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Chemical composition of essential oil of *Melissa officinalis L*. and antioxidant activity from Boa Vista-RR, Brazil

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In this work, the chemical composition of the essential oil of lemon balm (*Melissa officinalis* L.) was determined by CG-FID.In the essential oil of *M. officinalis,*there area total of 22 chemical constituents, among them are geranial (34.6%), neral (26.0%), *γ*-caryophyllene (7.5%), caryophyllene oxide (5.3%), μ-pinene (5.3%) and sabinene (3.6%); it also has antioxidant capacity and total phenolic compounds *in vitro*. The concentration of total phenolic compounds was 61.71 mgEAG g^{-1} for the essential oil and 7.81 mgEAG g^{-1} for the aqueous extract respectively. The inhibition percentage tested by different DPPP concentrations of 8, 20, 40 and 80 µg mL^{-1} was 17.12, 31.04, 48.24 and 68.12% respectively and the quercetin standard was used as a positive control.

Key words: Limon herb, Folin-Ciocateau, DPPH, medicinal plant.

INTRODUCTION

Mellissa officinalis L. popularly known as lemon balm, sweet balm or common balm, belongs to the *Laminaceae* family (Awad et al., 2009). This plant is of Asian and European origin; it is cultivated in Brazil for more than a century; a perennial and can vary from 20 to 80 cm and 30-100 cm in height, with its membranous leaves dark green in the upper and light green. On the underside, it has a large size, petiolate, opposite, lanceolate, oval, hairy and well protruding (Couto, 2006). Authors such as Osbaldeston (2000) point out that *M. officinalis* was used

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for medicinal purposes more than 2000 years ago. It is known in traditional European medicine aslemonbalm. Saad and Said (2011) point out that lemon balm was used in the Middle Ages to stop bleeding, treat toothache, earache, bent neck and baldness.

M. officinalis L. is a plant used as sedative, and tonic properties are attributed to the nervous system (Aghilikhorasani and Makhzan, 2008) as well as being implicated in relaxation processes and anti-anxiety, insomnia, anti-diarrheal, anti-ulcer properties (Lin et al., 2012; Shakeri et al., 2016; Ghsemi-dehkordiet al., 2002); it presents other functions such as improving benign palpitation and sexual dysfunction (Alijaniha et al., 2015).

In the food industry, according to Bisset and Wichtl (2001), it is a plant used to give fragrance to different foods and beverages; it is also used in the pharmaceutical industry. The essential oil is obtained from flowers with a light yellow color and citric odor, especially the presence of citral, geranial, neral and citronel acetate in their chemical composition (Dawson et al., 1988). Essential oil is responsible for the antibacterial and antifungal properties of the plant (Mimica-dukic et al., 2004). Essential oil of *M. officinalis* has antioxidant activity, mainly attributed to phenolic acids such as hydrocinamic acid and rosmarinic acid (Caniova and Brandsteterova, 2001). The objective of this work is to study the chemical composition of the essential oil of *M. officinalis* in the Roraima region (Brazil) in the northern Amazon as well as its antioxidant activity and total phenolic compounds

MATERIALS AND METHODS

Preparation of samples

Samples were collected in the Boa Vista-Roraima city (Brazil) and taken to the Laboratory of Environmental Chemistry of the Nucleus of Research and Postgraduate Studies in Technology. The essential oil is separated from the hydrolate and assembled in the amber and refrigerated bottle; it is made in different analyses where a part of the essential oil is sent to the Department of Chemistry of the Federal University of Minas Gerais (UFMG). It is carried out with chromatography analyses. Another part of the oil and hydrolatewere used to carry out the antioxidant activity and total phenolic compoundswere analyzed.

GC-FID analysis

The essential oil was analyzed on a HP 7820A Gas Chromatograph (GC) equipped with a flame ionization detector (FID) using a capillary column (HP5 30 m × 0.32 mm × 0.25 microns, Agilent): Column temperature: 50°C (0 min) at 3°C min-1 up to 230°C. Gun: 250°C Split (1:30). FID Detector: 250°C. Carrier gas: hydrogen at 3 mL min^{-1}. Vol injection: 1 µL. Essential oil was diluted at 1% in chloroform. Data acquisition software used was Compact EZChrom Elite (Agilent). The quantitative analysis was accomplished using Standard areas from the chromatograms obtained by GC-FID.

GM-MS analysis

A GCMS-QP2010 ULTRA (Shimadzu) was used. Column: Rxi-1MS dos Santos et al. (2014) 923 30 m × 0.25 mm × 0.25 microns (Restek). Column Temp: 50°C (3 min), 3°C min^{-1} to 250°C. Injector: 250°C Split (1:10), GC-MS interface at 250°C. MS detector (electron impact at 70 eV) temperature was 250°C. Carrier gas: helium at 1.5 mL min^{-1}. Vol injection: 1 µL. Essential oil was diluted at 0.1% in chloroform. Data acquisition software used was GC-MS Solution (Shimadzu) together with NIST11 library. Identification of peaks was made by comparison of the mass spectra obtained by GC-MS spectra with the NIST11 library and also by comparing the Kovatsíndices calculated by GC-FID and literature data.

Total phenolic compounds

The determination of total phenolic compounds was performed by the FolinCiocateau method, where a stock solution of 250 µg mL^{-1} of essential oil in methanol was initially prepared. Subsequently, 0.1 mL of this solution was transferred to a test tube and 0.1 mL of methanol was added. Then 2.5 mL FolinCiocateau and 2.0 mL 7.5% sodium carbonate were added to the test tube.The formed solution was taken to a 50°C water bath for 5 min and read on a 760 nm spectrophotometer. The calibration curve was made with a gallic acid standard at concentrations of 5, 10, 20, 40 and 80 µg mL^{-1} of each of the standard solutions was removed and placed in a test tube to which 2.5 mL of FolinCiocateau reagent and 1.5 mL of sodium carbonate solution were added and the absorbance readings at 760 nm (Nakashima, 1993).

Antioxidant activity

For the determination of antioxidant activity, the 2,2-diphenyl-2-picrylhydrazine (DPPH) reduction method was used, according to the methodology proposed by Kondo (2002), where primarily the crude samples of essential oil were solubilized in ethanol at concentrations of8, 20, 40 and 80 µg mL^{-1} and subsequently a 60uM DPPH ethanol solution was prepared. Samples were prepared by mixing 50 µL of DPPH solution and a control solution, where the sample volume was replaced with 50 uL of ethanol, and absorbance readings at 517 nm were taken. 30 min.

The determination of total phenolic compounds was performed by the FolinCiocateau method, where a Stock solution of 250 µg mL^{-1} of essential oil in methanol was initially prepared. Subsequently, 0.1 mL of this solution was transferred to a test tube and 0.1 mL of methanol was added. Then 2.5 mL Folin Ciocateau and 2.0 mL 7.5% sodium carbonate were added to the test tube. The formed solution was taken to a 50 C water bath for 5 minutes and read on a 760 nm spectrophotometer. The calibration curve was made with a gallic acid standard At concentration of 5, 10, 20, 40 and 80 µg mL^{-1} of each of the standard solutions was removed and placed in a test tube to which 2.5 mL of FolinCiocateau reagent and 1.5 mL of sodium carbonate solution were added and the absorbance readings at 760 nm (Nakashima, 1993).

RESULTS AND DISCUSSION

Table 1 presents the main constituents in the identified *M. officinalis* essential oil as well as the Retention time and the identified substances in total 22 compounds.A total of 22 chemical constituents were identified in *M.*

Table 1. Identification of constituents in the essential oil of *M. officinalis*.

Peat	Retentiontime(min)	Area	Conc* (%)	Retention time **	Probable substance***
1	3.891	425132	0.9	1002	Sulcatone
2	4.273	1629608	3.6	1013	Sabinene
3	4.322	2414211	5.3	1014	*β*-Pinene
4	5.79	841911	1.8	1054	*β*-Ocimene
5	7.424	702995	1.5	1098	Linalool
6	8.562	77756	0.2	1129	Citronelal
7	8.943	135585	0.3	1139	Canfora
8	9.211	112867	0.2	1147	Borneol
9	9.642	180491	0.4	1158	Pinocarvone
10	9.901	141541	0.3	1165	Terpinen-4-ol
11	10.313	329443	0.7	1176	α-Terpineol
12	12.532	11932918	26.0	1236	Neral
13	13.756	15840545	34.6	1270	Geranial
14	18.032	424954	0.9	1385	Acetato de geranila
15	18.872	3436001	7.5	1408	*β*-Cariofilene
16	20.142	301196	0.7	1443	α-Bisabolene
17	20.796	138710	0.3	1460	Humulene
18	21.376	1193889	2.6	1476	*β*-Selinene
19	21.737	365168	0.8	1486	*γ*-Gurjunene
20	22.096	805354	1.8	1495	Alloaromadendrene
21	22.513	165521	0.4	1507	A-Selinene
22	24.908	2412238	5.3	1572	Cariofilene oxid
		1836078	4.0		Other
% Monoterpenes			76.7		
% Sesquiterpenes			21.6		
% Other			1.7		

*****Results obtained by CG-FID chromatogram; **Retention Index Calculated Kovats Index; ***Identification confirmed by CG-MS.

officinalis essential oil (Figure 1), with GC-FID: geranial (34.6%), neral (26.0%), caryophyllene (7.5%), caryophyllene oxide (5.3%), β-pinene (5.3%) and sabinene (3.6%).

Compared to the values determined byKhalili et al. (2018), by hydrodistillation, the geraniale and neral values were 19.53 and 16.39%, respectively; they were lower than those found in this study, being the major constituent found by these authors for the study (caryophyllene oxide with 23.71%). Other authors such as Pirbalouti et al. (2019) studied the composition of the essential oil of *M. officinalis* by hydrodistillation; geranium values of 38.34% and neral of 31.93% were obtained; values close to those obtained in this work. The differences in the chemical composition of the different constituents of the essential oil influence environmental and genetic factors, as well as post-harvest plant processing factors (Lemos et al., 2017).

The main constituents were later identified by GC-MS (Figure 2).

Antioxidant activity

Table 2 shows the results of total phenolic Compounds and antioxidant activity for *M. officinalis* L. essential oil. Queiroz et al. (2014) evaluated the phenolic compounds in different extracts of *M. officinalis* L. where the aqueous extract presented total phenolic compounds concentrations of 817 $mgmL^{-1}$. Authors such as deMorais and Nascimento (2016) studied the total phenolic compounds in different phytotherapics from *M. officinalis* L. where the concentrations obtained from total phenolic compounds ranged from 18.541 to 75.16 mg AGEgrams of sample.

Essential oils are rich in phenolic compounds, with reducing properties that play an important role in free radical sequestration as well as chelation of transitionmetals (Sousa et al., 2007). The percentage of inhibition evaluated with DPPH increases with the concentration of essential oil. *M. officinalis* L. essential oil can be used as a pharmaceutical and nutritional

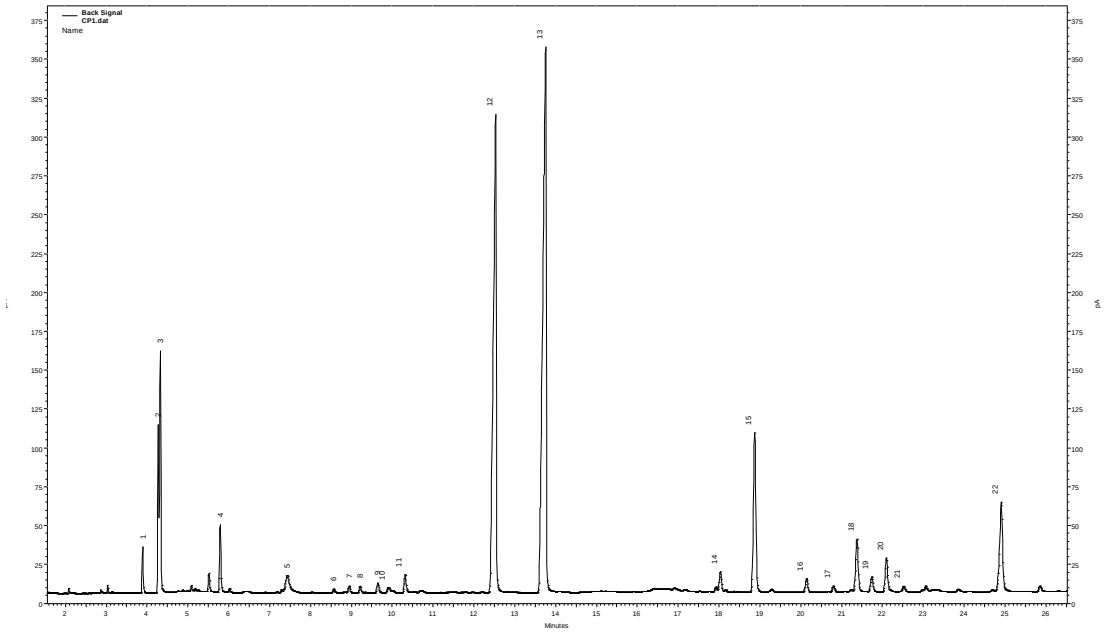
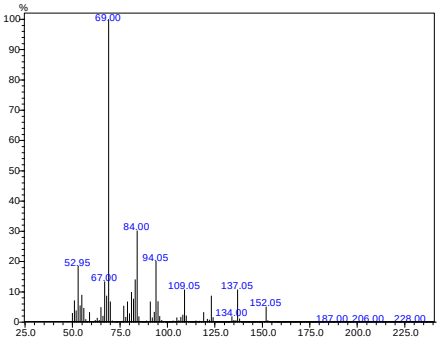
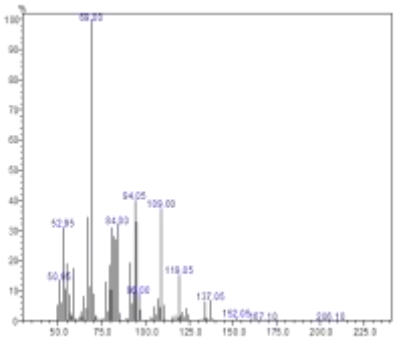


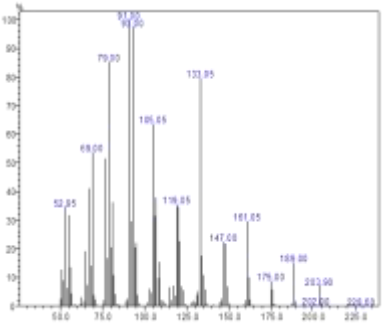
Figure 1. Chromatogram obtained by CG-FID of the essential oil of *M. officinalis*.



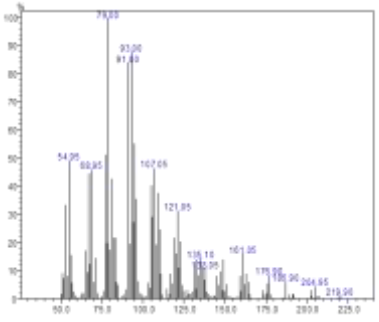
a) Geranial



b) Neral



c) β-cariofilene



d) Cariofilene oxid

Figure 2. Main constituents of *Melissa officinalis* L. by GC-MS.

Table 2. Antioxidant activity and total phenolic compounds of the essential oil of *Melissa officinalis* L.

Concentration (μg mL^{-1})	Antioxidant activityTotal phenolic compounds		
	% Inhibition E.O.	% Inhibition quercetine	Essential oil
8	17.12 ± 1.21	34.21 ± 2.17	61.71± 2.67
20	31.04 ± 1.16	57.64 ± 1.54	
40	48.24 ± 0.71	71.56 ± 1.15	
80	68.12 ± 0.71	88.92 ± 1.23	

product as a natural antioxidant source (Koksal et al., 2011). The inhibition percentage for the major concentration is 68.12% high compared to the quercetin standard used as positive standard with the inhibition percentage of 88.92%

Conclusion

The present work provides information on the chemical profile of 22 constituents of the *M. officinalis* L. essential oil by the hydro distillation method. It is a simple, fast and free of any residual solvent, being a method used to quantitatively determine constituents, volatile foods and medicine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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