CROP BREEDING AND APPLIED BIOTECHNOLOGY

NOTE

Chemical diversity of essential oils from native populations of *Eplingiella fruticosa*

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Abstract: *The objective of this work was to analyze the content and the chemical diversity of the essential oil (EO) of 22 Eplingiella fruticosa plants collected in Sergipe, Brazil. EOs were obtained from dry leaves by hydrodistillation and analyzed by GC/MS-FID. The mean EO contents ranged from 0.75 to 1.28%. The compounds found in greater amounts formed two clusters. The first cluster consisted of 15 plants and presented bicyclogermacrene (6.29-16.24%), spathulenol (7.59-15.23%), β-caryophyllene (5.77-12.97%), and caryophyllene oxide (5.00-11.90%) as major compounds. The second cluster consisted of seven plants and had 1,8-cineole (8.96-15.51%), α-pinene (5.46-13.77%), and camphor (4.08-11.40%) as major compounds. Results indicate chemical variability of the EO among E. fruticosa plants from the state of Sergipe. This information may assist in conservation strategies by providing data for investigation of biological activities of EO.*

Key words*: Lamiaceae, medicinal and aromatic plant, hydrodistillation, chemical diversity.*

INTRODUCTION

Eplingiella fruticosa Salzm. ex Benth, ex *Hyptis fruticosa* is a shrub popularly known as "alecrim-de-vaqueiro". It is distributed along the northeast coast of Brazil, and its leaves are used as an anti-inflammatory. Some studies have demonstrated the antimicrobial and antitumor activities of the essential oil and methanolic extract of *E. fruticosa*. Antinociceptive and larvicidal properties have also been identified in the chemical composition of the essential oil (EO) of this plant (Menezes et al. 2007, Silva et al. 2008), which intensified studies on the chemical characterization, biological properties, and conservation of the species.

The yield and chemical composition of the EO of aromatic plants are often related to climatic factors. In recent years, the chemical diversity of EO of plant species has been studied, enabling the obtainment of relevant information for the definition of strategies for the conservation and use of genotypes in breeding programs (Blank et al. 2015, Costa et al. 2015, Nizio et al. 2015). The knowledge on the distribution of the chemical diversity of *E. fruticosa* and on the factors that promote this distribution may assist in the obtainment of EOs with desirable characteristics.

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Authors have reported the presence of the major compounds bicyclogermacrene, 1,8-cineole, and β-caryophyllene in the EO in studies on the antinociceptive activity (Menezes et al. 2007) and on the larvicide activity (Silva et al. 2008) of *E. fruticosa*. Conversely, studies on phenotypic characterization have identified α-pinene, β-pinene, camphor, and limonene as major compounds in the EO extracted from leaves, flowers, and seeds of *E. fruticosa* (Franco et al. 2011a, Franco et al. 2011b). However, in these studies, plants were collected in only two locations, and thus, they do not represent all the chemical diversity of the species in the state of Sergipe.

Despite being widely distributed in the Brazilian northeast, no studies related to the chemical diversity of the EO of *E. fruticosa* have been found in the literature. The knowledge on the chemical diversity of the essential oil of native plants of *E. fruticosa* from the state of Sergipe may assist in the definition of conservation strategies of the species and will provide data for research studies on the biological activities of the EO. Thus, the objective of the present study was to analyze the content and the chemical diversity of the essential oil of *Eplingiella fruticosa* plants native to the state of Sergipe.

MATERIAL AND METHODS

Plant material

E. fruticosa plants were collected in 11 municipalities in the state of Sergipe, Northeast Brazil, totaling 22 plants, between August and October 2015, at flowering stage. Table 1 shows the identification and location of the 22 plants collected. Vouchers were deposited in the herbarium (ASE) of the Federal University of Sergipe for species confirmation.

Extraction and chemical characterization of essential oils

Leaves were dried in forced air circulation oven, at 40 ºC, for five days. EO was extracted by hydrodistillation in a modified Clevenger apparatus. Dried leaves samples (50g) were distilled for 140 minutes (Ehlert et al. 2006) in triplicate. EOs were collected and stored in amber bottles at -20 °C until chemical composition analysis. The following equation was used to calculate the EO content obtained from each sample:

Table 1. Identification and origin of *E. fruticosa* plants collected in municipalities of the state of Sergipe, Brazil

Plants	Origin (municipality)	Georeferenced information	Voucher/ASE
EPF0101	Areia Branca	10°46'03.4"S; 37°21'15.7"W	37921
EPF0201	Estância	11°12'37.6"S; 37°24'49.3"W	37922
EPF0202	Estância	11°14'38.5"S; 37°16'42.9"W	37923
EPF0203	Estância	11°13'53.3"S; 37°17'02.4"W	37924
EPF0301	Itaporanga d'Ajuda	10°59'15.9"S; 37°22'19.2"W	37925
EPF0302	Itaporanga D'ajuda	11°06'22.3"S; 37°15'56.8"W	37926
EPF0303	Itaporanga D'ajuda	11°10'03.8"S; 37°13'43.3"W	37927
EPF0401	Japaratuba	10°30'49.2"S; 36°56'49.5"W	37928
EPF0402	Japaratuba	10°38'16.4"S; 36°54'27.8"W	37929
EPF0501	Malhada dos Bois	10°21'36.5"S; 36°54'27.7"W	37930
EPF0601	Moita Bonita	10°33'50.8"S; 37°22'27.8"W	37931
EPF0701	Muribeca	10°25'59.1"S; 36°55'57.7"W	37932
EPF0801	Pirambú	10°36'56.3"S; 36°51'35.4"W	37933
EPF0802	Pirambú	10°37'22.0"S; 36°49'18.1"W	37934
EPF0803	Pirambú	10°37'39.2"S; 36°48'46.6"W	37935
EPF0901	Salgado	11°01'28.4"S; 37°28'17.1"W	37936
EPF0902	Salgado	11°00'34.0"S; 37°30'30.5"W	37937
EPF1001	Santo Amaro das Brotas	10°48'24.8"S; 37°00'24.4"W	37938
EPF1101	São Cristóvão	10°56'24.2"S; 37°11'38.1"W	37939
EPF1102	São Cristóvão	10°55'33.6"S; 37°11'55.9"W	37940
EPF1103	São Cristóvão	10°57'44.2"S; 37°09'51.1"W	37941
EPF1104	São Cristóvão	10°59'18.3"S; 37°11'10.1"W	37942

$$Content\ (\%, \frac{v}{m}) = \left(\frac{Volume\ of\ the\ essential\ oil\ extracted\ from\ the\ sample}{Mass\ of\ sample\ leaves} \right) \times 100$$

The chemical composition of *E. fruticosa* essential oil was analyzed using a GC-MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were accomplished using an Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane) of 30 m × 0.25 mm i.d., 0.25mm film thickness, at a constant helium (99.999%) flow rate of 1.2 mL min^{-1}. An injection volume of 0.5 µL (5 mg mL^{-1}) was employed, with a split ratio of 1:10. The oven temperature was programmed from 50°C (isothermal for 1.5 min), with an increase of 4 °C min^{-1}, to 200 °C, then 10 °C min^{-1} to 250 °C, ending with a 5 min isothermal at 250 °C.

MS and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). A 0.62 m x 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector; a 0.74 m x 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in the full scan mode (m/z of 40–350) at a scan rate of 0.3 scan/s using the electron ionization (EI) with electron energy of 70 eV. The injector temperature was 250 °C and the ion-source temperature was 250 °C. The FID temperature was set to 250 ºC, and the gas supplies for the FID were hydrogen, air, and helium at flow rates of 30, 300, and 30 **m**L min^{-1}, respectively. Quantification of each compound was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and arranged in order of GC elution.

Identification of essential oil compounds

Individual compounds of the essential oil were identified by computerized matching of the acquired mass spectra with those stored in the NIST21, NIST107 and WILEY8 mass spectral library of the GC-MS data system. A mixture of hydrocarbons (C9H20–C19H40) was injected under these same conditions, and the compounds were then compared with the spectra obtained with those of the equipment data bank and with the Kovats index, calculated for each component, as previously described (Adams 2007). Retention indices were obtained with equation proposed by Van Den Dool and Kratz (1963).

Statistical analyses

The experiment was conducted in a completely randomized design, evaluating the content and chemical composition of the essential oils of 22 plants. Three essential oil samples were obtained per plant, corresponding to the replications. The volume of essential oil solution (5 mg of essential oil per mL of ethyl acetate) of each sample injected into the chromatograph was 1.0 µL.

Data of the essential oil content were subjected to analysis of variance (ANOVA), and the means were compared by the Scott Knott test (P≤0.05), using the Sisvar® software.

From the data analysis of the chemical compounds of the EOs, two multivariate analyses were performed: cluster analysis and principal component analysis (PCA), using the Statistica® software. Subsequently, a dissimilarity matrix was constructed based on the chemical constitution of the EOs of each plant, using the Euclidean distance multivariate analysis. The dissimilarity matrix was simplified in dendrogram using the Ward's clustering method. Correlation analysis was carried out between all the chemical compounds of the EO of the sampled plants.

Graphs with the means of chemical compounds and standard deviations for each chemical cluster were constructed with the Graph Pad Prism® software.

RESULTS AND DISCUSSION

Analysis of essential oil contents

Similar organoleptic characteristics were observed for the essential oils obtained from different plants. The essential oils presented a yellow-translucent coloration, moderate viscosity, and strong odor.

E. fruticosa plants showed significant differences for essential oil content, and the highest EOs content was observed in plants collected in Itaporanga d'Ajuda: EPF0303 (1.33%), EPF0301 (1.20%), and EPF0302 (1.15%); together with three

Table 2. Content (%) of the chemical compounds of the essential oil *Eplingiella fruticosa* plants collected in Sergipe, Brazil

Compounds	IRRo	IRRl	Plants (EPF)																					
			0101	0201	0202	0203	0301	0302	0303	0401	0402	0501	0601	0701	0801	0802	0803	0901	0902	1001	1101	1102	1103	1104
α-Pinene	917	932	3.9	2.5	2.2	2.8	6.0	13.8	9.4	5.2	7.1	8.2	1.4	4.6	0.9	3.6	1.9	1.5	3.4	7.5	2.6	4.6	5.7	5.5
Camphene	931	946	1.0	0.5	0.9	1.5	1.4	1.6	2.7	1.0	1.7	2.8	0.6	0.8	0.3	1.2	0.9	0.6	0.9	2.1	0.7	0.7	1.0	1.1
Sabinene	955	969	0.5	0.4	0.2	0.2	0.7	0.9	0.5	0.5	1.1	0.6	0.3	0.7	0.3	0.7	0.4	0.2	0.5	0.7	0.3	0.5	0.6	0.5
β-Pinene	959	974	2.4	2.8	1.5	1.4	5.3	9.7	4.0	4.7	4.2	7.5	2.2	6.3	1.0	2.2	1.7	1.5	3.1	5.7	1.8	3.4	4.2	4.1
Limonene	1010	1024	5.2	1.5	1.2	2.1	2.7	4.4	3.1	2.2	2.4	2.1	1.3	1.6	1.2	3.5	2.3	1.4	2.3	4.2	1.4	1.7	2.1	2.0
1,8-Cineole	1013	1026	4.8	11.3	5.3	4.0	8.1	14.0	9.0	8.2	12.7	11.0	11.7	12.3	3.9	9.3	8.5	4.7	9.2	11.9	8.9	7.9	15.5	11.9
Camphor	1128	1141	8.9	2.0	6.8	5.9	5.4	4.1	11.4	4.2	6.0	8.8	2.6	2.5	0.7	2.2	3.6	3.6	4.7	11.0	4.8	3.0	9.3	6.1
Borneol	1149	1165	2.8	0.8	1.5	2.3	3.6	2.4	3.6	2.7	1.6	3.7	1.9	1.0	0.6	2.5	1.7	1.3	2.1	3.0	1.8	1.4	1.7	2.4
Isobornyl acetate	1266	1283	0.1	0.1	0.4	1.5	0.5	0.3	0.2	0.1	0.0	0.3	0.2	0.3	0.0	0.0	0.0	0.0	0.1	0.4	0.0	0.0	0.1	0.0
δ-Elemene	1318	1335	0.9	1.2	1.5	1.3	1.1	0.6	1.1	0.9	1.4	0.8	1.0	0.8	2.0	1.3	0.9	1.4	0.7	0.9	1.3	1.5	0.9	1.0
α-Cubebene	1330	1345	1.2	0.7	1.4	1.5	0.9	0.7	0.8	1.7	1.1	1.0	1.1	1.4	1.5	1.2	1.6	0.7	1.2	1.0	1.4	1.4	1.1	1.2
α-Copaene	1359	1374	2.2	2.6	2.5	2.9	2.0	1.7	2.0	3.3	2.0	2.3	2.0	2.4	2.8	2.3	3.0	1.5	2.2	2.0	2.6	2.4	2.2	2.2
β-Cubebene	1372	1378	1.0	1.2	1.1	1.2	0.8	0.0	0.7	1.1	1.1	0.7	0.8	0.8	1.7	1.1	1.4	1.3	1.0	0.7	1.4	1.4	0.8	0.9
β-Caryophyllene	1405	1417	7.6	11.6	10.9	9.5	8.5	5.6	6.4	9.3	6.9	6.1	5.8	7.3	12.6	10.0	8.0	13.0	7.2	7.0	8.8	9.0	4.9	6.3
cis-Muurol-3,5-diene	1434	1448	0.7	0.2	0.3	0.2	0.8	0.4	0.3	1.4	1.0	1.1	0.7	0.9	1.0	0.9	1.1	0.5	0.9	0.5	1.0	1.1	0.5	0.6
α-Humulene	1439	1452	1.3	1.8	1.8	1.7	1.5	1.1	1.2	1.5	1.3	1.2	1.1	1.4	2.3	1.8	1.5	2.1	1.3	1.3	1.4	1.5	1.0	1.2
cis-Cadina-1(6),4-diene	1447	1461	0.5	0.3	0.5	0.7	0.7	0.6	0.4	0.2	0.3	0.0	0.0	0.0	0.7	0.4	0.4	0.8	0.8	0.3	1.2	0.6	0.4	0.6
Germacrene D	1466	1484	2.2	5.3	2.6	3.8	2.0	1.2	1.8	1.7	2.7	1.3	2.2	1.4	3.9	2.6	3.4	4.7	2.8	1.6	3.6	3.3	1.8	2.4
trans-Muurol-4(14),5-diene	1478	1493	1.3	0.9	1.1	1.0	1.1	1.0	0.8	1.4	1.1	1.2	1.4	1.2	1.1	1.2	1.2	1.2	1.1	0.9	1.1	1.1	0.9	1.2
Bicyclogermacrene	1482	1500	7.6	9.8	9.9	8.9	7.5	4.4	7.3	8.0	9.1	5.4	8.6	7.1	16.2	11.0	8.2	11.9	6.3	6.5	11.4	9.8	6.2	7.2
Butylated hydroxytoluene	1489	1514	0.0	0.0	2.1	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	1.9
Cubebol	1498	1514	1.4	1.1	1.1	1.0	0.9	1.0	0.7	1.2	1.2	1.3	1.4	1.3	1.5	1.3	1.5	0.9	0.9	0.9	0.9	1.2	1.2	1.3
trans-Calamenene	1505	1521	2.9	1.5	1.7	2.0	3.1	2.5	1.8	4.3	2.9	3.7	3.4	4.0	3.1	3.0	3.4	2.4	3.6	2.0	3.4	3.4	2.5	2.6
α-Cadinene	1520	1537	2.5	0.4	0.8	2.1	3.1	2.0	1.3	0.2	0.3	0.3	0.0	0.8	2.5	1.4	1.2	3.1	3.5	0.7	4.2	1.5	0.8	1.6
Spathulenol	1565	1577	9.5	8.0	10.2	8.2	7.9	6.0	6.5	8.7	7.7	6.6	15.2	8.8	7.6	9.4	10.5	8.9	8.6	6.0	8.4	8.4	9.5	9.0
Caryophyllene oxide	1571	1582	8.0	10.4	8.7	5.5	6.0	4.4	4.4	8.9	3.5	4.8	11.9	8.1	5.9	6.3	8.1	8.0	7.5	4.0	5.0	7.1	5.6	5.1
epi-globulol	1580	1592	3.1	1.8	2.2	2.2	2.5	1.7	2.3	1.8	1.2	1.3	4.7	1.9	1.5	1.7	2.3	1.7	1.5	2.2	1.8	1.2	1.8	2.3
Humulene epoxide II	1597	1608	1.0	0.8	0.8	0.6	0.6	0.5	0.4	1.3	0.4	0.5	1.6	0.9	0.7	0.6	1.0	1.2	1.0	0.4	0.6	0.8	0.6	0.6
cis-Cadin-4-en-7-ol	1624	1635	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0
epi-α-Cadinol	1624	1638	0.0	3.4	3.6	2.3	1.2	0.0	1.7	1.5	2.1	2.0	2.0	2.2	2.3	2.6	2.7	1.4	1.3	1.6	1.1	0.0	1.4	1.5
β-eudesmol	1641	1649	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
α-Cadinol	1639	1652	2.1	0.7	2.0	2.8	3.2	3.3	1.9	0.0	1.3	1.9	0.0	1.5	1.9	1.7	1.4	2.9	3.7	1.5	3.6	2.5	2.1	2.3
Eudesm-7(11)en-4-ol	1684	1700	1.4	1.6	1.0	1.8	0.8	0.0	0.7	0.3	2.0	1.6	0.0	1.5	3.3	3.0	2.7	0.5	0.6	0.4	0.0	0.0	0.3	0.4
Essential oil content (%)			1.07b	1.11b	0.80d	0.75d	1.2a	1.15a	1.33a	1.20a	1.11b	1.02b	1.07b	1.02b	0.98c	1.07b	0.88c	1.15a	1.11b	1.11b	1.07b	1.15a	1.24a	1.15a

IRRo: Relative Retention Index - observed; IRRl: Relative Retention Index – literature. Means followed by the same letter in the row did not differ significantly from each other, by the Scott-Knott test ($p<0.05$)

plants from São Cristóvão: EPF1103 (1.24%), EPF1102 (1.15%), and EPF1104 (1.15%); one plant from Japaratuba: EPF0401 (1.20%); and one plant from Salgado: EPF0901 (1.15%). The lowest content was observed in two plants collected in Estância (EPF0202 and EPF0203), with means of 0.80 and 0.75%, respectively (Table 2). Results indicate variability among the plants in relation to the EO content of *E. fruticosa* from the state of Sergipe, possibly related to environmental and genetic differences (Regitano Neto et al. 2016).

When evaluating the EO content in *Hyptis pectinata*, the authors observed no significant differences between the six genotypes studied (mean of 0.5%) (Arrigoni-Blank et al. 2008). Conversely, for *Hyptis marrubioides*, variability was observed among the studied genotypes; however, the content obtained did not exceed 0.15% (Botrel et al. 2009). This scenario is possibly related to the physiological and morphological characteristics intrinsic to the species associated with the environmental characteristics, although both belong to the same family (Lamiaceae).

Analysis of the chemical composition of the essential oils

The regions where plants were collected have similar characteristics of relief, soil, and average annual temperature. The reliefs of the collection points are predominantly dissected in hills and inter-river tables. The soil is classified as yellow-red podzolic, with coarse texture surface horizon. The annual average temperature ranges from 24 °C (in Areia Branca, Malhada dos Bois, Moita Bonita, Muribeca, and Salgado) to 26 °C (in Itaporanga D'Ajuda, Japaratuba, Pirambú, Santo Amaro das Brotas, and São Cristóvão). The annual average rainfall is different between the regions, ranging from 920 mm in Malhada dos Bois, located in the hinterland of Sergipe, to 1465 mm in Estância, located in the east mesoregion of Sergipe.

By analyzing the chemical composition of the EO of the 22 *E. fruticosa* plants, 119 compounds were detected. Only 33 compounds were considered in the statistical analyses, and those with value lower than 1% were discarded (Table 2).

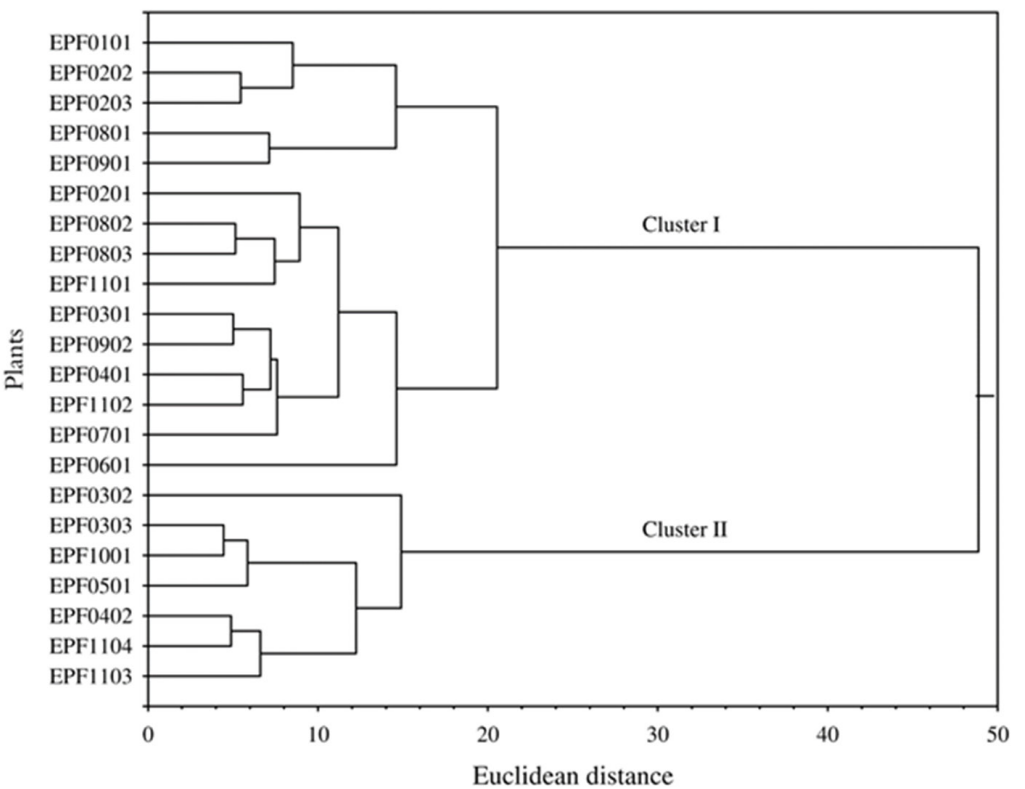

Figure 1. Bidimensional dendrogram representing the similarity of 22 *E. fruticosa* plants to the chemical composition of their essential oils.

The compounds 1,8-cineole, α-pinene, β-pinene, β-caryophyllene, bicyclogermacrene, camphor, spathulenol and caryophyllene oxide were found in greater amount, which defined the formation of two clusters, according to the cluster analysis (Figure 1). Cluster I, consisting of 15 plants (EPF101, EPF201, EPF202, EPF203, EPF301, EPF401, EPF601, EPF701, EPF801, EPF802, EPF803, EPF901, EPF902, EPF1101, and EPF1102), had bicyclogermacrene (6.29-16.24%), spathulenol (7.59-15.23%), β-caryophyllene (5.77-12.97%), and caryophyllene oxide (5.00-11.90%) as major compounds.

Cluster II, consisting of seven plants (EPF302, EPF303, EPF402, EPF501, EPF1001, EPF1103 e EPF1104), had 1,8-cineole (8.96-15.51%), α-pinene (5.46-13.77%), and camphor (4.08-11.40%) as major compounds (Figure 2).

The compounds bicyclogermacrene, β-caryophyllene, and 1,8-cineol were also identified as major compounds in several studies with *E. fruticosa* (Menezes et al. 2007, Silva et al. 2008, Franco et al. 2011a, Franco et al. 2011b), besides spathulenol (Silva et al. 2008), α-pinene and camphor (Franco et al. 2011a, Franco et al. 2011b). Caryophyllene oxide had been classified as minor compound in all previous studies with oil of *E. fruticosa* leaves. These variations are possibly related to the influence of the location and the collection period.

Considering that bicyclogermacrene and spathulenol were efficient in phytopathogenic fungi control (Mashigo et al. 2015), and that β-caryophyllene and caryophyllene oxide were efficient in tick repellency (Ashitani et al. 2015), the EOs of the plants of the first cluster may have potential biological activity and may contribute to other studies, such as those related to pest control. The same occurs to the EOs of the plants of the second cluster, since they present a mean of 12.29% of 1,8-cineole and 8.1% of camphor (Figure 2), and these compounds have already proven their insecticidal activity (Sriramavaratharajan et al. 2016, Tak et al. 2016).

According to the principal component analysis (Figure 3), the first principal component represented 31.03% of the total variation, and was positively related to the compounds β-cubebene (*r*=0.89), bicyclogermacrene (*r*=0.89), α-humulene (*r*=0.87), β-caryophyllene (*r*=0.86), germacrene D (*r*=0.84), and δ-elemene (*r*=0.78); and negatively related to the compounds α-pinene (*r*=-0.86), β-pinene (*r*=-0.84), camphene (*r*=-0.70), and 1,8-cineole (*r*=-0.70). The second principal component represented 18.15% of the total variation, and was positively related to α-cadinol (*r*= 0.72), and negatively related to β-eudesmol (*r*=-0.80), trans-muurola-4(14),5-diene (*r*=-0.78), humulene epoxide II (*r* =-0.75), and spathulenol (*r* =-0.73).

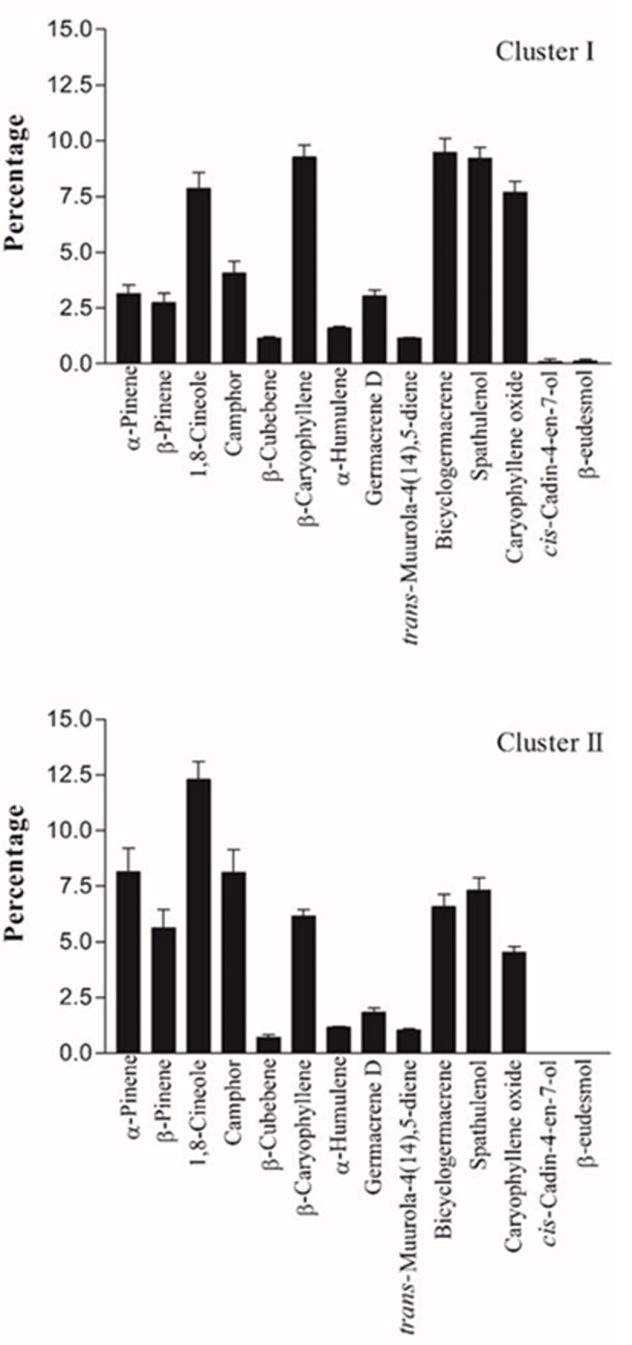

Figure 2. Means of the major chemical compounds of the essential oils of *E. fruticosa* plants, clusters 1 and 2.

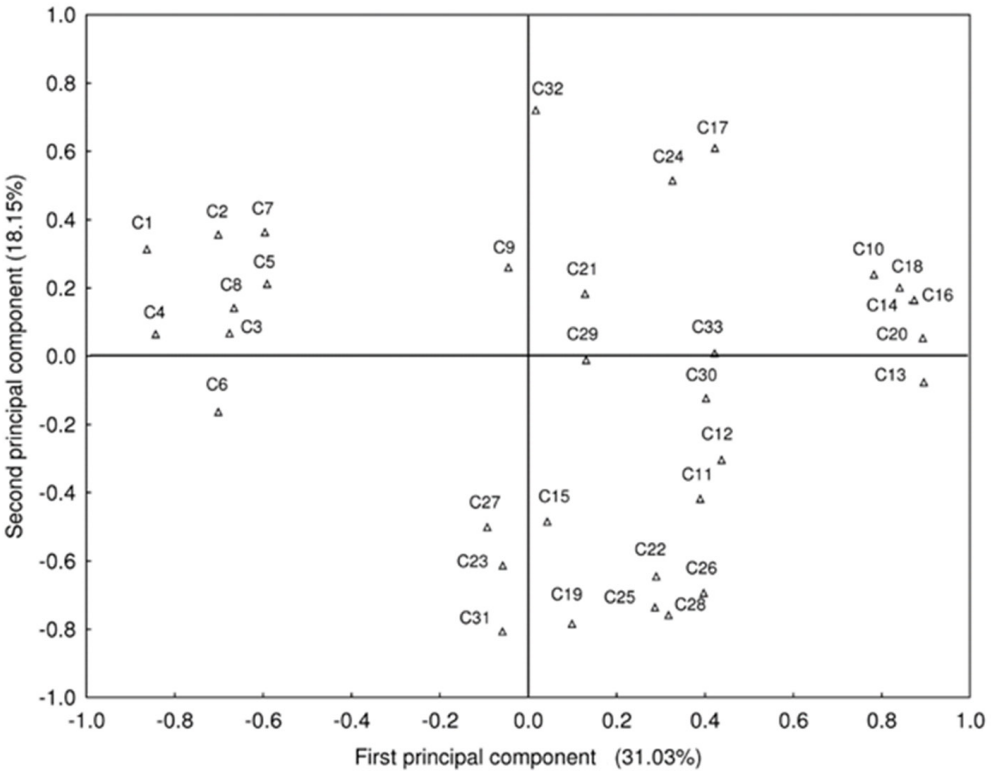

Figure 3. Distribution of the chemical compound of the essential oil from *E. fruticosa* samples in relation to the two principal components through the principal component analysis (PCA). (C1) α-pinene, (C2) camphene, (C3) sabinene, (C4) β-pinene, (C5) limonene, (C6) 1,8-cineole, (C7) camphor, (C8) borneol, (C9) isobornyl acetate, (C10) δ-elemene, (C11) α-cubebene, (C12) α-copaene, (C13) β-cubebene, (C14) β-caryophyllene, (C15) *cis*-muurola-3,5-diene, (C16) α-humulene, (C17) *cis*-cadina-1(6),4-diene, (C18) germacrene D, (C19) *trans*-muurol-4(14),5-diene, (C20) bicyclogermacrene, (C21) butylated hydroxytoluene, (C22) cubebol, (23) *trans*-calamenene, (C24) α-cadinene, (C25) spathulenol, (C26) caryophyllene oxide, (C27) epiglobulol, (C28) humulene epoxide II, (C29) *cis*-cadin-4-en-7-ol, (C30) epi-α-cadinol, (C31) β-eudesmol, (C32) α-cadinol, (C33) eudesm-7(11)en-4-ol.

The highest positive correlations were observed between α-humulene and β-caryophyllene (*r*=0.98); bicyclogermacrene and δ-elemene (*r*=0.91); α-cadinene and *cis*-cadina-1(6),4-diene (*r*=0,90); *trans*-calamenene and *cis*-muurola-3,5-diene (*r*=0.89); humulene epoxide II and caryophyllene oxide (*r*=0.89); α-pinene and β-pinene (*r*=0.88); α-cadinol and α-cadinene (*r*=0.84); besides bicyclogermacrene and α-humulene (*r*=0.84), β-cubebene (*r*=0.82) and β-caryophyllene (*r*=0.82) (Table 3).

The knowledge of the correlations between the chemical compounds in *E. fruticosa* can be useful in the selection of specific genotypes. For instance, genotypes with high content of α-humulene are likely to present high content of β-caryophyllene. This high positive correlation may be related to the same metabolic pathway in which the compounds present the same precursors (Barros et al. 2009).

Negative correlations were observed between β-cubebene and β-pinene (*r*=-0,83); β-cubebene and α-pinene (*r*=-0.82); 1,8-cineole and α-humulene (*r*=-0.74); β-pinene and biciclogermacrene (*r*=-0.73); 1,8-cineole and β-caryophyllene (*r*=-0.72); and β-pinene and germacrene D (*r*=-0.71) (Tabela 3). This negative correlation can also assist the selection of specific genotypes since it allows identifying plants with high content of the first compound to the detriment of the second compound. Negative correlation may be related to different metabolic pathways in which the compounds are synthetized. For instance, farnesyl cation diphosphate is one of the precursors of the pathway of β-cubebene synthesis and is not on the pathway of α-pinene synthesis (Barros et al. 2009).

Table 3. Correlation coefficients for the chemical compounds of the essential oils of *Eplingiella fruticosa* plants in Sergipe, Brazil

Compounds	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	**C18**	**C19**	**C20**	**C21**	**C22**	**C23**	**C24**	**C25**	**C26**	**C27**	**C28**	**C29**	**C30**	**C31**	**C32**	**C33**
C1	0.73	0.69	0.88	0.58	0.56	0.48	0.56	0.05	-0.51	-0.45	-0.41	-0.82	-0.62	-0.09	-0.63	-0.20	-0.68	-0.30	-0.69	-0.14	-0.39	-0.09	-0.19	-0.62	-0.61	-0.24	-0.56	-0.01	-0.44	-0.19	0.15	-0.32
C2		0.42	0.56	0.45	0.27	0.74	0.78	0.28	-0.34	-0.36	-0.25	-0.58	-0.50	-0.14	-0.48	-0.30	-0.56	-0.30	-0.56	-0.03	-0.41	-0.20	-0.27	-0.56	-0.64	-0.16	-0.62	-0.15	-0.06	-0.22	0.05	-0.05
C3			0.66	0.53	0.65	0.19	0.28	-0.21	-0.35	-0.27	-0.38	-0.53	-0.55	0.23	-0.52	-0.31	-0.55	-0.08	-0.47	-0.31	-0.02	0.15	-0.23	-0.36	-0.50	-0.24	-0.47	-0.04	-0.27	-0.19	-0.02	0.04
C4				0.41	0.65	0.28	0.44	0.03	-0.63	-0.36	-0.34	-0.83	-0.60	0.07	-0.60	-0.38	-0.71	-0.06	-0.73	-0.22	-0.24	0.16	-0.27	-0.51	-0.40	-0.21	-0.35	-0.03	-0.37	-0.07	0.05	-0.33
C5					0.14	0.47	0.57	0.04	-0.48	-0.25	-0.32	-0.53	-0.39	-0.14	-0.38	-0.06	-0.47	-0.08	-0.48	-0.24	-0.17	-0.14	0.04	-0.35	-0.35	0.11	-0.30	-0.13	-0.48	-0.20	0.12	-0.02
C6						0.17	0.09	-0.24	-0.56	-0.35	-0.31	-0.60	-0.72	0.02	-0.74	-0.46	-0.49	-0.17	-0.61	-0.10	-0.03	0.06	-0.46	-0.06	-0.20	-0.03	-0.24	-0.09	-0.08	0.10	-0.20	-0.32
C7							0.66	0.21	-0.35	-0.28	-0.27	-0.44	-0.52	-0.32	-0.54	-0.08	-0.50	-0.39	-0.55	0.23	-0.44	-0.36	-0.14	-0.34	-0.50	0.06	-0.43	-0.18	-0.18	-0.23	0.12	-0.30
C8								0.24	-0.50	-0.22	-0.20	-0.55	-0.50	0.01	-0.48	-0.10	-0.63	0.02	-0.59	-0.07	-0.39	0.04	-0.01	-0.28	-0.39	0.18	-0.28	-0.19	-0.30	0.05	0.12	-0.23
C9									-0.05	0.09	0.19	-0.21	-0.01	-0.46	0.03	0.01	-0.02	-0.19	-0.20	0.46	-0.33	-0.30	0.01	-0.14	-0.12	0.13	-0.19	-0.14	0.17	-0.06	0.17	0.04
C10										0.20	0.21	0.73	0.74	0.03	0.77	0.34	0.62	-0.12	0.91	0.16	0.19	-0.23	0.16	0.00	-0.05	-0.25	-0.11	0.23	0.34	-0.17	0.00	0.42
C11											0.79	0.50	0.14	0.56	0.18	0.02	-0.01	0.48	0.26	0.20	0.52	0.54	-0.01	0.30	0.17	-0.04	0.26	0.18	0.13	0.17	-0.16	0.28
C12												0.52	0.30	0.37	0.29	-0.06	0.23	0.24	0.28	0.15	0.40	0.29	-0.14	0.09	0.23	-0.14	0.17	0.02	0.39	0.14	-0.32	0.38
C13													0.70	0.32	0.69	0.35	0.75	0.16	0.82	-0.02	0.38	0.13	0.25	0.24	0.26	-0.18	0.25	0.27	0.29	-0.07	-0.06	0.43
C14														-0.06	0.98	0.37	0.76	0.05	0.82	-0.01	0.07	-0.19	0.28	-0.02	0.31	-0.24	0.21	0.07	0.37	-0.15	0.01	0.40
C15															-0.04	-0.11	-0.20	0.62	0.13	-0.46	0.47	0.89	-0.01	0.08	-0.01	-0.25	0.21	0.27	-0.22	0.25	-0.17	0.13
C16																0.34	0.72	0.05	0.84	0.02	0.15	-0.17	0.28	-0.01	0.26	-0.22	0.16	0.03	0.42	-0.18	0.02	0.51
C17																	0.44	-0.30	0.37	0.15	-0.42	-0.18	0.90	-0.19	-0.26	-0.23	-0.16	0.12	-0.33	-0.42	0.80	-0.18
C18																		-0.16	0.71	0.03	0.07	-0.32	0.32	0.11	0.31	-0.15	0.18	0.13	0.33	-0.19	0.09	0.30
C19																			0.05	-0.18	0.58	0.73	-0.16	0.52	0.49	0.29	0.68	0.04	-0.10	0.58	-0.38	0.05
C20																				-0.05	0.29	-0.05	0.29	0.16	0.18	-0.11	0.14	0.10	0.31	-0.02	-0.05	0.46
C21																					0.01	-0.44	-0.09	0.16	-0.07	0.06	-0.14	-0.10	0.29	-0.14	0.12	-0.08
C22																						0.41	-0.38	0.47	0.34	0.11	0.32	0.07	0.24	0.23	-0.52	0.54
C23																							0.04	0.23	0.17	-0.04	0.40	0.16	-0.28	0.37	-0.11	0.01
C24																								-0.18	-0.21	-0.14	-0.09	-0.01	-0.42	-0.40	0.84	-0.09
C25																									0.74	0.70	0.75	-0.03	0.21	0.66	-0.39	-0.06
C26																										0.51	0.89	0.05	0.27	0.58	-0.48	-0.04
C27																											0.51	-0.24	0.00	0.65	-0.35	-0.22
C28																												0.03	-0.00	0.71	-0.40	-0.19
C29																													-0.40	-0.07	0.10	-0.24
C30																														0.02	-0.45	0.56
C31																															-0.63	-0.30
C32																																-0.20

Compounds: (C1) α-pinene, (C2) camphene, (C3) sabinene, (C4) β-pinene, (C5) limonene, (C6) 1,8-cineole, (C7) camphor, (C8) borneol, (C9) isobornyl acetate, (C10) δ-elemene, (C11) α-cubebene, (C12) α-copaene, (C13) β-cubebene, (C14) β-caryophyllene, (C15) *cis*-muurol-3,5-diene, (C16) α-humulene, (C17) *cis*-cadina-1(6),4-diene, (C18) germacrene D, (C19) *trans*-muurola-4(14),5-diene, (C20) bicyclogermacrene, (C21) butylated hydroxy-toluene, (C22) cubebol, (23) *trans*-calamenene, (C24) α-cadinene, (C25) spathulenol, (C26) caryophyllene oxide, (C27) epiglobulol, (C28) humulene epoxide II, (C29) *cis*-cadin-4-en-7-ol, (C30) epi-α-cadinol, (C31) β-eudesmol, (C32) α-cadinol, (C33) eudesm-7(11)en-4-ol

Despite the variability observed in the composition and phenotypic diversity of the EO of the collected plants, environmental and geographic factors could not be related only to specific chemical clusters since plants collected in the same local/region were clustered separately. Part of this chemical variability is also due to genetic factors since plants collected in neighboring locations and subjected to similar environmental conditions formed different clusters.

Results indicated that: (i) the EO content varied among *E. fruticosa* plants, (ii) the compounds 1,8-cineole, α-pinene, β-caryophyllene, bicyclogermacrene, camphor, spathulenol and caryophyllene oxide were the major compounds detected in the EO of *E. fruticosa* plants from the state of Sergipe, and plants of this species were classified into two distinct clusters. Such knowledge will be useful for the establishment of strategies for the conservation and investigation of the potential biological activities of the essential oil of this plant.

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