrene D. The odor threshold is defined as the minimum concentration of a volatile compound that can allow its perception by the human olfaction. The lower odor threshold of a substance the greater its odorant potential (Mariano et al., 2019). Germacrenes, produced in various plant species, are known to act as insecticidal, antimicrobial, and insect pheromones (Bülow & König, 2000; Yang et al., 2005). This volatile organic compound has been observed in bryophytes, gymnosperms, and angiosperms. Interestingly, germacrene D plays an important role as a precursor in sesquiterpenes synthesis such as selinenes and cadinenes (Malik et al., 2019). Phytol was one of the major compounds in the oils, with the exception of the CS1 sample (3.1-20.1%). An odor threshold of 0.64 ppm infers that a green, weak floral-balsamic odor can be attributed to this compound (Guo et al., 2021; Butnariu, 2021). Phytol also presents interesting applications in cosmetics, fine fragrances, shampoos and is used as precursor for the manufacture of vitamin E and K1 (Vasudevan et al., 2014). Carvalho et al. (2020) showed that phytol has an anti-inflammatory activity in acute inflammation models, mainly by inhibition of neutrophil migration, owing to a reduction of IL-1β and TNF-α levels and oxidative stress (Carvalho et al., 2020).

β-caryophyllene appears in a relevant concentration (3.00-7.11%) and its low odor threshold 0.064 ppm (Niu et al., 2011), showing that it is a compound that contributes to the aroma of oils. To β-caryophyllene a dry, woody-spicy and somewhat oily odor is attributed (Jirovetz et al., 2006). β-caryophyllene has its use approved by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), as a flavor enhancer (Machado et al., 2018) in cosmetics (Gertsch et al., 2008). Francomano et al. (2019) published a review of the biological properties of β-caryophyllene in which they demonstrated (with a series of pre-clinical studies) the bioactive potential of this molecule, highlighting antioxidant, anti-inflammatory, neuroprotective, sedative and muscle relaxant activities.

Caryophyllene oxide and Spathulenol, also ubiquitous in all samples at concentrations ranging from 1.52 to 15.25% and 2.46 to 14.48% respectively, are two oxygenated sesquiterpenes well-recognized as presenting several biological activities. The first has some pharmacological potentials such as anticholinesterase, analgesic, anti-inflammatory, antifungal activities (Chavan et al., 2010; Yang et al., 2000), while the last possess several pharmacological potentials such as anti-inflammatory, antioxidant, antiproliferative, immunomodulator, and antimycobacterial (Nascimento et al., 2018). Regarding the contribution to the aroma of oils, there are no values in the literature for the odor thresholds of these compounds. However, an herbal aroma is attributed to spathulenol, while woody odor notes are related to the presence of caryophyllene oxide (Jirovetz et al., 2002; Jirovetz et al., 2004).

Although hexadecanoic acid was in significant quantities in the essential oils (2.05-32.13%), being a majority compound in CS1 and CS2, it is expected that its contribution to flavor would be negligible because of its high molecular weight. In fact, this compound shows a high odor threshold of 10,000 ppb (Pino & Quijano, 2012).

3.2 Multivariate analysis

PCA and HCA were performed from collected data to obtain an overview and understand the composition variability between essential oils from *in natura* and commercial samples.

Initially, PCA analysis was applied, and all groups among oil samples are shown by the scores in Figure 3. According to the results, three separation tendencies can be visualized in Figure 3A (2D plot) and five separation tendencies can be visualized in the Figure 3B (3D plot). The latter plot provides more information about oil samples because it has 87.08% of data variance explained. Thus, 3D plot can be used to explain separation among samples. The *in natura* samples, represented by IN on the Figure 3, was the most distinct group, when compared to the others. The commercial samples represented by CS1, CS2, CS3 and CS4 can also been distinguished as different brands. The brand CS3 is the most distinct among commercial samples.

Figure 4 shows the loadings plot. In this plot it can be identified the composition of essential oils related to the variability described in Figure 3. PC1 axis presented relevant information responsible for the separation of *in natura* samples from the commercial samples. In general, the IN group showed higher amounts of Germacrene D, Pythol, α-cadinol, β-caryophyllene and Caryophyllene oxide compounds than commercial groups. In relation to the commercial samples, the samples with the acronym CS3 are the most distinct. The difference is mainly due to the high concentration of the compounds caryophyllene

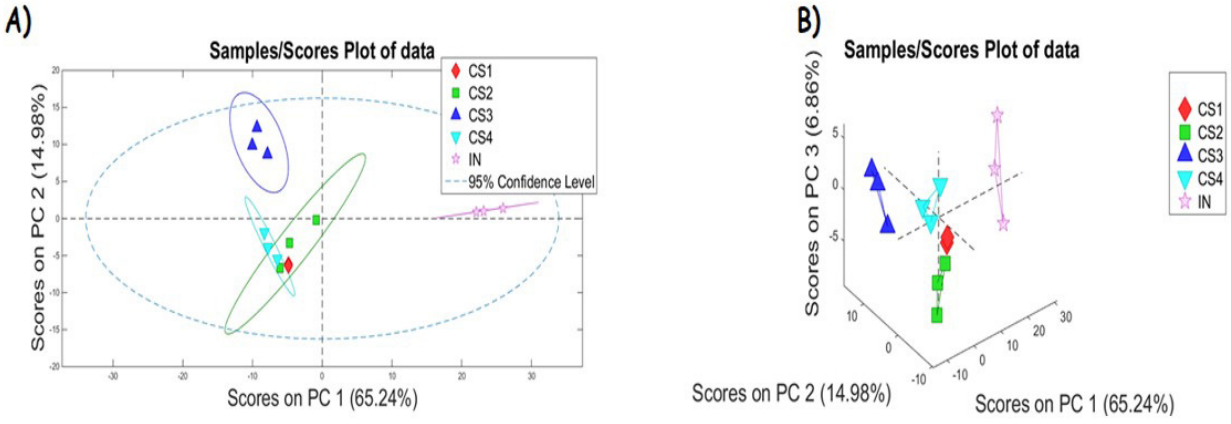

Figure 3. PCA results: (A) scores plot (2D plot) and (B) scores plot (3D plot).

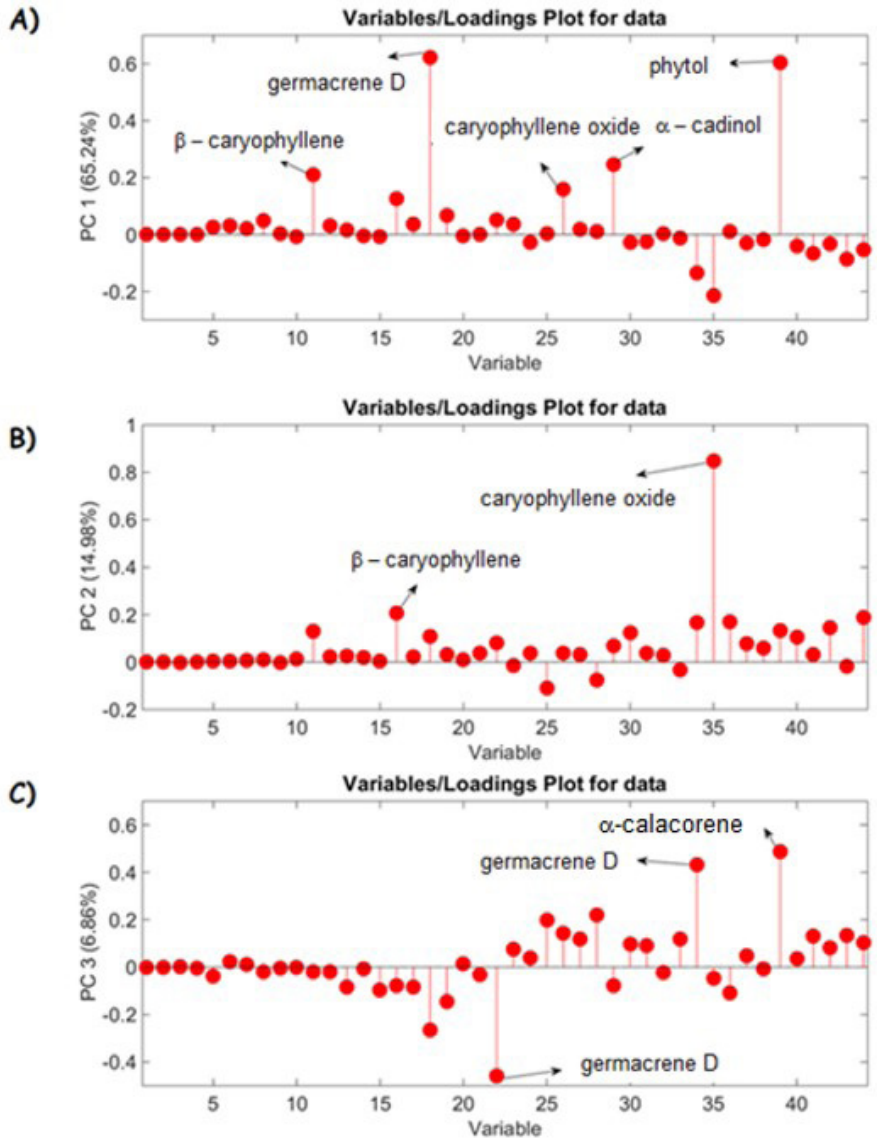

Figure 4. PCA results: loadings plots. (A) PC1 axis - information responsible for the separation of in natura samples from the commercial samples; (B) PC2 axis - information responsible for the separation of CS3 from the others commercial samples; (C) PC3 axis - information responsible for the separation of the most similar commercial samples CS1, CS2 and CS4.

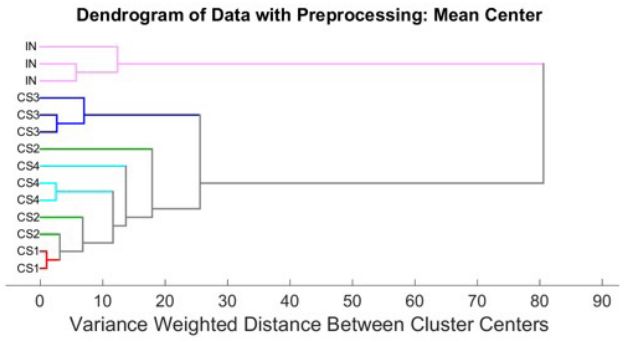

Figure 5. Dendogram representing the similarity relationship among the essential oils IN: *in natura* samples, CS1, CS2, CS3 and CS4 represent different brands.

oxide and β-caryophyllene, showed on Figure 4B, respectively, present in the CS3 group.

Commercial samples CS1, CS2 and CS4 are the most similar, but they are distinguished from each other, mainly due to the presence of the compounds showed in Figure 4C, Germacrene D and α-calacorene, in the CS4 samples. In addition, CS2 samples differ from CS1 and CS4 samples due to the high concentration of Germacrene D.

To corroborate the separation showed by PCA analysis, HCA analysis was applied to this data. The HCA results in Figure 5 showed the same results obtained by PCA analysis, i.e., IN samples are different from commercial samples and CS3 group was the most distinct group of the commercial samples.

4 Conclusion

The present study compared the chemical profile of fourteen essential oils extracted from of four commercial samples and one botanically identified considered a traceable authentic plant material of *B. forficata*. In total, 141 compounds of essential oils were detected in commercial and *in natura* samples, of which 116 were identified. The exploratory analysis of the data through PCA and HCA provided good separation of the five samples, thus indicating a distinction between them. It was also possible to identify the compounds responsible for the differences between *in natura* and commercial essential oils. The major and ubiquitous compounds in essential oils, namely β-caryophyllene, spathulenol, hexadecanoic acid, phytol, caryophyllene oxide, humulene epoxide II, δ-cadinene, germacrene D, α-humulene, β-cubebene (below 1% in CS1, CS3 and CS4) and isophytol (below 1%), could be established as a set of compounds as chemical markers for the species. Also, as *B. forficata* is mainly consumed in the form of infusion, these results help to better relate beneficial effects of the infusions in vivo studies, since some compounds with antioxidant action can migrate to infusion.

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