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Essential oils in aerial parts of *Myrcia tomentosa*: composition and variability

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Abstract: Species in the Myrtaceae family are used in folk medicine to treat gastrointestinal disorders, infectious diseases and hemorrhagic conditions and are known for their essential oil contents. Gas chromatography coupled with mass spectrometry (GC-MS) was used to characterize the chemical composition of essential oils of the leaves, stem bark and flowers of *Myrcia tomentosa* (Aubl.) DC., as well as to assess the chemical variability in the constituents of the essential oils of the leaf. Soil and foliar analyses were also performed to determine the mineral compositions. Principal component analysis (PCA) was used to examine the interrelationships between the obtained data. The most abundant component in the essential oils of the flowers was (2*E*,6*E*)-methyl farnesoate, whereas hexadecanoic acid was the most abundant essential oil component in the stem bark. The leaf essential oils showed seasonal variation in their chemical composition, with bicyclogermacrene and (2*E*,6*E*)-methyl farnesoate as the major chemical components. Forty-four constituents were identified, and only nine compounds were found in all of the samples. Sesquiterpenes were mainly produced in the flowers and leaves. The PCA showed a positive correlation between the oxygenated sesquiterpenes and the foliar nutrients Cu and P. Significant statistical correlations were verified between the climatic data, foliar nutrients and essential oil compositions.

Article

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Introduction

The Brazilian Savanna is recognized as the richest source of biodiversity the world, with more than 6500 plant species already cataloged, over 220 of which have medicinal uses (MMA, 2009). In Brazil, the Myrtaceae family is one of the most important floras, containing 23 genera and approximately 130 species, and several species are used in folk medicine for the treatment of gastrointestinal disorders, infectious diseases and hemorrhagic conditions. Among the species of the Myrtaceae family, *Psidium guajava* L. and species of *Eugenia*, such as *Eugenia punicifolia* (Kunth.) DC, *Eugenia jambos* L., *Eugenia uniflora* L. and *Eugenia dysenterica* DC (Rodrigues & Carvalho, 2001; Souza et al., 2002; Di Stasi et al., 2002; Pessini et al., 2003; Fiuza et al., 2008) are exemplary. This family includes several species that are characterized by the presence of essential oils (Rodrigues & Carvalho, 2001; Holetz et al., 2002; Gondim et al., 2006; Amaral et al., 2006).

Although biological activities have often been described for aromatic herbs, many Myrtaceae species do not show a relationship between the presence of certain essential oils and such biological activities; rather, numerous biological activities that have been attributed to these plants are the result of constituents acting synergistically (Cunha, 2005).

One activity frequently described for these essential oils is their antimicrobial activity, particularly antibacterial and antifungal properties. Examples of these species include the following: *Syzygium aromaticum* (L.) Merrirl et L. M. Perry, *Thymus* sp., *Lavandula* sp., *Origanum vulgare* L., *Rosmarinus officinalis* L. and *Eucalyptus globulus* Labill (Simões & Spitzer, 2004; Cunha, 2005).

Studies examining the composition and biological properties of the essential oils found in several species of the Myrtaceae family (Limberger et al., 2001; Franco et al., 2005; Zabka et al., 2009; Magina et al., 2009) have been reported, and their

antimicrobial properties have been emphasized. The work of Cerqueira et al. (2007) on seasonal variation and antimicrobial activity and Alarcón et al. (2009) on chemical composition and antibacterial activity are examples of such antimicrobial studies for the genus *Myrcia*. Souza (2009) analyzed the chemical composition of nine species of the Myrtaceae family, two of which belonged to the genus *Myrcia*, Limberger et al. (2004) analyzed the composition of nine species of the genus *Myrcia*, and Zoghbi et al. (2003) analyzed three species.

The genus *Myrcia* has 300 species (Judd et al., 2009), for which a range of pharmacological activities have been described (Hecht, 1984; Almeida, 1993; Cerqueira et al., 2007; Xu et al., 2011). Within this genus, *Myrcia tomentosa*, popularly known as "goiaba-brava", can be found from Panama, northern Venezuela and Guyana to southeast Brazil (McVaugh, 1969) and is often cited in works on the flora, phytosociology and characterization of the Savanna/Cerrado (Mahmoud et al., 2003; Teixeira et al., 2004; Morais & Lombardi, 2006). However, few works report pharmacognostic or phytochemical studies for this species (Dianese et al., 1993; Cardoso & Sajo, 2006; Rossatto et al., 2009, Cardoso et al., 2009), and no reports have been found concerning its popular use.

Considering the wide distribution of volatile oils in higher plants and that the Myrtaceae family is considered rich in volatile oils, this study aimed to study the chemical composition of essential oils extracted from the aerial parts (leaves, flowers and stem bark) of *M. tomentosa* and to evaluate the seasonal variation in leaf constituents over the course of a year.

Materials and Methods

Plant material

Samples of *Myrcia tomentosa* (Aubl.) DC., Myrtaceae, were collected in Hidrolândia-GO, Brazil (16°53'59.4"S 49°13'29.4"W) and identified by Prof. José Realino de Paula. A voucher specimen was deposited in the Herbarium of the Federal University of Goiás (code number 41318). Six leaf samples were collected every two months, from June 2009 to April 2010. One sample of stem bark (October 2008) and one sample of the flowers (October 2009) were also collected. The leaves were air-dried at room temperature, and the stem bark was dried in a forced air oven at 40 °C; both tissue types were pulverized in a knife mill and then used for extraction of the essential oils. Fresh material was used for the extraction of essential oils from the flowers.

Essential oil extraction

The essential oils of the aerial parts of *M. tomentosa* were obtained by hydrodistillation in a modified Clevenger-type apparatus (2 h). After extraction, each essential oil sample was dried over anhydrous sodium sulfate and stored at -20 °C for further analysis.

GC-MS analysis of essential oils

The essential oils obtained were analyzed using a gas chromatograph interfaced with a mass selective detector (CG-MS), Shimadzu QP5050A, using an ionization voltage of 70 eV. A fused silica capillary column was utilized (CBP - 5; 30 m x 0,25 mm x 0,25 μm) and helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The temperature program used was as follows: ramp up from 60 to 240 °C at 3 °C min^{-1}, increase to 280 °C at 10 °C min^{-1}, and complete with 10 min at 280 °C. The injection volume was 1 μL diluted with CH_2Cl_2 at a ratio 1:5. The essential oil constituents were identified by comparing their mass spectra with those from the National Institute of Standards and Technology (NIST, 1998), as well as by comparing the mass spectra and calculated linear retention indices (RI) with values in the literature (Adams, 2007). Retention indices were obtained by co-injection with a mixture of linear hydrocarbons, C_9-C_{22} (Sigma, USA) and calculated using the equation of Van Den Dool & Kratz (1963). The percentage of each component was calculated to normalize for the area in the chromatogram obtained using a Varian gas chromatograph (FID) equipped with a ZB-5 fused silica capillary column that was 30 m x 0.25 nm with 0.25 μm film thickness (5% phenylmethylpolysiloxane). The following temperature program was used: increase from 60 to 240 °C at 3 °C min^{-1}, followed by an increase to 280 °C at 10 °C min^{-1}, and complete with 10 min at 280 °C. The carrier gas was N_2, at a flow rate of 1.0 mL/min; the injector port and detector temperatures were 220 °C and 240 °C, respectively. Samples were injected by splitting, and the split ratio was 1:20.

Soil and foliar nutrient analyses

The analyses of the chemical composition of the soil and leaf nutrients were performed by the Laboratory of Soil and Foliar Analysis, School of Agronomy, Federal University of Goiás, according to the methodology of Silva (2009).

Approximately 1 kg of soil from the site of collection was taken from a depth of 30 cm in four locations around the specimens of *M. tomentosa*. For the analysis of foliar nutrients, leaf samples (15 g) were

used. Three replicate measurements were performed per plant sample.

Statistical analyses

Principal component analysis (PCA) was used to examine the interrelationships between the climatic data, foliar nutrients and essential oil composition. Cluster analysis (CA) was used to examine the similarity of the samples in their constituent distribution, and hierarchical clustering was performed according to Ward's variance minimizing method (Ward, 1963). Prior to the multivariate analysis (PCA and CA), the data were preprocessed by auto-scaling and mean centering. A Pearson correlation analysis was employed to determine the association between the climatic data, foliar nutrients and essential oil composition obtained from leaves of *M. tomentosa*. All analyses were performed using the software Statistica 7 and Past 2.12.

Results and Discussion

The percentage yields (v/w) of the leaf essential oils were 0.54%, which was greater than the yield found in other species, such as *Eugenia brasiliensis* Lamarck, *E. beaurepaireana* (Kiaerskou), *E. umbeliflora* (Berg.) and *Myrcia fallax* (Rich.) DC, which contained 0.07, 0.20, 0.33 and 0.25%, respectively, in studies by Magina et al. (2009) and Alarcón et al. (2009). The yield of essential oils in the fresh flowers was 0.31% (v/w), a value close to that obtained by Alarcón et al. (2009) for Myrcia fallax flowers. The yield of bark essential oils was 0.10% (v/w).

Thirty-one compounds were identified in the essential oils obtained from the bark and thirteen compounds were identified in the essential oils obtained from the flowers, representing 76.27 and 72.17% of all isolated compounds, respectively (Tables 1 and 2). (2*E*,6*E*)-Methyl farnesoate (14.39%) and hexadecanoic acid (22.05%) were the main compounds found in the stem bark, while in the flowers, the main compounds were espathulenol (7.36%), (2*Z*,6*Z*)-farnesol (10.65%) and (2*E*,6*E*)-methyl farnesoate (14.28%). Sesquiterpenes were predominant in the flowers, as has previously been found in *Myrcia fallax*; however, guaiol (27.5%) and aristolone (24.5%) were the primary compounds found in *Myrica fallax,* while monoterpenes, represented by α-pinene were the main components (62-87.3%) present in *Myrcia myrtifolia* DC (Cerqueira et al., 2007; Alarcón et al., 2009).

As shown in Table 3, 44 components were identified, representing 95.3 to 99.24% of the total essential oil content; however, only nine compounds were found in all six leaf samples (β-elemene, (*E*)-caryophyllene, (*E*)-β-farnesene, bicyclogermacrene, germacrene B, spathulenol, globulol, α-cadinol and (2*E*,6*E*)-methyl farnesoate) and their concentrations varied.

Table 1. Percentages of chemical constituents of *Myrcia tomentosa* bark oils.

RI	Constituent	Amount (%)
1100	*n*-nonanal	2.36
1177	terpinen-4-ol	0.55
1201	*n*-decanal	2.15
1376	α-copaene	0.96
1455	geranyl acetone	1.04
1456	(*E*)-β-farnesene	0.52
1523	δ-cadinene	1.95
1545	α-calacorene	0.63
1566	dodecanoic acid	1.00
1578	spathulenol	2.05
1583	caryophyllene oxide	2.14
1642	*epi*-α-Muurolol	1.19
1646	α-muurolol (=Torreyol)	1.11
1654	α-cadinol	1.80
1676	cadalene	1.16
1784	(2*E*,6*E*)-methyl farnesoate	14.39
1800	*n*-octadecane	0.54
1807	2-ethyhexyl-salicylate	0.77
1829	isopropyl tetradecanoate	1.96
1875	*n*-hexadecanol	0.75
1900	*n*-nonadecane	1.53
1913	(5*E*,9*E*)-farnesyl acetone	1.50
1960	hexadecanoic acid	22.05
1988	1-eicosene	1.96
2000	*n*-eicosane	1.03
2077	*n*-octadecanol	0.58
2100	*n*-heneicosane	2.83
2133	linoleic acid	2.59
2189	1-docosene	0.49
2200	*n*-docosane	0.72
2300	*n*-tricosane	0.93
	oxygenated monoterpenes	0.55
	sesquiterpene hydrocarbons	6.26
	oxygenated sesquiterpenes	9.06
	others	60.4
	total	76.27

(*E*)-β-farnesene was identified in all samples comprising 6.14% of the total essential oil content in August and 8.49% in October. Bicyclogermacrene was also found in all samples, ranging from 4.73% of the total

essential oil content in October to 14.71% in June. The amount of (2*E*,6*E*)-methyl farnesoate in the samples also varied over the year, making up less of the total essential oil content in April (5.33%) and more in October (47.29%).

Table 2. Percentages of chemical constituents of *Myrcia tomentosa* flower oils.

RI	Constituent	Amount (%)
1455	geranyl acetone	0.87
1578	spathulenol	7.36
1590	globulol	5.97
1592	viridiflorol	0.31
1654	α-cadinol	3.61
1684	(2*Z*,6*Z*)-farnesal	6.86
1698	(2*Z*,6*Z*)-farnesol	10.65
1713	(2*E*,6*Z*)-farnesal	4.40
1715	(2*E*,6*Z*)-farnesol	2.17
1741	(2*E*,6*E*)-farnesal	5.36
1784	(2*E*,6*E*)-methyl farnesoate	14.28
1865	benzyl salicylate	5.99
2043	kaurene	3.47
	sesquiterpene hydrocarbons	0.87
	oxygenated sesquiterpenes	46.69
	others	24.61
	total	72.17

(2*E*,6*E*)-Methyl farnesoate was the major component in samples collected in August, October, December and February comprising between 33.10% and 47.29% of the total essential oil content. In the April sample, *epi*-α-bisabolol was the major component, comprising 42.39% of the sample, while in June the main components were germacrene D (18.96%), (2*E*,6*E*)-methyl farnesoate (17.99%) and bicyclogermacrene (14.71%).

The component γ-muurolene was not found in August and December, however, it comprised of 4.18% and 18.46% of the total essential oil content in February and April, respectively. These results show the high variability of the composition of the essential oils, which may be due factors including the time of collection, collection site, growing conditions, plant age, climatic conditions, season, soil composition and storage period (Oliveira et al., 1998; Farias, 2004; Cunha, 2005).

Although (2*E*,6*E*)-methyl farnesoate was found in all of the essential oil samples, there is a difference in the chemical composition of the oils obtained from the bark, flowers and leaves of *M. tomentosa*, which can be seen in the dendrogram shown in Figure 2. Simões & Spitzer (2004) reported that the volatile oils obtained from different tissues of the same plant may differ in their chemical composition.

Results obtained from the Principal Component (PCA) and Cluster analyses showed a high level of chemical variability within the oils of *M. tomentosa*. Figure 1 shows the relative position of the samples according to the two first axes originated in the PCA. The majority of the data could be represented on two first axes, which explains 62.83% of the total variance (Component 1=36.16% and Component 2=26.67%; Figure 1). The strong positive correlation between the oxygenated sesquiterpenes and the foliar nutrient variables Cu and P is clear; however, sesquiterpene hydrocarbons show the opposite behavior.

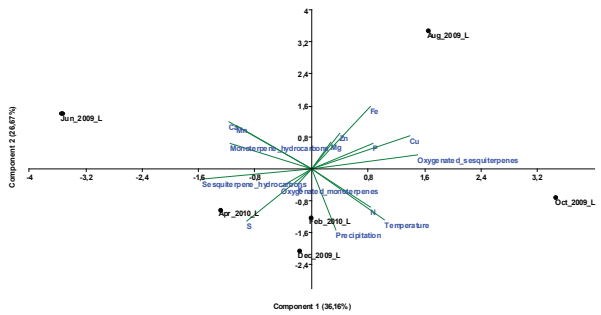

Figure 1. Scatterplot of samples of *Myrcia tomentosa* leaves collected in Hidrolândia-GO; Axes refer to scores from the samples; between parentheses refer to the explained variance on each Principal Component from PCA.

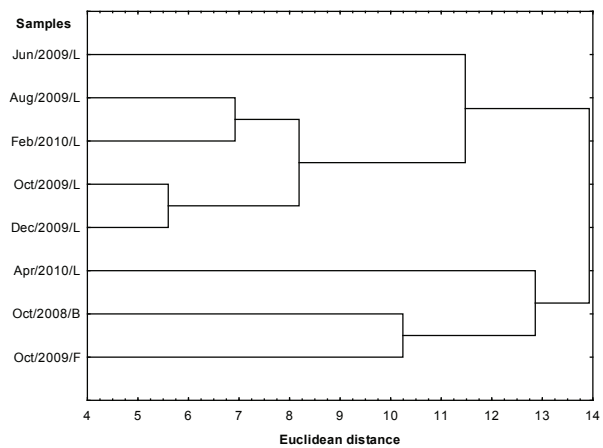

Figure 2. Dendrogram representing the chemical composition similarity relationships of *Myrcia tomentosa* oils (L: leaf; I: inflorescence; B: barks) according to Ward's variance minimization method (Ward, 1963).

Two samples (Oct/2008/B and Oct/2009/F) were added to the Cluster Analysis (Figure 2), and it was verified that the composition of oil obtained from the bark and flowers showed little similarity to the leaf essential oils. The dry months (Jun/2009/L and Aug/2009/L) were more similar than the months with higher rainfall.

Table 3. Percentages of chemical constituents of *Myrcia tomentosa* leaf oils.

RI	Components	Amount (%)					
		Jun/2009	Aug/2009	Oct/2009	Dec/2009	Feb/2010	Apr/2010
990	myrcene	0.23	-	-	-	-	-
1037	(*Z*)-β-ocimene	0.35	-	-	-	-	-
1096	linalool	-	-	-	-	-	0.16
1338	δ-elemene	-	-	-	-	-	0.25
1376	α-copaene	0.52	-	0.67	0.55	0.48	0.40
1388	β-bourbonene	0.21	-	-	0.30	0.37	-
1390	β-elemene	1.24	0.67	0.77	0.30	0.72	0.20
1419	(*E*)-caryophyllene	5.23	3.57	6.46	5.74	4.57	3.43
1432	β-copaene	0.34	-	-	-	-	-
1434	α-*trans*-bergamotene	0.46	-	0.47	0.33	0.31	0.31
1441	aromadendrene	0.37	-	-	-	-	
1442	(*Z*)-β-farnesene	-	-	-	0.30	-	0.68
1454	α-humulene	0.69	-	0.77	0.84	0.62	0.38
1456	(*E*)-β-farnesene	8.01	6.14	8.49	8.30	6.94	7.85
1460	*allo*-aromadendrene	0.74	-	0.48	0.78	-	-
1466	9-*epi*-(*E*)-caryophyllene	-	-	-	-	0.61	-
1466	*cis*-muurola-4(14),5-dieno	-	-	-	-	-	0.27
1479	γ-muurolene	0.47	-	0.39	-	18.04	18.46
1485	germacrene D	18.96	13.88	8.62	14.88	-	-
1500	bicyclogermacrene	14.71	9.57	4.73	10.14	11.51	8.19
1500	α-muurolene	-	-	0.62	0.68	0.49	0.41
1505	(*E*,*E*)-α-farnesene	3.34	-	-	-	-	-
1505	β-bisabolene	-	-	-	-	-	1.33
1509	germacrene A	-	-	0.45	0.80	-	0.37
1512	δ-amorphene	-	-	-	-	-	3.47
1513	γ-cadinene	0.44	-	-	0.28	-	-
1523	δ-cadinene	2.64	1.35	0.88	2.36	1.78	-
1531	(*E*)-γ-bisabolene	-	-	-	-	-	0.39
1561	germacrene B	1.12	0.87	0.77	0.71	0.78	0.39
1563	(*E*)-nerolidol	-	-	-	-	-	0.46
1568	palustrol	0.92	-	-	-	-	-
1578	spathulenol	1.21	4.24	6.41	4.45	3.29	0.32
1590	globulol	2.36	3.95	0.97	2.86	2.87	0.69
1592	viridiflorol	1.80	2.54	-	1.55	1.33	-
1600	rosifoliol	0.66	-	-	-	0.53	-
1631	muurola-4,10(14)-dien-1-β-ol	-	0.96	0.28	-	0.37	-
1642	*epi*-α-muurolol	0.90	1.29	0.83	-	1.39	0.61
1646	α-muurolol (=Torreyol)	0.24	-	-	-	0.44	0.35
1646	cubenol	-	-	-	0.89	-	-
1654	α-cadinol	1.13	1.84	1.15	1.33	2.08	0.78
1684	epi-α-bisabolol	10.01	5.49	-	-	-	42.39
1713	(2*E*,6*Z*)-farnesal	0.64	3.97	1.59	0.96	1.18	-
1741	(2*E*,6*E*)-farnesal	0.83	5.66	2.21	1.17	1.59	-

1784	(2*E*,6*E*)-methyl farnesoate	17.99	33.10	47.29	37.87	36.95	5.33
	monoterpene hydrocarbons	0.58	-	-	-	-	-
	oxygenated monoterpenes	-	-	-	-	-	0.16
	sesquiterpene hydrocarbons	59.49	36.05	34.57	47.29	47.22	46.78
	oxygenated sesquiterpenes	38.69	63.04	60.73	51.08	52.02	50.93
	total	98.76	99.09	95.3	98.37	99.24	97.87

In the Pearson correlation analysis, we found two strong significant correlations ($p<0.05$) between the foliar Cu and sesquiterpene hydrocarbons (R=-0.87) and between foliar Cu and oxygenated sesquiterpenes (R=0.84). The observed strong negative correlation between foliar Cu and sesquiterpene hydrocarbons is consistent with inhibition of Cu by some sesquiterpene hydrocarbons, such as germacrene D and B and bicyclogermacrene, because the formation of sesquiterpenes from farnesyl diphosphate by germacrene D synthase in ginger is inactive in the presence of Cu^{2+} ions. This inhibitory effect was found in *Zingiber officinale* Roscoe, Zingiberaceae (Picaud et al., 2006). Thus, the data obtained from *M. tomentosa* showed that Cu seems to inhibit sesquiterpenes through this enzyme.

Sesquiterpenes were found predominantly in *M. tomentosa* leaves, as was found previously for *Myrcia splendens* (Sw.) DC (Souza, 2009), *M. laruotteana* Camb (Stefanello et al., 2007) and nine other *Myrcia spp* (Limberger et al., 2004). There appears to be variation in the major components, as was also observed for *Myrcia macrocarpa* DC (Souza, 2009).

The physicochemical properties of the soil are shown in Table 4 and indicate that it was dystrophic (V<50%) and moderately acidic (pH 5.4), with a low cation exchange capacity, and calcium and potassium were the principal cations in exchange with soil. For foliar nutrients in the leaves (Table 5), it was observed that the elements N, P, K, Mg, S and Mn showed little variation over the time course of the study. In October, the Ca level was lowest (0.60 dag/kg), while Cu was at its greatest level of all the samples (31.0 mg/kg). In August, the nutrients Cu (29.0 mg/kg), Zn (100.0 mg/kg) and Fe (527.0 mg/kg) exhibited high levels in the leaves of *M. tomentosa*, and Fe decreased in December (82.0 mg/kg).

The PCA showed a positive correlation between oxygenated sesquiterpenes and the foliar nutrient variables Cu and P, which was verified by significant statistical correlations between the climatic data, foliar nutrients and essential oil composition.

Table 4. Levels of mineral nutrients and fertility parameters of soil.

Parameter	Soil sample	Parameter	Soil sample
Cu (mg/dm^3)	1.6	H+Al (cmol/dm^3)	3.9
Fe (mg/dm^3)	79.1	CEC (cmol/dm^3)	6.0
Mn (mg/dm^3)	62.8	m (%)	0.0
Zn (mg/dm^3)	1.3	V (%)	34.7
O.M. (%)	1.3	Ca/Mg	2.8
pH (Ca/Cl^2)	5.4	Mg/K	2.8
P (mg/dm^3)	5.1	Ca/K	7.9
K (mg/dm^3)	69.0	Ca/CEC (%)	23.4
Ca (cmol/dm^3)	1.4	Mg/CEC (%)	8.4
Mg (cmol/dm^3)	0.5	K/CEC (%)	3.0
Al (cmol/dm^3)	0.0		

(H+Al): potencial acidity; CEC: cation exchange capacity; O. M.: organic matter; V: base saturation; m: aluminum saturation.

Table 5. Levels of foliar macronutrients in the leaves of *Myrcia tomentosa* over the period from June 2009 to April 2010.

Nutrients	Jun/09	Aug/09	Oct/09	Dec/09	Feb/10	Apr/10
N (dag/kg)	1.26	1.34	2.02	1.40	2.12	1.60
P (dag/kg)	0.154	0.176	0.243	0.14	0.05	0.08
K (dag/kg)	0.96	0.86	0.96	0.60	1.70	1.28
Ca (dag/kg)	1.90	1.70	0.60	1.14	1.30	1.40
Mg (dag/kg)	0.20	0.30	0.20	0.21	0.29	0.25
S (dag/kg)	0.06	0.01	0.01	0.12	0.09	0.12
Cu (mg/kg)	4.0	29.0	31.0	7.0	5.0	6.0
Fe (mg/kg)	184.0	527.0	300.0	82.0	121.0	162.0
Mn (mg/kg)	441.0	420.0	110.0	191.0	310.0	420.0
Zn (mg/kg)	13.50	100.0	10.4	18.0	67.0	19.0

The results obtained in this work show that oil samples from *M. tomentosa* can vary in composition, depending on sampling period and the part of the plant from which they are extracted. *M. tomentosa* is a species that shows great potential to produce essential oils, and creating new perspectives for research into biological activities related to its essential oils.

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