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Artigo

Seasonality Effect on Essential Oil Yield and Chemical Composition of Four Accessions of *Schinus molle* L.

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Efeito da Sazonalidade Sobre o Rendimento e a Composição Química dos Óleos Essenciais de Quatro Acessos de *Schinus molle* L.

Resumo: O objetivo deste estudo foi determinar o rendimento e a composição química dos óleos essenciais das folhas secas de quatro acessos de pimenta (*Schinus molle* L.) coletados em quatro locais diferentes na cidade de Volta Redonda, Rio de Janeiro, Brasil, em quatro estações do ano. Após extração por hidrodestilação e análise química dos óleos essenciais por cromatografia em fase gasosa com detector por ionização em chama (CG-DIC) e cromatografia em fase gasosa acoplada à espectrometria de massa (CG-EM), o biciclogermacreno e o sabineno foram os principais constituintes químicos dos óleos essenciais obtidos durante o verão (34,34 % e 26,54 %), outono (23,63 % e 45,4 %) e primavera (21,06 % e 45,39 %), enquanto mirceno e sabineno foram os principais constituintes encontrados no inverno (21,97 % e 27,32 %), respectivamente. Observou-se que o perfil metabólico de *Schinus molle* tem forte influência da sazonalidade, uma vez que este foi variável ao longo do ano.

Palavras-chave: Sazonalidade; aroeira; monoterpeno; sesquiterpeno.

Abstract

The objective of this study was to determine the yield and chemical composition of the essential oils of the dry leaves of four accessions of false pepper tree (*Schinus molle* L.) collected at four different sites in the city of Volta Redonda, Rio de Janeiro, Brazil, in four seasons of the year. After hydrodistillation extraction and chemical analysis of the essential oils by GC-FID and GC-MS, bicyclogermacrene and sabinene were found to be the major chemicals in the essential oils obtained during the summer (34.34 % and 26.54 %), autumn (23.63 % and 45.4 %) and spring (21.06 % and 45.39 %), while myrcene and sabinene were the main chemicals found during winter (21.97 % and 27.32 %) respectively. It was observed that the metabolic profile of *Schinus molle* has strong influence of seasonality, since this was variable throughout the year.

Keywords: Seasonality; *Schinus molle* L.; Anacardiaceae.

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Seasonality Effect on Essential Oil Yield and Chemical Composition of Four Accessions of *Schinus molle* L.

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1. Introduction

Schinus molle L. (Anacardiaceae) is a native species in Brazil, found mainly in the South region, and popularly known as aroeira, aroeira-salsa, pimenta-americana and pimenta-da-árvore (while in English it is variously called peppertree or false peppertree, among others). [1]

The chemical composition of the essential oil of *S. molle* is complex, varying in number of substances and their percentages, being constituted mainly of hydrocarbons of monoterpenes and sesquiterpenes, with smaller concentrations of oxygenated forms.[2-4]

Different studies have indicated a large diversity of *S. molle* chemotypes, with the essential oil variously containing α-pinene, β-pinene, α-phellandrene, β-phellandrene, myrcene, limonene, terpineol, myrtenol, β-caryophyllene, bicyclogermacrene, spathulenol and cubenol as major substances.[4-7]

The chemical composition of the essential oil of a species can vary depending on the genetic characteristics of the specimen, the organs or parts used in the extraction, the location, growing and collection conditions, climatic factors and also the different seasons of the year that contribute to the edaphoclimatic characteristics of a locale.[8,9]

The quality and quantity of active constituents extracted from plants is not constant throughout the year for practically all secondary metabolites, such as essential oils, phenolic acids, flavonoids and alkaloids [9], which makes it interesting to carry out studies to find plant specimens that have stable yield and quality of the chemical profile among traits of interest.

2. Materials and Methods

2.1. General

The leaves of *Schinus 3ole* were collected in the city of Volta Redonda, Rio de Janeiro state, Brazil, at four different sites: accession 1 (22°31'36'S; 44°04'31''W), accession 2 (22°31'42'S; 44°04'32''W) accession 3 (22°31'22'S; 44°07'30''W), and accession 4 (22°31'14'S; 44°07'07''W), and in the four seasons of the year: summer (January 16, 2017), fall (April 14, 2017), winter (July 25, 2017) and spring (October15, 2017). A specimen was deposited in the herbarium of the Institute of Botany of Rio de Janeiro Federal Rural University (UFRRJ) and was identified by Dr. Pedro Germano Filho with the code RBR 35791. After the collections, the leaves were separated from the stems and dried in a forced-air oven at 40 °C until reaching constant weight (72 hours). The essential oil was obtained from a sample composed of leaves, identified by accession and season of the year. Leaf drying and extractions of the essential oils were carried out at the Aromatic and Medicinal Plants Laboratory (Department of Plant Science/UFRRJ) and the chemical analysis at the Multiuser Analytical Center (Institute of Chemistry/UFRRJ).

2.2. Extraction, content (% m/m) and chemical characterization of essential oils

The essential oils were extracted from the dried leaves in triplicate by hydrodistillation in a Clevenger apparatus for three hours. The recovered distillate was partitioned with CH_2Cl_2 (2 x 5 mL), dried with anhydrous Na_2SO_4 and the CH_2Cl_2 was eliminated with N_2 gas to constant weight. The essential oils obtained were deposited in amber bottles, identified by accession/replicate/season and stored in a freezer at -10 °C. The yield of essential oil was calculated by the equation: Yield (%) = MEO H100/MDL, where MEO is the mass (g) of essential oil and MDL is the mass (g) of dry leaves.

Gas chromatography was carried out with a Hewlett-Packard 5890 II system (Palo Alto, USA) equipped with a flame ionization detector (FID) and a split/splitless injector, and a 1:20 split ratio was used to separate and detect essential oil constituents. Substances were separated into a VF-5ms fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent J&W). The oven, injector and detector temperatures were programmed as reported in the literature[6]. The carrier gas used was He (1 mL/min) and the injected volume was 1 µL at a 1:20 split ratio. Percentages of essential oil compounds were calculated from the relative area of each peak analyzed by FID. Essential oils were also analyzed with a GC/MS QP-2010 Plus (Shimadzu, JPN). Carrier gas flow, capillary column and temperature conditions for GC/MS analysis were the same as those described for GC/FID and reported by Adams (2007).[10] Mass spectrometer operating conditions were ionization voltage of 70 eV and mass range of 40-400 *m/z* and 0.5 scan/s. The compounds' retention indices were calculated based on co-injection of samples with a *n*-C_8-C_{20} hydrocarbon mixture, as reported by Van den Dool and Kratz (1963).[11] Constituents were identified by comparison of

their mass spectra with the NIST library (2008) and with those reported by Adams (2007).[10]

2.3. Statistical analysis

The standard error around the mean and the graphs were calculated and constructed with the aid of the GraphPad Prism program, version 6.01 (Graph Pad Software Inc., San Diego, USA). Principal component analysis and hierarchical grouping were performed using the PAST program, version 3.13.[12] The data used for the multivariate analyses were the dependent variables (substances of the essential oils and class of substances, which were: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes), while the independent variables were essential oil samples based on treatments: accession and season.

3. Results and Discussion

3.1. Yield analysis (%)

The essential oil obtained from the dried leaves had a pale yellowish color and aroma similar to fruit of the Brazilian peppertree (*Schinus terebinthifolius*), regardless of the accession or season when the leaves were collected. The yields of essential oils relative to the accessions and the seasons can be observed in Table 1. Accession 2 presented the highest yield of essential oil (3.26 %) in autumn and spring and accession 1 presented the lowest yield of essential oil (1.30 %) in summer and spring.

On average, the plants collected in the summer had lower essential oil content (1.53 %) compared to the other seasons (Figure 1A). The accessions showed no difference in the average essential oil content during the seasons (Figure 1B).

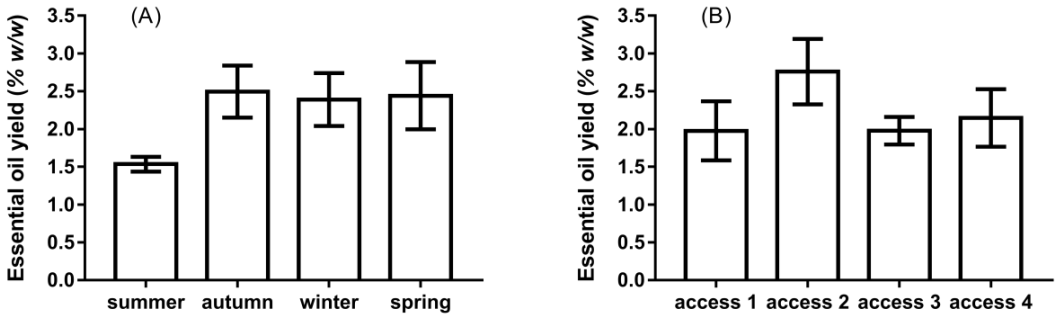

Figure 1. Yield (% m/m) of essential oil of the dried leaves of *S. molle* collected in the city of Volta Redonda, Brazil. Average of the four accessions per season (A) and average of the four seasons per accession (B). The bars represent the standard error around the mean

3.2. Analysis of the chemical profile of essential oil

The minimum and maximum concentrations of 32 substances identified in the essential oil of *S. molle* are presented in Table 1 and 2 based on the seasons and the accessions. The monoterpenes α-pinene, sabinene, β-pinene, myrcene and sesquiterpene bicyclogermacrene were the major substances in the essential oils analyzed.

Table 1. Maximum and minimum concentration of the main substances identified in *Schinus molle* essential oils by GC-MS and quantified by GC-FID* in the four seasons of the year, in Volta Redonda, Brazil

Substances	**AI_c**	**AI_t**	**Seasons of the year**							
			Summer		autumn		winter		Spring	
			%							
			min	max	min	max	min	max	min	max
α-pinene	934	932	0.6	15.1	1.7	13.7	1.8	19.7	1.5	15.0
sabinene	977	969	1.3	26.5	2.9	45.5	2.3	45.5	1.3	45.4
β-pinene	981	974	0.6	21.3	1.5	19.7	1.6	26.9	1.2	21.9
myrcene	990	988	0.5	8.1	0.3	20.6	0.2	22.0	0.6	15.9
limonene	1031	1024	0.4	6.9	0.8	8.9	0.3	11.7	1.0	10.6
(*E*)-β-ocimene	1046	1044	0.1	0.2	0.1	1.6	0.1	1.4	0.0	0.0
γ-terpinene	1060	1054	0.6	0.6	0.4	0.8	0.0	0.0	1.1	1.1
cis-sabinene hydrate	1073	1065	0.2	0.3	1.5	1.5	0.5	1.0	0.6	0.6
trans-sabinene hydrate	1098	1098	0.1	1.1	0.3	0.3	0.2	0.2	0.0	0.0
terpinen-4-ol	1185	1174	0.9	1.1	0.6	1.1	0.1	0.1	1.3	1.3
α-terpineol	1186	1186	0.0	0.0	0.0	0.0	0.7	1.2	1.4	1.4
α-cubebene	1349	1345	0.7	0.7	0.5	0.6	0.6	0.6	0.7	0.7
α-yangene	1379	1373	0.0	0.0	0.0	0.0	0.2	0.9	3.0	3.1
α-copaene	1379	1374	0.1	3.2	3.0	3.7	0.2	3.3	0.0	0.0
β-cubebene	1390	1387	1.1	1.3	1.0	1.1	0.2	1.3	1.3	1.3
β-caryophyllene	1425	1417	4.0	14.7	5.8	9.7	3.4	10.6	3.8	10.2
α-humulene	1457	1452	0.3	0.8	0.4	0.9	0.5	0.7	0.6	0.6
allo-aromadendrene	1461	1458	1.3	3.0	1.6	2.3	0.2	2.6	1.9	2.6
germacrene D	1486	1484	1.6	7.2	3.2	6.2	3.0	5.6	1.9	5.1
bicyclogermacrene	1502	1500	5.6	34.3	8.6	23.6	7.4	20.9	5.4	21.1
γ-cadinene	1520	1513	5.0	5.0	2.1	4.0	0.0	0.0	4.8	4.8
10-epi-cubebol	1523	1533	0.6	16.1	1.0	13.8	1.0	14.8	5.4	8.5
spathulenol	1586	1577	3.9	5.9	2.0	5.4	1.7	2.4	2.2	6.8
caryophyllene oxide	1591	1582	2.8	3.9	1.3	4.6	0.7	1.5	1.4	2.8
gleenol	1601	1586	1.0	2.5	0.6	1.0	0.0	0.0	0.0	0.0
ledol	1613	1602	0.5	0.6	0.4	0.4	0.6	0.6	0.5	0.5
1,10-di-epi-cubebol	1623	1623	2.0	2.0	0.3	1.6	0.4	0.4	1.8	1.8
1-epi-cubenol	1641	1627	0.2	1.4	1.0	1.4	1.4	1.4	0.0	0.0
cis-cadin-4-em-7-ol	1635	1635	0.0	0.0	0.0	0.0	0.8	0.9	2.1	2.1
epi-α-cadinol	1654	1638	15.2	15.2	0.6	1.1	0.4	8.9	0.7	13.4
cubenol	1650	1645	0.6	0.7	12.7	12.7	0.6	0.6	0.0	0.0
α-cadinol	1664	1652	0.1	0.9	0.8	0.8	0.4	0.4	0.0	0.0
Monoterpene hydrocarbons			30.4	41.2	31.5	52.5	52.4	58.4	39.4	52.4
Oxygenated monoterpenes			1.2	7.2	0.0	2.9	0.0	2.5	0.0	2.8
Sesquiterpene hydrocarbons			20.9	57.9	24.5	40.8	20.4	38.4	20.3	36.4
Oxygenated sesquiterpenes			7.3	30.5	4.2	27.6	4.9	19.7	4.5	22.2
Total			95,8	98,7	97,6	98,9	97,6	98,5	94,0	96,0

*Concentration relative to the area of the peaks in the chromatographic analysis (CG-FID). Calculated and tabulated arithmetic retention index (AI_C and AI_T)

Table 2. Maximum and minimum concentration of the main substances identified in essential oils by GC-MS and quantified by GC-FID* in the four accessions of *Schinus molle*, collected in the city of Volta Redonda, Brazil

Substances	**AI_c**	**AI_t**	**Accessions**							
			1		2		3		4	
			%							
			min	max	min	max	min	max	min	max
α-pinene	934	932	0.6	2.6	1.8	13.7	8.0	18.2	2.3	19.7
sabinene	977	969	26.5	45.5	4.9	45.5	1.3	2.9	1.9	23.5
β-pinene	981	974	0.6	3.7	1.6	19.7	12.7	25.3	3.3	26.9
myrcene	990	988	0.5	22.0	0.4	15.9	0.3	0.6	0.2	20.6
limonene	1031	1024	0.3	2.0	0.4	8.9	6.9	11.7	0.8	10.6
(*E*)-β-ocimene	1046	1044	0.1	1.4	0.1	0.6	0.3	0.5	0.2	1.6
γ-terpinene	1060	1054	0.6	1.1	0.6	0.6	0.0	0.0	0.4	0.4
cis-sabinene hydrate	1073	1065	0.2	1.5	0.3	1.0	0.0	0.0	0.0	0.0
trans-sabinene hydrate	1098	1098	0.1	0.3	0.2	1.1	0.0	0.0	0.0	0.0
terpinen-4-ol	1185	1174	0.9	1.3	0.1	1.1	0.0	0.0	0.6	0.6
α-terpineol	1186	1186	0.7	0.7	1.2	1.4	0.0	0.0	0.0	0.0
α-cubebene	1349	1345	0.0	0.0	0.6	0.6	0.5	0.7	0.6	0.7
α-yangene	1379	1373	0.0	0.0	0.0	0.0	0.2	3.0	0.9	3.1
α-copaene	1379	1374	0.1	0.1	0.1	3.7	3.0	3.2	3.2	3.3
β-cubebene	1390	1387	0.0	0.0	0.2	1.1	1.0	1.3	1.3	1.3
β-caryophyllene	1425	1417	6.7	14.7	5.2	10.6	3.4	6.8	3.8	6.9
α-humulene	1457	1452	0.3	0.9	0.5	0.7	0.4	0.6	0.6	0.8
allo-aromadendrene	1461	1458	1.3	2.6	0.2	3.0	1.6	2.2	1.8	2.3
germacrene D	1486	1484	3.1	7.2	1.6	5.6	3.0	6.2	2.5	3.6
bicyclogermacrene	1502	1500	7.4	34.3	5.4	20.9	9.0	19.0	8.1	9.5
γ-cadinene	1520	1513	0.0	0.0	2.1	5.0	0.0	0.0	4.0	4.0
10-*epi*-cubebol	1523	1533	0.6	4.3	1.0	1.9	8.5	15.6	2.3	16.1
spathulenol	1586	1577	2.1	3.9	2.4	4.8	1.7	6.8	1.7	5.5
caryophyllene oxide	1591	1582	1.4	1.6	1.2	3.9	1.3	4.6	0.7	2.8
gleenol	1601	1586	2.5	2.5	0.6	1.0	1.0	1.0	0.6	0.6
ledol	1613	1602	0.6	0.6	0.5	0.6	0.4	0.5	0.5	0.5
1,10-di-epi-cubebol	1623	1623	0.4	0.4	1.8	2.0	0.3	0.3	1.6	1.6
1-epi-cubenol	1641	1627	0.2	1.4	1.0	1.0	1.3	1.4	1.4	1.4
cis-cadin-4-em-7-ol	1635	1635	0.0	0.0	0.0	0.0	0.9	2.1	0.8	0.8
epi-α-cadinol	1654	1638	0.7	8.9	0.4	15.2	0.6	2.0	1.0	1.0
cubenol	1650	1645	0.0	0.0	0.0	0.0	0.6	0.7	0.6	12.7
α-cadinol	1664	1652	0.1	0.4	0.9	0.9	0.0	0.0	0.8	0.8
Monoterpene hydrocarbons			30,4	57,2	41.2	52.4	31.5	58.4	40.3	52.8
Oxygenated monoterpenes			1,2	2,9	0.0	3.2	0.0	7.2	0.0	2.6
Sesquiterpene hydrocarbons			20,4	57,9	20.3	40.8	22.6	38.6	24.5	29.5
Oxygenated sesquiterpenes			4,2	19,7	4.9	30.5	16.6	27.7	14.5	27.4
Total			94,6	98,8	95,8	98,2	96,0	97,6	94,0	98,9

*Concentration relative to the area of the peaks in the chromatographic analysis (CG-FID). Calculated and tabulated arithmetic retention index (AI_C and AI_T).

The highest concentration of α-pinene (19.7 %) was found in the essential oil of accession 4 in winter, of sabinene (45.5 %) in accessions 1 and 2 in autumn and winter, of β-pinene (26.9 %) in accession 4 in winter, of myrcene (22.0 %) in accession 1 in winter and bicyclogermacrene (34.3 %) in accession 1 in summer (Table 1 and 2).

In general, the predominant classes of terpenes found in the essential oil of *S. molle* were monoterpene hydrocarbons, followed by sesquiterpene hydrocarbons. The oxygenated sesquiterpenes presented the greatest variability between the seasons and between the accessions (Table 1 and 2).

The principal component analysis presented in Figure 2 allowed observing the dispersion of three distinct groups. On the axis of principal component 1, representing 54 % of the observed variance, sabinene, α-pinene and β-pinene presented the greatest contribution to the formation of the groups. On the axis of principal component 2, representing 25 % of the observed variance, the sesquiterpene hydrocarbons, bicyclogermacrene, myrcene and monoterpene hydrocarbons contributed most to the dispersion (Figure 2).

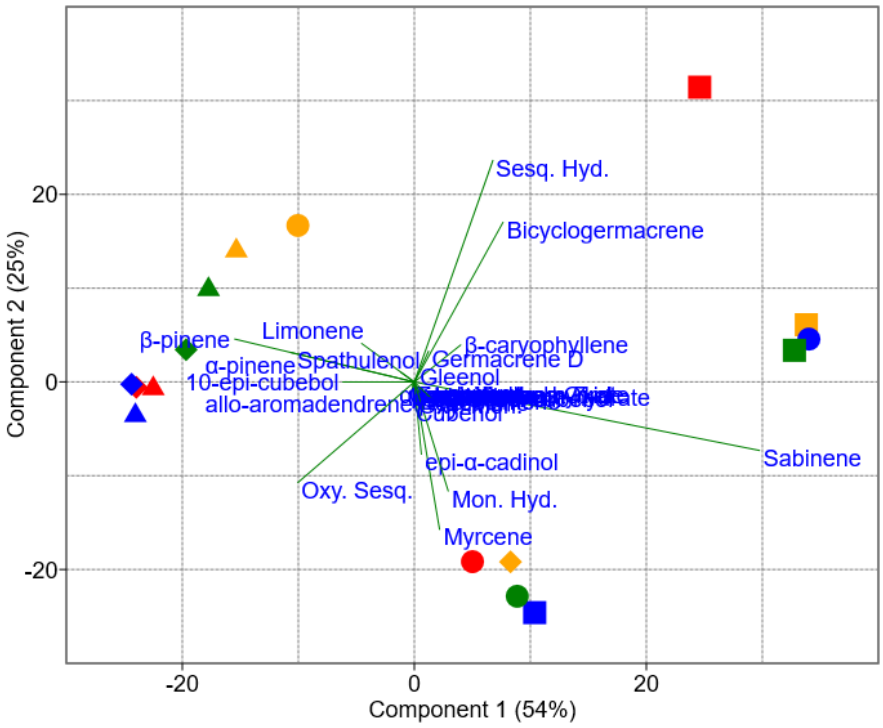

Figure 2. Biplot graph of principal component analysis based on 16 essential oil samples from four *S. molle* accessions collected during four seasons of the year in the city of Volta Redonda, Brazil (scores) and 32 identified substances plus four chemical classes (loading) obtained from chromatographic analyses (GC-FID and GC-MS). Symbols and colors: summer (red), autumn (orange), winter (blue), spring (green), accessions 1(□), 2 (○), 3 (△) and 4 (◇)

The hierarchical cluster analysis presented in Figure 4 allowed visualizing three groups formed based on the chemical profile of the essential oils: group 1) accession 1 collected in summer, autumn and spring and accession 2 collected in the winter; group 2) accession 3 collected in the four seasons, accession 4 collected in the summer, winter and spring and accession 2 collected in the fall; group 3) accession 2 collected in summer, accession 4 collected in autumn and accession 1 collected in winter.

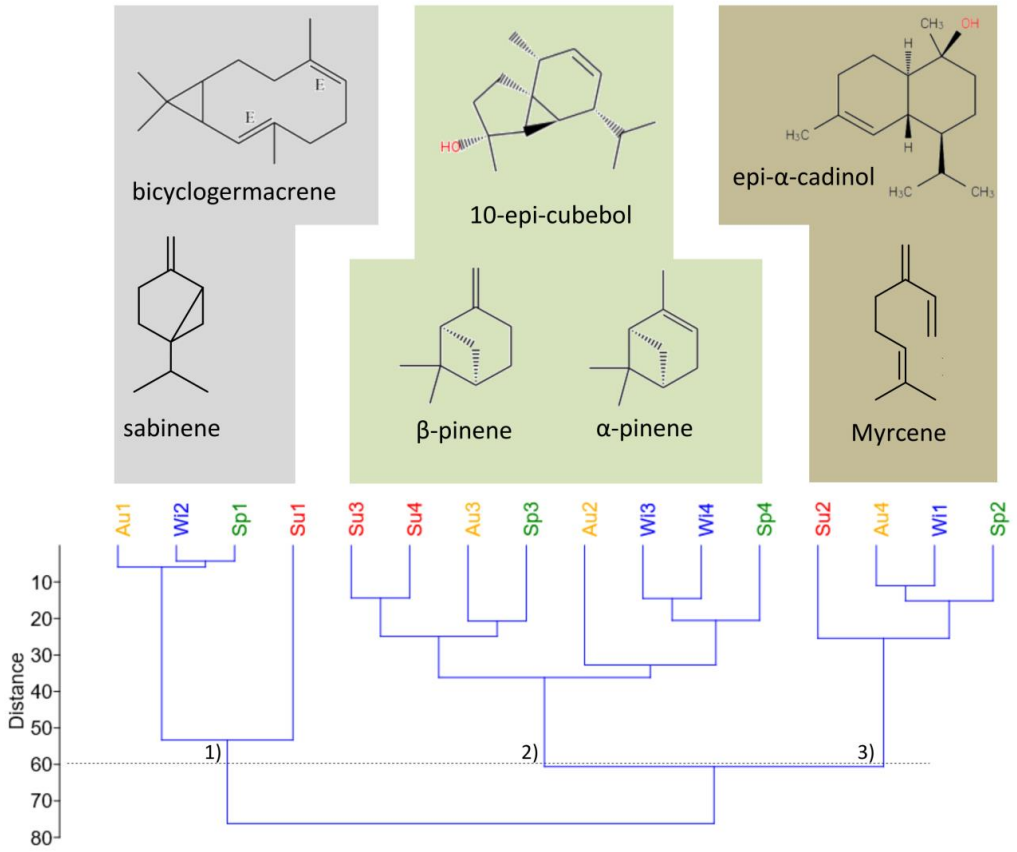

Figure 4. Hierarchical cluster analysis chart based on the chemical profile of 16 essential oil samples obtained from chromatographic analyses (GC-FID and GC-MS) of four accessions of *S. molle* collected during four seasons of the year in the city of Volta Redonda, Brazil

The chemical substances that most contributed to the formation of group 1 were sabinene and bicyclogermacrene, which are monoterpene and sesquiterpene hydrocarbons respectively. The chemical substances that contributed most to the formation of group 2 were α-pinene and β-pinene, which are monoterpene hydrocarbons, and 10-epi-cubebol, an oxygenated sesquiterpene. Finally, the chemical substances that contributed most to the formation of group 3 were myrcene and epi-α-cadinol, which are a monoterpene hydrocarbon and oxygenated sesquiterpene, respectively (Figure 4).

The essential oil content (% m/m) obtained from the different accessions and in the different seasons were similar to other data previously published.[3, 13]

Muhdl *et al.* (2005) [14], who studied 11 populations of *S. molle* collected in different locations in the state of Rio Grande do Sul (Brazil) and Uruguay, found four predominant chemical groups: sabinene, α-pinene/β-pinene, cadinol and myrcene, similar to our study.

Turkey *et al.* [15] investigated the composition of the essential oil from fruits and leaves of *S. molle* and verified the existence of a-phellandrene, b-phellandrene and limonene chemotypes.

In Tunisia a study of the essential oil profile of *Pistacia lentiscus* L., from the same family as *S. molle*, was carried out, showing chemical groupings according to the bioclimatic system, generating two distinct populations: the first, belonging to the semi-arid bioclimate, containing the major constituents a-pinene,

sabinene, b-pinene, myrcene, limonene, and terpinen-4-ol; and the second, from the moist and sub-humid bioclimate, containing essential oil rich in germacrene D, cadinene, a-cadinol and b-cadinol.[16]

Genetic factors determine the chemical composition of essential oils, although other factors also contribute significantly to the production of secondary metabolites, such as environmental and nutritional conditions, water and light availability, pest infestation, disease, competition with other plants and agricultural management.[17-23]

4. Conclusion

From the point of view of the samples, the *Schinus molle* accession number 3 was the most stable in the seasonality study among the others, and its major constituents were concentrated in the same region regardless of the season, obtaining similar values during the year. The *Schinus molle* accession number 4 presented good stability because like *Schinus molle* accession number 3, it was concentrated in a certain region, having only one accession out of order (autumn). It was possible to observe similarity between *Schinus molle* accession number 3 and 4, although one site was outside the region of highest concentration. The *Schinus molle* accession number 1, with the exception of winter, presented good stability, as well as, *Schinus molle* accession number 4, concentrated in the same region. The *Schinus molle* accession number 2 presented the least chemical stability, not having a defined region, being similar only in summer and spring. In each season it presented a different composition. This study showed that plants from each collection site had a higher or lower concentration of certain components such as bicyclogermacrene, beta-caryophyllene and sabinene, and the presence or absence of others depending on the season. Although they are the same species, they were grown in different soils under different climatic conditions, and different external factors may have influenced these results. These factors were not evaluated in this study.

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