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Artigo

SEASONAL VARIATION IN THE COMPOSITION OF VOLATILE OILS FROM *Schinus terebinthifolius* RADDI

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Essential oils from leaves, ripe and unripe fruits of *Schinus terebinthifolius* growing in Brazil were investigated. Oil content from either ripe or unripe fruits was similar (4.65% and 3.98%, respectively). Sesquiterpenes (from 78.0% to 90.4%) dominated the oil content of both leaves and unripe fruit. The essential oils were tested *in vitro* for their allelopathic activity on germination and radicle growth of *Lactuca sativa* and *Cucumis sativus* at 1,000 and 10,000 μg mL^{-1}concentrations. The three samples tested were more active in inhibiting the radicle growth for *L. sativa* (88.6-92.4%) than for *C. sativus* (50.5-84.5%) at 10,000 μg mL^{-1} concentration.

Keywords: *Schinus terebinthifolius*; essential oils; allelopathic activity.

INTRODUCTION

Schinus terebinthifolius Raddi (Anacardiaceae) is a perennial tree indigenous to the coast of Brazil, and has been introduced into other South American countries, parts of Central America, Bermuda, the Bahama Islands, the West Indies, Florida, Southern Arizona, California, Hawaii, Mediterranean Europe, North Africa, Southern Asia and South Africa.[1-3] It is known by a variety of common names including "aroeira-vermelha", "aroeira-pimenteira", Brazilian pepper, Christmas-berry, pink-pepper, poivre rose.[1,4,5] In Brazil, *S. terebinthifolius* dried fruits are also marketed as a substitute for black pepper and is occasionally found as a pink seeded adulterant in *Piper nigrum* (Black Pepper) in other countries. Many medicinal properties have been attributed to this plant, such as antioxidant,[6-8] wound-healing,[9] antitumor[10] and antimicrobial[11,12] activities. In Brazil, the extract of stem bark is widely used as an anti-inflammatory and to heal over or cicatrize wounds.[13] Specifically, the crushed, dried leaves are applied as antiseptic poultices upon skin ulcers. Relief from bronchitis and other respiratory ailments is treated by leaf infusions. Interestingly, the juice of macerated roots is considered effective in treating ganglionic tumors. Although this plant is widely used for medicinal purposes, mutagenic (cytotoxic) activity detected within extracts from its stem bark[14] as well as hypersensitivity to the volatile oil warrant considerable caution in indiscriminate use of this natural remedy until a full toxicological profile is available.

Despite the aforementioned benefits, the Brazilian pepper tree is often undesirable outside its native range. Since its introduction into the United States in the late 19th century as an ornamental tree, it has been recognized as an invasive, exotic plant widely found in Florida and Hawaii that out competes native species.[1,2,15,16] Therefore, *S. terebinthifolius* has been considered one of the most serious biological threats to the Everglades upland ecosystem, and classified in Category I of the Florida Exotic Pest Plant Council's List of Invasive Species. A plant in that category is defined as being able to alter the structure and function of native communities.[17] In a recent, study the allelopathic effects of Brazilian pepper aqueous extracts upon germination and growth of selected Florida native plants has been demonstrated.[15]

The occurrence of deleterious biochemical interactions among higher plants, known as allelopathy, is generally considered a significant ecological factor in determining the structure, variety and composition of plant communities.[18] A variety of allelochemicals are known, including components from essential oils, that both inhibit seed germination as well as plant growth.[18-22] Some of the essential oils considered to present allelopathic effects can be extracted from *Tagetes minuta* L. (Compositae), *Schinus areira* L. (Anacardiaceae),[18] *Ruta graveolens* L. (Rutaceae),[19] *Rosmarinus officinalis* L., *Thymus vulgaris* L. (Labiatae) *Satureja montana* L (Lamiaceae)[20] and *Conyza albida* Willd. (Compositae).[21]

The essential oils from leaves, flowers and fruits of *S. terebinthifolius* from different locations have been previously investigated and some variation on their chemical composition have been observed.[10,23-26] Most of the oil samples analyzed revealed α-pinene (15.01-51.82%) as the major component, especially those originating from India.[23-26] Other major constituents were α-phellandrene, elixene, germacrene D, limonene and *p*-cymene.

This investigation details the essential oil composition of both leaves and fruits from *S. terebinthifolius* collected in Viçosa - Brazil. In addition, the allelopathic properties of the oil extracts obtained upon seed germination and radicle growth of *Lactuca sativa* (lettuce) and *Cucumis sativus* (cucumber) are reported.

EXPERIMENTAL

Plant material and commercial oil

The aerial parts (leaves and fruits) of *S. terebinthifolius* were collected from wild plants found in the campus of Universidade Federal de Viçosa, Minas Gerais State (Brazil). A voucher specimen (VIC 30839) was deposited in the Herbarium of the Botany

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Department at the same university. Plant samples were collected at intervals of 30 days, starting in October 2004 and ending in September 2005. In October 2004, when the plants started flowering, two samples of leaves were collected, one from branches with flowers (ILO) and another one from branches without flowers (LO). In December 2004 and February 2005, samples (100 g each) of both unripe and ripe fruits were collected, respectively. A sample of commercial oil (CO), extracted by supercritical fluid extract of fruits of *S. terebinthifolius* (trade mark FLAVEX®), was procured from French commercial sources and analyzed as a comparator.

Essential oil extraction

Oil extraction of fresh samples of both leaves and fruits of *S. terebinthifolius* (20 g of each component), over a three hour period, was achieved using a Clevenger apparatus.[27] The resulting oils obtained were weighed and the yields were expressed relative to the dry matter content of either leaves or fruits. Leaf dry weight was calculated by drying each sample (2 g, held at 103 ± 2 ºC for 24 h) according to published methods.[28] Each determination was carried out in triplicate.

In order to evaluate the influence of the extraction time on the oil composition, a sample of ripe fruit (20 g) was subjected to the aforementioned oil extraction procedure and the oil was collected at intervals of 20 min, over a three hour period. This experiment was also carried out in triplicate.

Gas chromatography-mass spectrometry (GC-MS)

The essential oil samples were analyzed by both gas chromatography (Shimadzu GC–17A equipped with a flame ionization detector (FID) and by gas chromatography-mass spectrometry (GC-MS); Shimadzu GCMS-QP5050A apparatus, equipped with an ion trap detector, operating in electron impact mode (70 eV); scan speed 1000; scan interval 0.50 and fragments were scanned between 45 to 450 Da. Identical chromatographic conditions were used in both analyses: fused silica capillary column (30 m x 0.22 mm) with a DB5 bonded phase (0.25 µm film thickness); under the following conditions: carrier gas N_2 (GC) or He (GC-MS), flow rate 1.8 mL min^{-1}; injector temperature 220 °C, detector temperature 240 °C; column temperature was programmed to hold at 60 °C (isothermal for 2 min), then ramped by 3 °C min^{-1}, to 240 °C, then isothermal at 240 °C for 15 min; injection volume was 1.0 µL (1% solution in CH_2Cl_2), in split mode, with ratio of 1:10. Each component was identified by comparison of acquired mass spectrum with reference data from a commercially available database (Wiley 330.000), literature data[29] and also by its experimental Kovat's retention index (KI) calculated from a C_9-C_{24} *n*-alkanes series.[29,30] The chemical components amounts were calculated from the GC–17A peak area, and the results presented are the average of three replicate experiments.

Germination and growth assay

Bioassays were carried out as previously described,[31] with seeds of *Lactuca sativa* and *Cucumis sativus.* Essential oils solutions from fresh leaves, unripe and ripe fruits were prepared at concentrations of 1,000 and 10,000 µg mL^{-1} in dichloromethane. Assays were conducted in a 90 x 15 mm glass Petri dishes lined with 1 sheet of Whatman Nº 1 filter paper and sealed with Parafilm®. To each dish was added 3 mL of each solution and the solvent was evaporated before addition of 3 mL of water followed by 20 seeds of *L. sativa* or *C. sativus.* Assays were carried out at 25 ºC under artificial fluorescent light (8 x 40 W) in an incubator for 3 days. Subsequently, after which germination was scored and the radicle length was measured. Seeds were considered to have germinated if a radicle protruded at least 1 mm. A control experiment was carried out under the same conditions described but using only water instead of the test oil. Each bioassay was replicated 5 times in a complete randomized design. The percentage of root growth inhibition was calculated with respect to the root length of the water-treated control. The results were analyzed by the Tukey's test at 0.05 probability level.

RESULTS AND DISCUSSION

A preliminary study was undertaken to ascertain optimal distillation time on both the yield and composition of the oil obtained. Consequently, hydrodistillation of the ripe fruits was carried out during a period of 3 h and the oil obtained was collected every 20 min. After 3 h of extraction, the amount of oil obtained corresponded to 4.65% w/w, in relation dry fruit weight. A major proportion (78%) of the total oil present in the fruits was extracted within 20 min (Figure 1), but the last 20 min (from 160 to 180 min) only provided a small amount (2%) of the total oil extracted.

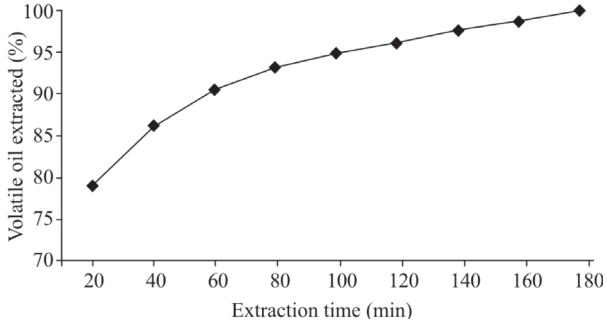

***Figure 1**. Yield of volatile oil with extraction time*

The 27 major constituents positively identified in each oil fraction are shown in Table 1. For the first two fractions (20 and 40 min) the compounds identified corresponded to more than 90%, relative to the total chromatogram area. With longer extraction time, the amount of oil extracted decreased and the percentage of compounds identified was also smaller, reducing to around 80% of the total chromatogram peaks areas for the last fraction (160 to 180 min).

Conveniently, the oil components can be grouped into four major classes: hydrocarbon monoterpenes (HM); oxygenated monoterpenes (OM); hydrocarbon sesquiterpenes (HS) and oxygenated sesquiterpenes (OS). The percentage of area corresponding to each compound class was calculated for each fraction (Figure 2). In general, the lighter compounds (HM) were extracted almost completely during the first 20 min (94%), and a smaller amount (22%) was obtained during the last 20 min of extraction.

The percentage of OM was very small in all fractions (<3%). The HS and OS respectively were present in 5% and 0.8% respectively in the oil extracted during the first 20 min, but was higher (22 and 53%) in the last fraction (160 to 180 min).

From the results presented in Figure 1 and 2, a total extraction time of 180 min appeared optimal and was chosen for all subsequent experiments, as it allowed most, if not complete extraction of the less volatile oxygenated sesquiterpenes.

Using this extraction procedure (3 h) for the leaves collected from branches without flowers (LO), the oil content was 0.44% w/w, and for those from branches that were flowering (ILO), the amount found

Table 1. Major terpenoid components identified in the essential oil extracted from ripe fruits. The total extraction time was 180 min and samples were collected every 20 min

Compounds	IK*	Time (min)								
		20	40	60	80	100	120	140	160	180
		Chromatographic area (%)								
α-Thujene	933	0.20	0.14	0.15	0.14	0.10	0.16	0.20	0.16	0.14
α -Pinene	940	**14.31#**	**8.28**	**6.48**	**5.51**	**3.12**	**2.54**	**2.56**	**2.13**	**1.80**
Sabinene	977	3.49	1.34	0.93	1.28	0.55	0.42	0.39	0.39	0.26
β-Pinene	980	3.47	2.07	1.67	1.81	0.99	0.82	0.87	0.74	0.70
β-Myrcene	991	5.69	3.59	2.80	3.83	1.63	1.29	1.30	1.08	0.96
α-Phellandrene	1004	**12.94**	**9.16**	**7.45**	**9.12**	**4.58**	**3.68**	**3.76**	**3.17**	**2.88**
Δ^3-Carene	1011	**30.09**	**21.74**	**17.15**	**14.27**	**10.23**	**7.87**	**7.80**	**6.42**	**5.82**
p-Cymene	1026	1.32	0.95	0.85	0.84	0.69	0.60	0.74	0.60	0.61
β-Phellandrene	1031	**18.51**	**13.85**	**11.17**	**7.73**	**7.07**	**5.62**	**5.79**	**4.79**	**4.49**
α-Terpinolene	1088	1.05	1.04	0.89	0.79	0.61	0.51	0.53	0.47	0.44
Terpin-4-ol	1177	0.17	0.86	1.11	1.23	1.57	1.75	2.18	2.17	2.24
δ-Elemene	1339	0.15	0.59	0.57	0.43	0.42	0.37	0.28	0.32	0.29
α-Copaene	1376	0.13	0.34	0.35	0.33	0.34	0.31	0.33	0.32	0.30
β-Elemene	1391	0.49	0.88	0.96	1.88	0.89	0.86	0.69	0.80	0.74
(*E*)-Caryophyllene	1418	1.23	3.76	3.80	1.87	3.32	3.17	2.60	2.79	2.54
α-Humulene	1454	0.09	0.40	0.47	4.28	1.09	1.05	0.84	0.97	0.85
Germacrene D	1481	**2.16**	**8.67**	**8.73**	**5.49**	**7.39**	**6.55**	**4.86**	**5.22**	**4.39**
α-Muurolene	1499	0.30	1.65	1.76	3.57	2.64	2.68	1.70	1.77	1.62
γ-Cadinene	1513	0.14	0.42	0.67	0.78	0.98	1.04	0.93	1.07	1.02
δ-Cadinene	1524	0.29	2.93	4.87	6.34	6.92	7.05	6.37	6.77	6.33
Elemol	1548	**0.31**	**3.43**	**6.32**	**5.64**	**11.33**	**12.93**	**12.80**	**13.64**	**13.62**
Germacrene B	1557	0.09	0.59	0.76	0.77	0.85	0.88	0.77	0.79	0.69
Caryophyllene oxide	1581	0.06	0.37	0.62	0.67	0.86	0.89	0.81	0.83	0.77
Germacrene D-4-ol	1631	0.07	0.58	1.38	1.94	3.19	4.02	4.59	5.09	5.33
epi-α-Cadinol	1642	0.05	0.53	1.31	3.19	3.04	3.76	4.17	4.50	4.56
α-Cadinol	1655	**0.09**	**1.48**	**3.79**	**4.74**	**9.38**	**12.07**	**14.12**	**15.50**	**16.26**
α-Bisabolol	1684	0.08	0.65	0.87	0.79	0.89	0.78	0.94	0.69	0.43
Total identified		96.97	90.29	87.88	89.26	84.67	83.67	82.92	83.19	80.36

*IK: Kovats retention index. # Bold values indicate compounds present in larger quantities.

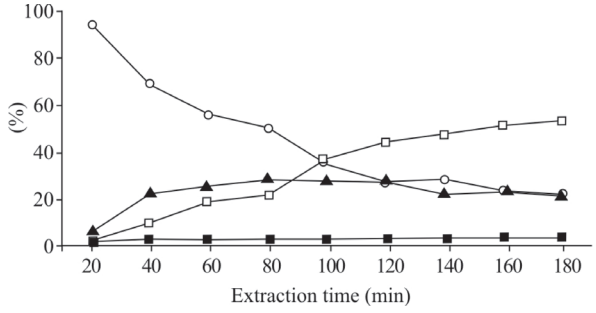

***Figure 2**. Percentage of the various classes of compounds present in the volatile oil from ripe fruits at different extraction times: HM (○), OM (■), HS (▲) and OS (□)*

was reduced to 0.11% w/w. We suggest that reduction in the oil content in the leaves is due to a change in metabolism, with the plant expending more energy and resources during the flowering process, for a parallel can be dawn the perennial rosemary (*Rosmarinus officinalis*), whose leaves show some variation in the oil content during the flowering stage.[32]

Analyses revealed that the unripe and ripe fruits contained 3.98 and 4.65% w/w of oil, respectively. Chromatographic and spectrometric analysis of the four oil samples obtained (LO, ILO, UFrO, RFrO) allowed the identification of a total of 57 compounds (Table 2). Both quantitative and qualitative differences were detected between samples. For instance, within sample LO, the major constituents were germacrene D (33.80%), (*E*)-caryophyllene (12.25%), β-pinene (5.18%) and (*Z*)-β-ocimene (5.16%); for sample ILO they were bicyclogermacrene (20.82%), germacrene D (16.06%), and β-elemene (5.92%). In contrast, the oil from unripe fruits had the following major constituents: α-cadinol (20.60%), δ-cadinene (15.48%), β-pinene (10.21%) and epi-α-muurolol (9.89%).

Oil compositions between unripe and ripe fruits differed (Table 2). Compounds were grouped into monoterpenes and sesquiterpenes and their percentage in each sample are presented in Figure 3.

Sesquiterpenes are major secondary metabolites within these leaves (83.60 and 90.40% for LO and ILO samples, respectively) and unripe fruits (78.0%). A sample of commercial oil of *S. terebinthifolius* (FLAVEX®) was analyzed for comparative purposes and the results are shown in Table 2 and Figure 3. Ripe fruits (RFrO) and the commercial oil (CO) are both rich in monoterpenes (90.00 and 79.50%, respectively). However, a closer inspection of Table 2 reveals major differences in their relative chemical composition. For instance, RFrO sample has 29.22% of Δ^3-carene while the commercial oil (CO) has only 6.32%. In contrast, α-pinene, α-phellandrene and germacrene D are higher in the CO sample (18.82, 23.55 and 11.89%, respectively) compared to the RFrO sample (12.94, 13.04 and 3.09%, respectively). These results suggest that essential oils of the leaves and unripe fruits cannot be used as

Table 2. Chemical composition of the essential oil from aerial parts[#]

Compounds	KI*	LO	ILO	UFrO	RFrO	CO
			Area (%)			
α-Thujene	933	-	-	-	0.21	0.20
α-Pinene	940	1.07	-	3.06	12.94	18.82
Sabinene	977	0.08	-	0.40	3.25	2.33
β-Pinene	980	5.18	0.09	10.21	5.02	2.54
Myrcene	991	0.05	-	0.41	-	1.99
α-Phellandrene	1004	1.14	-	0.14	13.04	23.55
Δ^3-Carene	1011	-	-	-	29.22	6.32
p-Cymene	1026	0.06	-	-	0.20	4.03
β-Phellandrene	1031	0.12	0.07	2.49	18.08	16.88
(*Z*)-β-Ocimene	1040	5.16	-	-	-	-
γ-Terpinene	1061	0.70	-	0.10	0.25	-
Terpinolene	1088	0.12	-	0.10	-	0.64
Linalool	1097	0.20	0.11	-	1.04	-
Borneol	1165	0.05	0.22	0.02	-	-
Terpin-4-ol	1177	0.81	1.17	0.62	0.29	-
α-Terpineol	1189	3.05	5.35	1.47	-	-
δ-Elemene	1339	2.61	-	-	-	-
α-Copaene	1376	0.53	0.34	0.29	-	0.32
β-Bourbonene	1384	0.12	0.29	-	-	-
β-Elemene	1391	1.16	5.92	1.15	0.32	-
α-Gurjunene	1409	0.08	0.08	1.80	-	-
(*E*)-Caryophyllene	1418	12.25	2.93	4.78	1.45	2.34
β-Gurjunene	1429	0.24	0.23	-	-	-
γ-Elemene	1433	0.15	0.21	-	-	-
Aromadendrene	1439	0.04	0.77	-	-	-
α-Humulene	1454	1.20	0.65	1.26	0.19	0.14
(*E*)-β-Farnesene	1458	-	0.23	0.62	-	-
Alloaromadendrene	1461	-	0.86	0.85	-	-
β-Chamigrene	1475	0.43	1.29	-	-	-
γ-Muurolene	1476	-	-	0.80	-	0.07
Germacrene D	1481	33.80	16.06	5.19	3.09	11.89
β-Selinene	1487	0.17	1.43	-	-	-
Bicyclogermacrene	1495	4.59	20.82	-	0.57	1.40
α-Muurolene	1499	0.95	-	2.85	-	-
Germacrene A	1504	0.78	4.81	0.63	-	-
γ-Cadinene	1513	0.55	0.31	1.58	-	-
δ-Cadinene	1524	2.95	3.11	15.48	1.22	0.32
Cadina-1,4-diene	1532	0.13	0.20	1.07	-	-
α-Cadinene	1537	0.21	0.09	0.37	0.15	-
α-Calacorene	1542	0.18	0.65	-	-	-
Elemol	1548	-	-	-	-	1.88
Germacrene-B	1557	2.58	-	-	0.22	0.64
(*E*)-Nerolidol	1563	0.17	0.32	-	-	-
Ledol	1567	-	0.72	0.53	-	-
Germacrene D-4-ol	1574	-	-	2.36	-	-
Spathulenol	1576	0.10	4.04	-	0.21	1.11
Caryophyllene oxide	1581	-	-	0.21	-	0.31
Globulol	1583	0.58	3.14	-	-	-
Viridiflorol	1591	0.69	2.69	0.98	-	-
Rosifoliol	1600	-	1.20	-	-	-
1-epi-Cubenol	1627	0.19	0.56	0.62	-	-
Isospathulenol	1628	1.84	-	-	-	-
γ-Eudesmol	1631	0.35	0.44	-	-	-
epi-α-Muurolol	1642	2.45	-	9.89	0.35	-
α-Muurolol	1646	2.44	2.98	1.58		-
α-Cadinol	1655	2.73	5.32	20.60	1.21	-
α-Bisabolol	1684	0.80	0.31	0.42	0.26	-
TOTAL		**95.83**	**90.01**	**94.93**	**92.78**	**97.72**

*KI - Kovats retention index; # Oils from fresh leaves from LO, ILO, UFrO, RFrO and CO.

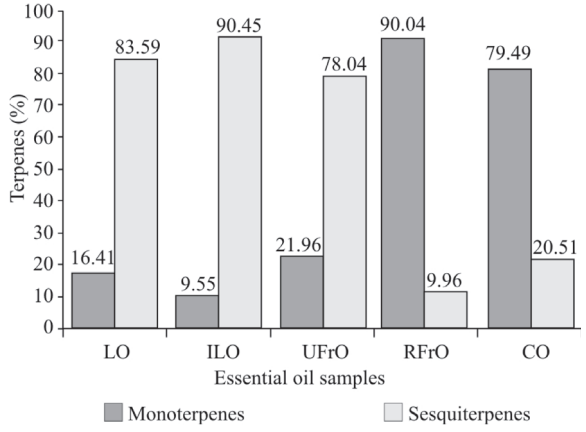

***Figure 3**. Percentage of monoterpenes and sesquiterpenes in the essential oil obtained from LO, ILO, UFrO, RFrO and CO*

commercial substitutes for FLAVEX®. Conversely, the oil from ripe fruits, obtained by steam distillation, from plants native to the Viçosa region in Brazil, has a potential commercial value. However care should be taken in order not to mix unripe with ripe fruits during the extraction process, since their chemical compositions are entirely different.

Following the discovery that oils obtained from leaves collected from branches, with and without flowers, were significantly different, a further study was carried out to ascertain the effect of any seasonal variation on leaf essential oil composition.

The oil content in the leaves of *S. terebinthifolius* shows some minor changes throughout the course of one year but these changes were usually statistically insignificant (Figure 4). Oil content peaked (0.65-0.69%) between March to September, and dipped (0.45-0.55%) between October to February, which coincided with flowering and fruiting.

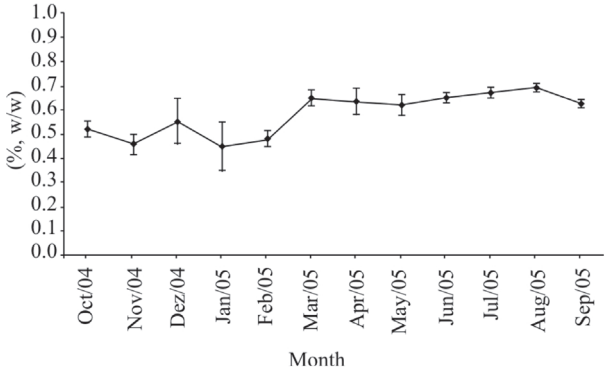

***Figure 4**. Changes in the essential oil content in the leaves during the course of one year, from October 2004 to September 2005*

Seasonal variation in the oil composition of the leaves was also investigated and the results presented in Figures 5 and 6. The two major classes of compounds were the HM and HS. The percentage of HM varied greatly during the year, with a minimum during the months of March and April (around 12%) and October and November (around 16.50%). A maximum quantity of HM occurred in December (36.84%), January (37.20%) and July (42.05%).

The HS also showed a considerable seasonal variation with a minimum content (approximately 47% in July) coinciding with the maximum of HM (Figure 5). In general, as observed in Figure 5, an increase in the amount of HM is accompanied by a decrease in the HS content, and *vice-versa*. This observation is concordant with literature precedent[33] which shows that the metabolism of these two classes of compounds is interconnected.

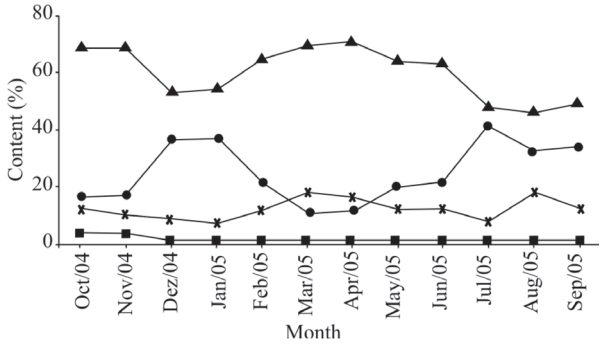

***Figure 5**. Changes in the relative percentage of HM (●), OM (■), HS (▲) and OS (✖) in the essential oil from the leaves during the course of one year*

The OS corresponded to the third most predominant group of compounds, with minimal production in the summer (7.35% in January) and maximum in the winter (18.73% in August). The OM were minor constituents from this oil, with levels ranging from 0.88% in April to 3.37% in October.

The variation in the relative abundance of the five major terpenes (*p*-cymene, (*Z*)-β-ocimene, (*E*)-caryophyllene, germacrene D and bicyclogermacrene) from the volatile oil from leaves during the course of one year is represented in Figure 6.

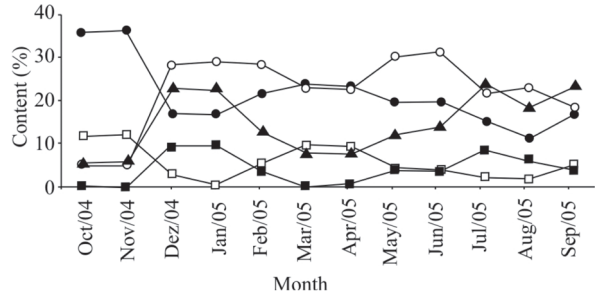

***Figure 6**. Changes in the relative percentages of p-cymene (■), (Z)-β-ocimene (▲), (E)-caryophyllene (□), germacrene D (●) and bicyclogermacrene (○) in the volatile oil from the leaves during the course of one year*

As the amount of germacrene D and (*E*)-caryophyllene decreases, there is a corresponding increase in the content of bicyclogermacrene, particularly from November to December (Figure 6). These results suggest that the biosynthesis of these terpenes is interrelated and a possible biosynthetic pathway for the formation of bicyclogermacrene, germacrene D and (*E*)-caryophyllene is presented in Figure 7. As reported in the literature,[33-35] farnesylpyrophosphate (**1**, FPP) can be converted into the non-classical carbocation **2**, that can led to tertiary carbocations **3** or **9**. Isomerization of **3** to **6**, followed by intramolecular cyclopropane formation and proton ejection results in the formation of the more thermodynamically stable bicyclogermacrene (**8**). The intermediate **9** can be transformed into **10**, and a subsequent elimination of H^+ results in the formation of (*E*)-caryophyllene (**11**). From this biosynthetic pathway, an increase in the formation of **8** has to be accompanied by a decrease in the accumulation of **5** and **11**.

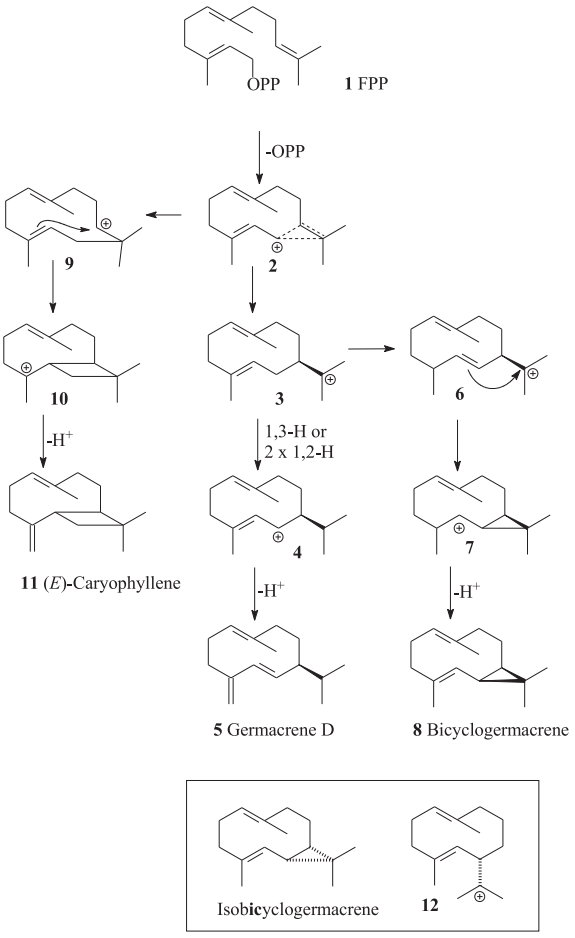

Figure 7**. Proposed biosynthetic pathways[33] for compounds **5**, **8** and **11** and structures of isobicyclogermacrene and the intermediate biogenetic cation **12

Allelopathic effect

The effects of essential oils from fresh leaves (LO), unripe (UFrO) and ripe (RFrO) fruits were evaluated upon radicle growth and seeds germination of *Lactuca sativa* (lettuce) and *Cucumis sativus* (cucumber). At the concentration of 1,000 μg mL^{-1}, samples LO and UFrO caused small root inhibition on *C. sativus* (22.4 and 33.1%, respectively) and no effect on *L. sativa*. Sample RFrO had no effect on both species. None of the samples caused any significant inhibition on seed germination on both plants tested. However, at the concentration of 10,000 μg mL^{-1}, all samples caused significant inhibition on the radicle growth of both species and, in general, the three samples tested were more active in inhibiting the radicle growth for *L. sativa* (88.6-92.4%) than for *C. sativus* (50.5-84.5%). For *L. sativa*, no significant differences were observed in the activity upon application of the three samples (LO, UFrO, RFrO), by Tukey's test at 0.05 probability level.

For *C. sativus*, using the same test, no significant difference was observed between the activity of samples LO and UFrO. However, sample RFrO was significantly less active than the other two.

Sample LO, consists mainly of sesquiterpenes (83.60%), included germacrene D, bicyclogermacrene and (*E*)-caryophyllene, caused 88.6 and 78.7% inhibition on the radicle growth of *L. sativa* and *C. sativus*, respectively (at 10,000 μg mL^{-1}). Also at 10,000 μg mL^{-1}, sample UFrO, also rich in sesquiterpenes (78.00%), including α-cadinol, β-cadineno and epi-α-muurolol), caused 90.1 and 84.5% radicle growth inhibition of *L. sativa* and *C. sativus*, respectively. However, sample RFrO, behaved differently from the two previous terpene fractions, is composed mainly of monoterpenes (90%), with major components being Δ^3-carene, α-phellandrene and β-phellandrene, α-pinene and β-pinene. This sample caused considerable radicle growth inhibition in *L. sativa* (92.4%), but its effect on *C. sativus* was halved (50.5 %).

At 1,000 μg mL^{-1} dose none of the three samples (LO, UFrO and RFrO) caused any significant inhibition on the germination of neither *L. sativa* nor *C. sativus*. In contrast, at the higher dose of 10,000 μg mL^{-1}, no significant inhibition was observed for *C. sativus.* At the same concentration, samples LO and UFrO, dominated mainly by sesquiterpenes, presented the same biological activity (75% of germination inhibition), while the RFrO sample, composed mainly of monoterpenes, was significantly more active (90% of germination inhibition).

The allelopathic activity of both monoterpenes[36,37] and sesquiterpenes[38,39] is thoroughly documented. Amongst the active monoterpenes, there are a variety of oxygenated compounds especially nerol, citronellol, geraniol, linalool, terpinen-4-ol, α-terpineol, borneol, carvone, fenchone, pulegone, camphor, 1,8-cineol, 1,4-cineol, carvacrol and ocimenone.[18,20,22,36,40,41]

It seems that the allelopathic activity showed by the oil of ripe fruits of *S. terebinthifolius* is not due to oxygenated monoterpenes, since these are present in very low concentration in this oil, with respect to the hydrocarbon monoterpene content. In the case of *S. terebinthifolius*, both essential oils rich in sesquiterpenes (LO and UFrO) and that rich in monoterpenes (RFrO) showed significant radicle growth inhibition for *L. sativa* and *C. sativus*, and significant inhibition of germination of *L. sativa.* Although it is not yet possible to attribute such activities to any specific constituent of these oils, it is relevant that major components, or combination of components, from the oil of unripe fruits are sesquiterpenes derived from cadinene (α-cadinol, β-cadineno, δ-cadineno, γ-cadineno, epi-α-muurolol), the same compounds that presented allelopathic activity as reported in the literature.[38]

In conclusion, although we were unable to locate any literature on the chemical composition requirements for the commercial volatile oil of *S. terebinthifolius*, we believe that the oil obtained by hydrodistillation from *S. terebinthifolius* ripe fruits, found in Viçosa (Brazil), can be used as a substitute for the commercial oil obtained by supercritical fluid extraction. Care should be taken in the processing stage in order to avoid the mixture of ripe and unripe fruits *S. terebinthifolius*, as their chemical constitution are very different. Importantly, the reported allelopathic activity observed for *S. terebinthifolius* can, in part, be explained by the volatile oil produced in their leaves and fruits.

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