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GC-MS analysis of esterified fatty acids obtained from leaves of wild and cultivated specimens of *Leonotis nepetifolia*

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Leonotis nepetifolia **(L.) R. Br. is an African bush that belongs to the Lamiaceae family which was introduced as ornamental plant in all the continents. In Brazil, the plant is known as "cordão de são francisco" or "cordão de frade". The plant has a traditional use in folk medicine for the treatment of some human diseases. In this study, it was realized for the first time, the extraction and characterization by gas chromatography–mass spectrometry (GC-MS) of the fixed oils from the leaves of specimens of *L. nepetifolia* collected in wild and cultivated environments. The esters were identified by comparing the mass spectra obtained with those of the equipment database. In the fixed oil of the wild specimen were identified 16 compounds, totaling 95.13%. Methyl linoleate (46.98%) was the majoritary compound. For the sample of cultivated fixed oil, 21 compounds were identified, representing 88.76%. Among these, two pairs of isomers, propanoic acid 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester (31.97%) and propanoic acid 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester (22.78%) reported here for the first time for this species, were identified as majoritary constituents. The results support the affirmation that the environment can modify the metabolic reply of plant specimens.**

Key words: *Leonotis nepetifolia,* fixed oils, environments, esters, Caatinga.

INTRODUCTION

The Lamiaceae family also called Labiatae, has about 300 genera and more than 7500 species with cosmopolitan distribution. The species of this family are important to humans for their utilities in the culinary, folk

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medicine, pharmaceutical and cosmetic industries (Kruppa and Russomano, 2008). Another striking feature of the species of this family is the production of essential oils, compounds belonging to the class of secondary metabolites are credited with some biological activities such as antioxidant, antibacterial, antifungal and insecticidal activity (Lima and Cardoso, 2007).

Phytochemical studies on species of this genus have identified the presence of alkaloids, saponins, tannins, flavonoids and diterpenes. In the folk medicine, the species are used in the treatment of hemorrhoids, diabetes, hypertension, anemia, eczema and other skin irritations, as well as purgative (Habtemariam et al., 1994; Maroyi, 2013). *Leonotis nepetifolia* is an African bush that was introduced as ornamental plant in all the continents (Cruz et al., 2011). In South Africa and West Indies, it is known as Klip Dagga, Lion's ear, Christmas and Candlestick (Ashish et al., 2011). In Brazil, this species is known as "cordão de São Francisco" and "cordão de frade", and it is considered as a weed of waste land and cultivated areas. This species has a traditional use in folk medicine, especially as invigorating and for the treatment of bronchial asthma, gynecological diseases, parasitic infections (Cruz et al., 2011), to treat coughs, fever, stomachache, skin infections, rheumatism, dysmenorrhea and kidney dysfunction (Li et al., 2012; Udaya et al., 2013). Phytochemical study with extracts of this species collected in India revealed the presence of volatile oils, carbohydrates, terpenoids, saponins, flavonoids, proteins, amino acids, phytosterols, alkaloids and fixed oils (Trivedi et al., 2011). In this study, of preliminary nature, no constituents of the studied classes were identified. In this way, fixed oils characterization becomes a complementary on phytochemical characterization of the species under study.

Some studies on this plant evaluated biological activities such as antibacterial, larvicidal, pesticidal, cyto-toxic, hypotensive, anti-inflammatory and antiplasmodial. Several compounds have been isolated from this plant, such as diterpenes, coumarins, iridoids, saponins, condensed tannins, flavonoids, alkaloids and steroids in extracts of leaves, stems and roots (Cruz et al., 2011; Li et al., 2012; Narayan, 2012; Imran et al., 2012; Udaya et al., 2013). However, no study by gas chromatography–mass spectrometry (GC-MS) identified the constituents present in the fixed oil of the species. Lipids are biological compounds insoluble in water and soluble in organic solvents. The fixed oils belong to a class of lipids constituted by saturated and unsaturated fatty acids. This class has emollient properties when incorporated in dermatological formulations and researches of biological activity reveal insecticidal potential and larvicidal for these compounds (Pereira et al., 2005; Pereira et al., 2008). This is the probable explanation for the larvicidal activity found by Udaya et al., (2013).

Some authors affirm that situations of stress conduct the plant to express different defense components called secondary metabolites, as those found in the fixed oils. Recent studies have shown that the variations of total lipid contents and fatty acids are affected by the species, age, water temperature, nutritional condition and seasonal variation, and that the environmental condition is a main factor for this variation (Emara and Shalaby, 2011).

The aim of this study was to investigate the phyto-constituents present in the fixed oil from the leaves of *L. nepetifolia* collected in two different environments: wild and cultivated. The chemical composition was analysed by GC-MS.

MATERIALS AND METHODS

Plant material

The leaves of the specimens of *L. nepetifolia* (L.) R. Br. were collected in the city of Petrolina (Coordinates: S 9°45'17''; W 40°58'02'' for wild specimen, and S 9°39'36''; W 40°54'65" for cultivated specimen), State of Pernambuco, Brazil, in June of 2013. The samples were identified by a botanist from Centro de Recuperação de Áreas Degradadas da Caatinga (CRAD) and the voucher specimen (5266) was deposited at the Herbário Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley.

Extraction

The dried and powered leaves of wild and cultivated specimens (25.04 g and 4.30 g, respectively) were extracted with petroleum ether in the Soxhlet apparatus for 2 h. The extractive solution was concentrated under vacuum in a rotatory evaporator at 40°C, producing 0.96 g for cultivated sample and 0.29 g for wild samples, respectively.

Saponification

The methodology used with some adaptations, was described by Matos et al., (1992). The samples of fixed oils were subjected to saponification with potassium hydroxide (KOH) under reflux with methanol for 30 min. Subsequently, the solutions were concentrated under vacuum in a rotatory evaporator for extraction of solvent. A bracket of the water was added and the non-saponified fractions were extracted with petroleum ether.

Methylation of saponified fraction

The aqueous solutions of soaps were acidified at pH 2 with HCl aqueous solution 10%, and the fatty acids were extracted with petroleum ether. The residual water was removed with anhydrous sodium sulfate and the solvent was evaporated under vacuum in a rotatory evaporator. Successively, the fatty acids were esterified in apparatus of reflux for 2 minutes with methanol acidified with drops of concentrated hydrogen chloride. The methyl esters were extracted with hexane and dried over sodium sulfate after addition of water. The solvent was evaporated under vacuum in a rotatory

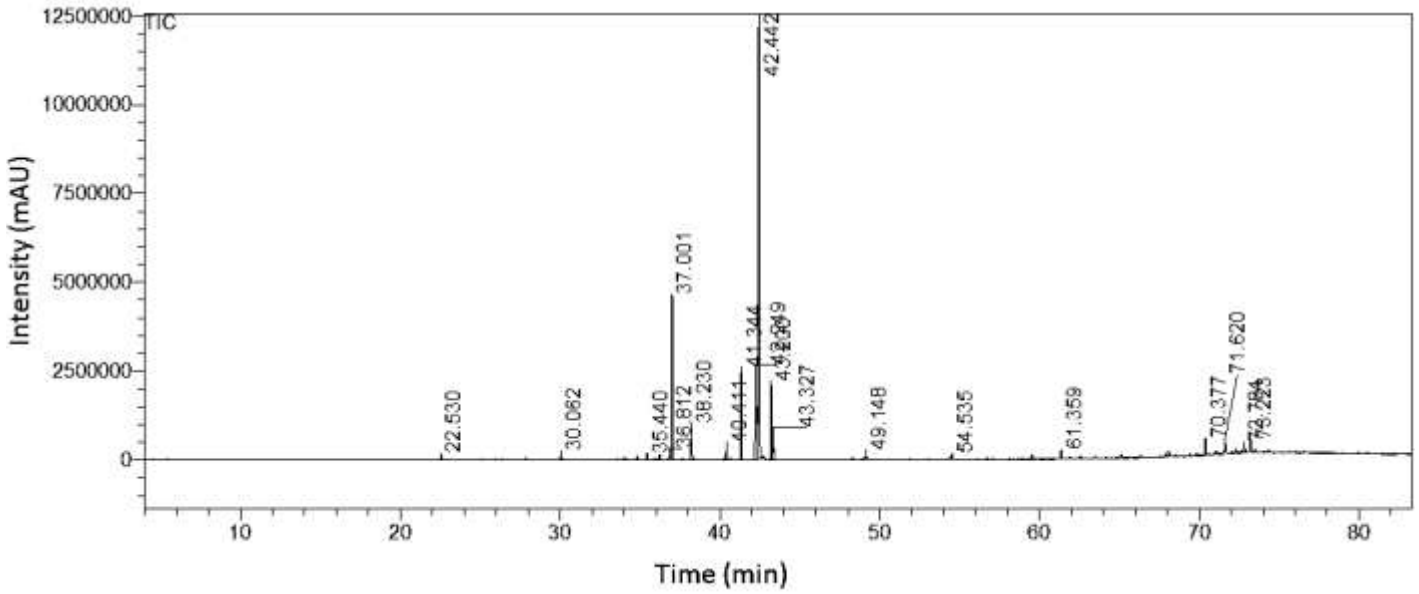

Figure 1. TIC of chemical constituents of the fixed oil from the leaves of wild *L. nepetifolia.*

evaporator.

GC-MS analysis

The substances present in the fixed oil of *L. nepetifolia* were investigated on a Shimadzu QP-2010 GC-MS. The following conditions were used: ZB-5MS column Phenomenex Zebron (30 m x 0.25 mm x 0.25 mm); helium (99.999%) carrier gas at a constant flow of 1.1 ml/min; 1 µl injection volume; injector split ratio of 1:40; injector temperature 240°C; electron impact mode at 70 eV; ion-source temperature 280°C. The oven temperature was programmed from 100°C (isothermal for 5 min), with an increase of 10°C/min to 250°C (isothermal for 5 min) and 10°C/min to 280°C (isothermal for 15 min). A mixture of linear hydrocarbons (C_9H_{20}–$C_{40}H_{82}$) was injected under the same experimental conditions as samples, and identification of the constituents was performed by comparing the mass spectra obtained with those of the equipment databases Wiley 7 lib and Nist 08 lib (Carvalho et al., 2013).

RESULTS AND DISCUSSION

The total ion chromatograms (TIC) are showed in Figures 1 and 2. In the Tables 1 and 2 a list of the identified compounds and their quantification in the fixed oils are presented according to their retention times.

The results obtained from the identification of compounds present in fixed oils demonstrated the modification of the metabolic response as a function of the environment. In the wild specimen, a total of 16 compounds were identified and 9,12,15-octadecatrienoic acid methyl ester also known as methyl linoleate (46.98%) was the majoritary compound.

In humans, the precursor acid of this compound [α-linolenic acid (9.21%)] together with the linolenic acid are necessary to keep normal conditions, the cellular membranes, brains functions and transmission of nerve impulses. These acids are precursors of the arachidonic acid and docosahexaenoic acid, responsible for the development and operation of brain and retina. It participates also of the transfer of atmospheric oxygen to a blood plasma, in hemoglobin synthesis and cell division, being called essential and consumables required (Martin et al., 2006).

Among other compounds identified further highlight the presence of the esters of palmitic acid (13.92%) and 6-octadecynoic acid also known as tarric acid (6.49%), a fatty acid often considered according to its triple bond in position 6. Biological activity studies have identified the potential antifungal against strains of *Candida albicans*, *C. neoformans*, *Aspergillus* spp. and *Trichophyton* spp. (Li et al., 2008), and their potential for inhibiting hepatic fibrosis *in vitro* (Ohtera et al., 2013).

For the cultivated specimen, 21 compounds were identified and in this, two isomers: propanoic acid 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester (31.97%) and propanoic acid 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester (22.78%), were the majoritary compounds. These compounds were obtained from exudates of tubers of *Solanum tuberosum* after inoculation with the pathogen *Fusarium coeruleum* and *Phytophthora infestans* and identified by GC-MS

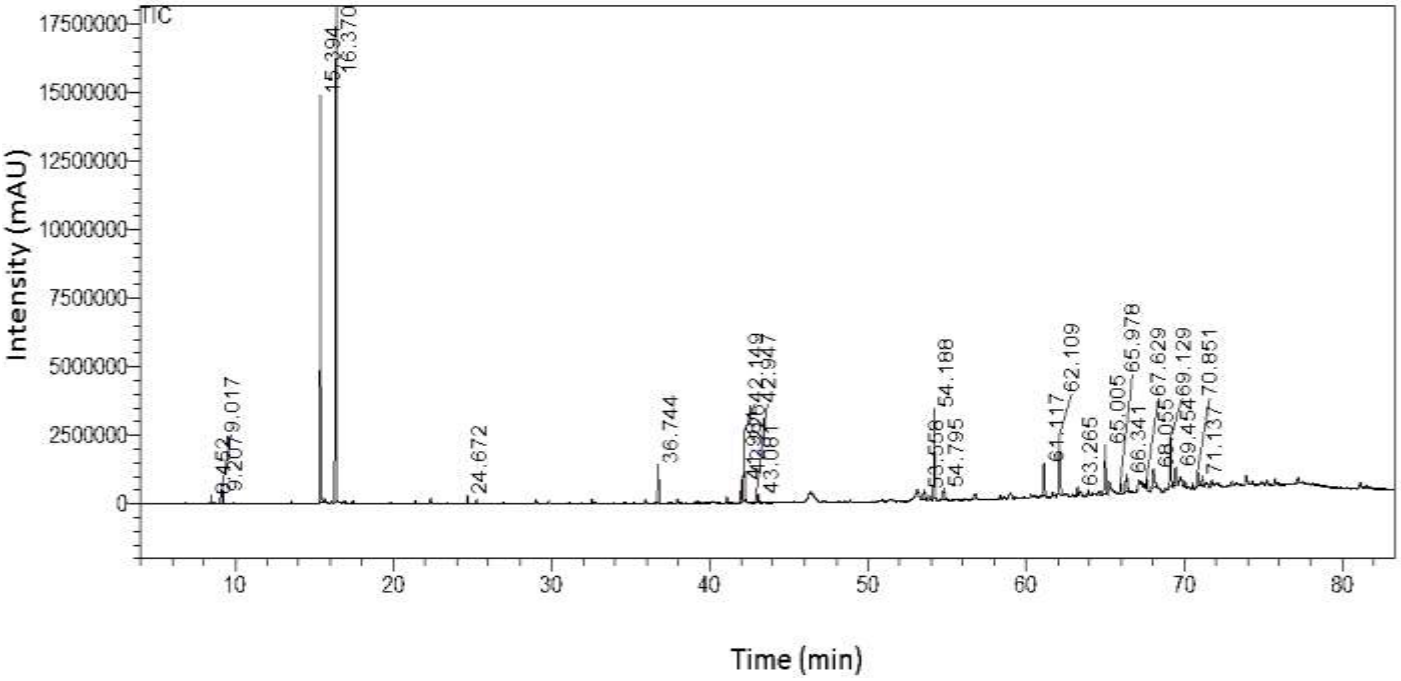

Figure 2. TIC of chemical constituents of the fixed oil from the leaves of cultivated *L. nepetifolia.*

Table 1. Chemical constituents of the fixed oil from the leaves of wild *L. nepetifolia.*

Peak	RT (min)	Compound	(%) GC-MS
1	22.53	Methyl laurate	0.38
2	30.06	Methyl myristate	0.64
3	35.44	Phytol	0.49
4	36.81	NI	0.87
5	37.00	Methyl palmitate	13.67
6	38.23	NI	2.92
7	40.41	γ-Undecanolide	1.32
8	41.34	γ-Decanolide	7.67
9	42.25	9,12-Octadecadienoic acid methyl ester	9.21
10	42.44	Methyl linoleate	46.98
11	43.20	6-Octadecynoic acid methyl ester	6.49
12	43.33	Methyl stearate	2.79
13	49.15	Arachidic acid methyl ester	0.74
14	54.54	Docosanoic acid methyl ester	0.45
15	61.36	Squalene	0.62
16	70.38	Stigmasterol	1.62
17	71.62	Stigmast-5-en-3β-ol	1.11
18	72.78	Stigmast-7-en-3β-ol	0.95
19	73.22	NI	1.07
Total	-	-	**95.13**

RT = retetion time; NI = not identified.

Table 2. Chemical constituents of the fixed oil from the leaves of cultivated *L. nepetifolia.*

Peak	RT (min)	Compound	(%) GC-MS
1	8.45	NI	0.29
2	9.01	1,3-Diisopropyl cyclohexane	0.12
3	9.21	1,4-Diisopropyl cyclohexane	0.41
4	15.40	Propanoic acid 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester	22.78
5	16.37	• Propanoic acid 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester	31.97
6	24.67	Propanoic acid 2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	0.34
7	36.74	Palmitic acid methyl ester	2.00
8	41.98	9,12-Octadecadienoic acid methyl ester	1.21
9	42.149	Linolenic acid methyl ester	3.89
10	42.22	11-Octadecenoic acid methyl ester	1.23
11	42.95	6-Octadecynoic acid methyl ester	0.57
12	43.08	Stearic acid methyl ester	0.46
13	53.56	Squalene	1.16
14	54.19	1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester	5.05
15	54.80	3-Methylheptadecane	1.51
16	61.12	NI	2.32
17	62.11	NI	6.18
18	63.26	Docosane	0.40
19	65.00	Hentriacontane	4.45
20	65.98	NI	0.87
21	66.34	Cycloartenol	1.93
22	67.63	Pentacosane	0.48
23	68.05	β-Amyrin	2.58
24	69.13	Nonacosane	4.20
25	69.45	α-Amyrin	2.04
26	70.85	NI	0.92
27	71.14	NI	0.66
Total	-	-	88.76

RT = retetion time; NI = not identified.

(Costello et al., 2001). And in essential oils obtained from straw of *Oryza sativa* L. collected in three Japanese cities (Miyazawa et al., 2008), in the species *Leonotis nepetifolia* these compounds were described for the first time. The cultivated specimen had the largest amount of compounds when compared to wild specimen. Squalene is present in both specimens.

The presence of these compounds in the studied plants is important phytochemically. However, the chemical potential of *L. nepetifolia* must be investigated and biological properties should be evaluated.

Conclusion

This study described for the first time the identification and quantification of components obtained from fixed oil from the leaves of *L. nepetifolia*. The analysis showed differences both qualitatively as well as quantitatively. The isomers found in the cultivated fixed oil were recorded by the first time as natural product in this species. Future studies are necessary to verify the biological properties of these fixed oils, and to examine if the differences in the chemical composition of these fixed oils are able to promote different biological activities.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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