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Analysis of Essential Oil from Brazilian *Mentha x piperita* L. Commercial Samples

Análise do Óleo Essencial de Amostras Comerciais Brasileiras de Mentha x piperita L.

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The present work aimed to evaluate the content and composition of the essential oil from different Brazilian commercial samples of *Mentha x piperita* L (peppermint). The essential oil yield of the studied samples ranged from 0.12 to 0.62%. The gas chromatographic analysis of the essential oil showed 28 different compounds, which were obtained by hydrodistillation of the six samples. Samples **1**-**4** presented menthol as the major compound (49.3-82.0%), and menthone, isomenthone and menthyl acetate as secondary compounds. Samples **5** and **6** presented carvone as the major compound (64.4-65.5%) and pulegone was the second compound in higher proportion (6.1-8.6%). According to the Brazilian Pharmacopoeia, only samples **1**-**4** are constituted by *Mentha x piperita* L in agreement with the composition of the essential oils. The results obtained for samples **5** and **6**, which conferred essential oil rich in carvone, suggest that these samples are not comprised by *Mentha x piperita* L but other species of *Mentha*, which can affect the therapeutic actions expected by people consuming the samples as an herbal medicine.

Keywords: Peppermint; Gas Chromatography-Mass Spectrometry; tea bags.

1. Introduction

Mentha x piperita L., native to Europe and Asia, is found along the coast and mountains of the Brazilian sea and is popularly known as "peppermint".[1] *Mentha x piperit*a L. is a natural hybrid between *Mentha aquatica* and *Mentha spicata* and is detached among the most popular tea ingredients.[2]

The essential oils from some *Mentha* species are potential candidates for antimicrobial, antioxidant, radical scavenging and cytotoxic activities. Commercially, *Mentha* essential oils and their compounds are widely applied in food, cosmetics, fragrances, tobacco and medicine industries.[3] The essential oil of *Mentha x piperita* L. (peppermint oil) is commonly used in folk medicine to treat respiratory diseases, as expectorant and anti-congestive,[4-6] and also, as relief from general symptoms of irritable bowel syndrome in humans,[7] as well as digestive disorders such as flatulence and gastritis.[8] Externally, it can also be used to treat myalgia and headache.[9,10]

The volatile oil of *Mentha x piperita* L., is compounded by at least 35.0% menthol. As main characteristics, *Mentha x piperita* L. essential oil is presented as a colourless, pale yellow or pale greenish-yellow liquid, with a peculiar odor and taste, followed by a freshness sensation.[11]

The genus *Mentha* (Lamiaceae) comprises approximately 25-30 species and due to the variety of essential oil produced, it receives great economic importance in the pharmaceutical and food industries.[12] Mentha is intensively cultivated in temperate and tropical regions of the world for oil production.

Variation in the essential oil composition is due to different factors such as stress during growth, humidity, temperature, light intensity, photoperiod and also drying and storage after harvest.[13,14] The study of the chemical composition of the essential oil is important, as any variation can naturally affect its commercial value and the therapeutic activities expected from its use. Thus, the objective of this work was to determine the content and composition of the essential oil in different commercial samples of *Mentha x piperita* L. teas.

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2. Material and Methods

2.1. Samples acquisition

Six commercial samples of *Mentha x piperita* L. (**1** to **6**) were acquired at supermarkets and local markets in Montes Claros - Minas Gerais State, Brazil. Five of these samples corresponded to tea bags (**1** to **5**) and one sample corresponded to a plastic package (**6**). The material in all the samples was dried and ground.

2.2. Loss on drying and essential oil contents

Three Petri dishes were used (with approximately 1.0 g of sample, each - **1** to **6**) to determine the loss on drying content of each sample. Samples were submitted to thermal treatment for 24 hours in an oven at 105 °C. Then, the samples were duly collected into desiccators, where they remained for approximately 60 minutes to interrupt the process of water loss and achieve thermal equilibrium. The samples were weighed on analytical balance and the loss on drying content determined.

The essential oil was obtained by hydrodistillation. To determine the hydrodistillation time, 20.0 g of sample **1** was transferred to a 500 mL round-bottom flask on a heating mantle, coupled to a Clevenger extractor. Sufficient amount of water was added to this system until it reached about 250 mL of the flask level. Approximately 80 mL of hydrolate (water + oil) was collected at each hour, during eight hours. The oil was extracted from the aqueous phase using pentane as solvent (3 x 15 mL). Anhydrous magnesium sulphate was added in excess to the organic phase, in order to remove the water present in the solvent. The organic phase was filtered and the pentane removed on a rotary evaporator. The oil mass was measured and the oil content determined.

About 10.0 g of each sample (commercial samples **1**-**6**) was weighed for the essential oil hydrodistillation. The assays were performed in triplicate for each sample.

2.3. Qualitative analysis of the essential oil compounds from *Mentha x piperita* L. by GC-MS

Five mg of essential oil from one triplicate of each sample was diluted in 1 mL of spectroscopic grade hexane, providing a solution at 5000 ppm. Then, 1 µL of this solution was injected into the Gas Chromatograph coupled to the Mass Spectrometer to identify the chemical compounds. A Shimadzu equipment (model QP-PLUS-2010) was used, with capillary column Rtx-5MS (30 m of length and 0.25 mm inner diameter, 0.25 µm film thickness), and helium as the carrier gas. The temperature of the injector was of 220 °C and the detector's temperature was of 300 °C. Temperature was increased from 60 to 240 °C at a rate of 3 °C/minute. The split ratio was of 1:5, and the solvent cut-off time was of 5 minutes. To determine the chemical compounds of the essential oil of *Mentha x piperita* L., the mass spectra obtained was compared to those from the apparatus library (Willey 7), data from other studies and to the linear retention indices (LRI).[15]

To calculate the Linear Retention Index (LRI), 1 µL of a standard mixture of C8-C40 n-alkanes (Sigma-Aldrich), solubilized in HPLC grade methanol, was injected into the GC-MS under the same conditions as the essential oil analysis. The alkane retention times and the retention times of each compound of interest were used. The LRI for each compound was calculated using Equation 1:

$$LRI = 100\left(\frac{t_c - t_n}{t_{n+1} - t_n} + n\right) \quad (1)$$

where: LRI - linear retention index; t_c - retention time of the target compound; t_n - retention time of the reference n-alkane hydrocarbon eluting immediately before the target compound; t_{n+1} - retention time of the reference n-alkane hydrocarbon eluting immediately after the target compound; n - number of carbon of the n-alkane hydrocarbon eluting immediately before the target compound.

The calculated LRI values were compared with values found in the literature for columns of the same polarity.[15]

2.4. Quantitative analysis of essential oil compounds from *Mentha x piperita* L. by GC

Five mg of essential oil from one triplicate of each sample was diluted in 1 mL of spectroscopic grade hexane, providing a solution at 5000 ppm. Then, 1 µL of this solution was injected into the Gas Chromatograph (GC) (Shimadzu, model CG-2010 Plus) equipped with flame ionization detector and capillary column Rtx-5MS (30 m of length and 0.25 mm inner diameter, 0.25 µm film thickness). Nitrogen was used as the carrier gas (flow rate of 2.65 mL/min). The oven temperature programming was the same used in the GC-MS analysis. The injector temperature was of 240 °C, the detector temperature of 250 °C, split ratio of 1:5 and 1 µL of the sample was injected.

3. Results and Discussion

To determine the required time to distill the essential oil of *Mentha x piperita* L. samples using the hydrodistillation with Clevenger apparatus, we submitted sample **1** to an eight-hour process of hydrodistillation. The condensate was collected, at each hour, for extraction and determination of the essential oil content. After three hours of distillation, just a little amount of essential oil from the species is distilled (Figure 1). As the hydrodistillation of all the essential oil from all the samples in this work could have been time and energy consuming, three hours was established as the time of hydrodistillation.

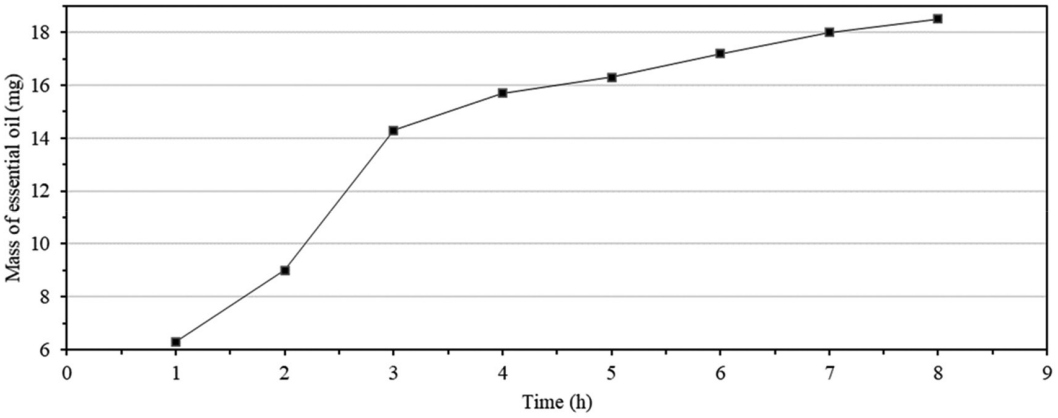

Figure 1. Essential oil content (mg) of sample **1** during the eight-hour hydrodistillation

The assay to determine the moisture content for the six commercial samples of *Mentha x piperita* L., was carried out by a 24-hour heat treatment (Table 1).

According to ANVISA (Brazilian National Health Surveillance Agency), which elaborates Brazilian Pharmacopoeia,[11] the moisture content of commercial samples of *Mentha x piperita* L. should be at most 12% indicating that the commercial samples analysed in this work are in accordance with current legislation. Relation between the part of the plant used and the moisture content was not noticed.

Following the same Brazilian Pharmacopoeia specifications, the minimum essential oil content of *Mentha x piperita* L. is of 0.9% in processed leaves. None of the commercial samples are in accordance with ANVISA.[11] We must also consider that some samples were constituted by leaves and others by leaves and stems. Tea bag samples presented half or less than half of the essential oil content presented by leaves from the plastic package. During the teabags preparation, the gridding process is responsible for the exposure of the essential oil and its volatilization due the heat generated. Thus, some samples could have been more ground than the others.[16]

The drying methods, which these commercial samples **1**-**6** were submitted must be considered. Beigi et al.[17] revealed that in general, increasing drying temperature decreased the essential oil content of *Mentha x piperita* L.

The identification of the individual compounds from the essential oil can be performed by comparing the mass spectra of each compound obtained through Gas Chromatography-Mass Spectrometry and the mass spectra library. The Linear Retention Index (LRI), which relates the retention time of the compounds with the retention time of saturated hydrocarbons is used to eliminate possible misidentification.[15]

Compounds quantified in each essential oil from the six samples analysed by Gas Chromatography are shown at Table 2. The normalization method was used for the quantification. The total value of the areas of the peaks is considered 100% and the percentage of each signal is calculated by its area.[18] Twenty eight different compounds were identified and quantified in the essential oil of the samples analysed.

Although the great variation in the essential oils composition, the presence of two different groups based on the major compounds of each oil was verified. Group one is compounded by samples **1**-**4**, and presents menthol as the major compound (49.3%-82.0%), besides menthone, isomenthone and menthyl acetate as secondary compounds. Group two is comprised by samples **5** and **6** with carvone as the major compound (64.4%-65.5%) and pulegone as the second compound in higher proportion (6.1%-8.6%). Neither menthone, isomenthone, menthol, and menthyl acetate were found in group 2, as found in group 1 (Figure 2). The chemical structures of these compounds are presented at Figure 3.

According to the Brazilian Pharmacopoeia,[11] the percentages of some of the major compounds of

Table 1. Average values of essential oil content (%) and moisture content (%) in tea bag products of *Mentha x piperita* L. commercialized in Brazil

Sample	Sample origin	Part of plant used[a]	Moisture content (%)	Oil Content (%, dry matter)[b]
1	Montes Claros, Tea bag	leaves and stem	5.30±0.26	0.24±0.04
2	Montes Claros, Tea bag	leaves	3.64±0.02	0.12±0.03
3	Montes Claros, Tea bag	leaves and stem	4.36±0.30	0.32±0.04
4	Montes Claros, Tea bag	leaves	5.68±0.33	0.24±0.04
5	Montes Claros, Tea bag	leaves and stem	5.59±1.00	0.42±0.09
6	Montes Claros, Plastic package	leaves	6.38±0.28	0.62±0.09

[a] According to the manufacturer; [b] Values are expressed as mean ± standard deviation (SD); Averages followed by the same letter do not differ among themselves, by Tukey test, at 5% probability.

Table 2. Chemical composition of volatile oils obtained from commercial *Mentha x piperita* L. samples

Peak	Compound	RT (min.)	LRI[b] Literature	LRI Experimental	Relative peak area (%)[a]					
					1	2	3	4	5	6
1	1,8-Cineole	13.26	1026	1028						0.9
2	Isopulegol	18.85	1145	1144		0.6				
3	Menthone	19.33	1148	1152	14.4	2.0	13.8	11.1		
4	Isomenthone	19.81	1158	1164	8.5	3.5	7.6	8.9		
6	Terpinen-4-ol	20.41	1174	1176						0.5
7	Menthol	20.54	1167	1177	49.3	82.0	50.9	49.3		
8	Isomenthol	20.83	1179	1184	0.7		0.7	0.7		
9	α-Terpineol	21.16	1186	1192			0.8			
10	Dihydro carveol	21.43	1192	1198					0.4	4.5
11	Neo-dihydrocarveol	21.49	1193	1199					1.5	
12	*trans*-Carveol	22.80	1215	1227						1.0
13	Pulegone	23.48	1233	1241	2.3		2.6	6.0	8.6	6.1
14	Carvone	23.86	1239	1246	1.0		4.2	4.7	65.1	64.4
15	Piperitone	24.20	1249	1256	1.4	2.4	1.7	1.7	0.5	2.6
16	Menthyl acetate	26.13	1294	1298	11.8	2.7	9.6	9.6		
17	Carvacrol	26.20	1298	1300						1.1
18	*cis*-Carvyl acetate	29.24	1365	1369					0.4	0.8
19	β-Bourbonene	30.07	1387	1387					1.3	0.9
20	β-Elemene	30.42	1389	1395					0.7	0.5
21	*trans*-β-Caryophyllene	31.58	1417	1422	2.7		2.3	1.7	1.3	1.2
22	*cis*-Calamenene	36.01	1528	1529					1.8	1.0
23	Spathulenol	38.25	1577	1585	1.0	0.6	0.8	0.6	2.0	2.4
24	Caryophyllene oxide	38.45	1582	1590	1.7		1.2	2.1	2.9	1.2
25	Viridiflorol	38.80	1592	1599	1.5		1.7	1.4		
26	Cubenol	39.74	1618	1624					1.6	0.9
27	Torreyol	40.73	1644	1651					0.6	0.5
28	α-Cadinol	41.28	1652	1665					1.3	0.9
	Total area identified				96.3	93.8	97.9	97.8	90.0	91.4

[a] Average of two repetitions. The variation coefficient was less than 5%; [b] LRI = Linear Retention Index.

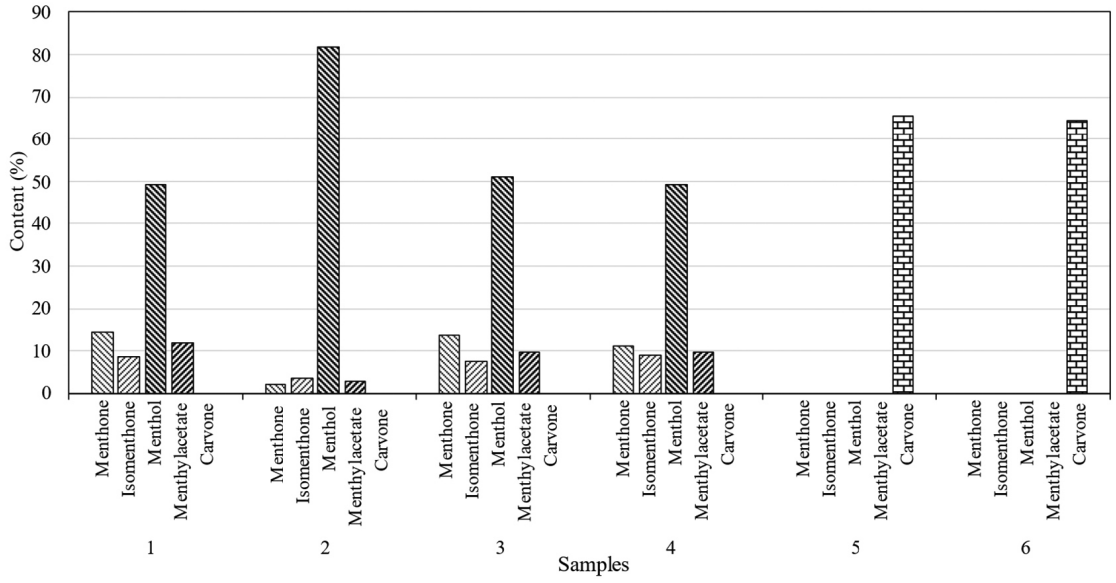

Figure 2. Variation in the menthone, isomenthone, menthol, menthyl acetate, and carvone contents (%) from the essential oil of samples **1**-**6**.

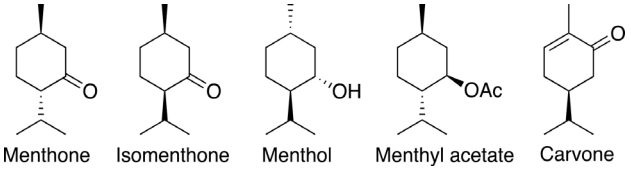

Figure 3. Chemical structures of menthone, isomenthone, menthol, menthyl acetate and carvone

Mentha x piperita L. essential oil follow the ranges: menthol (35.0-79.0%); menthone (6.0-30.0%); isomenthone (2.0-10.0%); menthyl acetate (3.0-10.0%) and 1,8-cineole (0.5-13.0%). According to these characteristics, only samples **1**-**4** could be classified as *Mentha x piperit*a L. even though some values can be out of the range stablished and 1,8-cineole was not identified in these samples (Table 3).

The syntheses and composition of the essential oils, in several aromatic plants, depend on the climate conditions, harvesting season, planting time, plant age, mineral fertilization.[19-22] These factors are responsible for the variation observed in the chemical composition of the essential oils from samples **1**-**4**.

Samples **5** and **6** present similar chemical composition. The compound carvone ranged from 64.4-65.5% in these two samples and they do not present either menthol or menthone. Similar composition of the essential oil was found by Hussain et al.[21] for *Mentha spicata* essential oil (spearmint), in which carvone varied from 59.8 to 63.24% in samples collected during the summer and winter in Pakistan. Menthol was not identified, at the same work, and the maximum of menthone found was of 0.61% (Table 3). The content of carvone found by Kizil et al.[23] in samples of *Mentha spicata* collected in Turkey was of 50.3%. Although, high concentrations of carvone (51.4-57.6%) were found in four *M. longifolia* L. samples.[24] According to Lawrence,[25] different *Mentha* species and hybrids possess essential oil rich in carvone. These results may suggest that samples **5** and **6** are not *Mentha x piperita* L.

Uses of peppermint leaves as described in pharmacopeias and in traditional systems of medicine are for symptomatic treatments of nausea, flatulence, intestinal colic, bronchitis and inflammation of the oral mucosa.[26-30]

In Brazil, *Mentha x piperita* L. is considered an herbal medicine by ANVISA[31] and the essential oil distilled from the leaves needs to present 30-55% of menthol and 14-32% of menthone to perform the therapeutic actions (expectorant, carminative and antispasmodic).[31]

Since samples **5** and **6** do not present the amount of menthol and menthone requested by ANVISA, many people could not obtain the therapeutic actions using these samples.

4. Conclusion

This study shows the importance of the chemical composition control of products from natural sources. The results revealed that none of the commercial samples evaluated presented their essential oils compounds according to the Brazilian National Health Surveillance Agency. Based on the essential oil composition, four of the samples correspond to *Mentha x piperita* L. The same analyses showed that two other samples present carvone as major compound and they do not have any amount of menthol and menthone, so they do not correspond to *Mentha x piperita* L. as declared by the manufacturer.

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Table 3. Variation in content and chemical composition of the essential oils from leaves of two *Mentha* species according to literature data[15]

Compounds[a]	LRI[b]	Composition (%)	
		***Mentha x piperita* L.**[c]	***Mentha spicata***[d]
Limonene	1024	0.5 – 5.0	9.09 - 10.44
1,8-Cineole	1026	0.5 – 13.0	3.51 - 6.36
Menthone	1148	6.0 – 30.0	0.00 - 0.06
Isomenthone	1158	2.0 – 10.0	0
Neo-menthol	1161	2.0 – 3.5	0
Menthol	1167	35.0 – 79.0	0
Pulegone	1233	maximum of 2.0	0
Carvone	1239	maximum of 1.0	59.5 – 63.2
Menthyl acetate	1294	3.0 – 10.0	0
Piperitenone oxide	1249		0

[a] Compounds are listed in order of LRI; [b] LRI = Linear Retention Index in literature[15]; [c] Brazilian Pharmacopoeia; [d] Hussain *et al*., 2010

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