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Chemical Characterization, Antibacterial and Antioxidant Activities of Essential Oils of *Mentha viridis* L. and *Mentha pulegium* L. (L)

Lucilene Fernandes Silva[1], Maria das Graças Cardoso[1*], Luís Roberto Batista[2], Marcos de Souza Gomes[1], Leonardo Milani Avelar Rodrigues[2], Danúbia Aparecida de Carvalho Selvati Rezende[1], Maria Luisa Teixeira[1], Marcos Schleiden Sousa Carvalho[3], Juliana de Andrade Santiago[1], David Lee Nelson[4]

[1]Department of Chemistry, Federal University of Lavras, Lavras, Brazil
[2]Department of Food Science, Federal University of Lavras, Lavras, Brazil
[3]Department of Agriculture, Federal University of Lavras, Lavras, Brazil
[4]Federal University of Vales de Jequitinhonha e Mucuri, Diamantina, Brazil
Email: [*] mcardoso@dqi.ufla.br

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Abstract

The essential oils from *Mentha viridis* L. and *Mentha pulegium* L. were characterized by gas chromatography coupled to mass spectrometry (GC-MS). These oils were obtained by hydrodistillation and presented linalool (40.70%), carvone (13.52%) and α-terpinene (8.56%) as the principal constituents in the essential oil from *Mentha viridis* L. Pulegone (50.01%), menthol (31.90%) and menthone (16.56%) were the principal constituents in the essential oil from *Mentha pulegium* L. These essential oils (in concentrations ranging from 3.91 to 500 µL·mL^{-1}) showed satisfactory activities against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella choleraesuis* and *Staphylococcus aureus*. The antioxidant activities with 2-deoxyribose and phosphomolybdenum and the reducing power (in concentrations ranging from 0.78 to 1000 µL·mL^{-1}) were determined. The antioxidant activity was observed for the two oils evaluated by the phosphomolybdenum and 2-deoxyribose methods, whereas the essential oil from *M. viridis* presented low antioxidant activity in the reducing power assay.

Keywords

Mentha, Linalool, Pulegone, Volatiles

[*]Corresponding author.
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1. Introduction

Essential oils are composed of a number of compounds of different biosynthetic origins ranging from terpenoid hydrocarbons to sulfur compounds [1], and such compounds are naturally present in different concentrations [2]. The essential oils of the *Mentha* genus have a high commercial value, because they usually contain the monoterpene menthol in their constitutions, and menthol is widely used in pharmaceutical, cosmetic, personal care and food products [3].

Within the broad spectrum of action of the essential oils, one can highlight the study of their activities against bacteria [4]. Several methods have been used by mankind with the intention of obtaining produce uncontaminated by these microorganisms. Essential oils constitute a viable alternative because of the fact that they are substances of low molecular weight, are volatile and usually exhibit low toxicity to mammals. Bacteria have caused many problems that result mainly from the proliferation of resistant microorganisms. They are among the most serious threats to public health and the success of anti-bacterial treatment. Recently, the emergence of antibiotic-resistant bacterial strains has increased dramatically, so the search for natural substances to control these microorganisms is of paramount importance [4]. The changing lifestyle of the population is concomitant with the phenomenon of bacterial resistance. Stress, pollution, poor diet, physical inactivity and other factors are evidence that bad habits can cause serious human health problems. One of the effects posed by these bad habits is the oxidative stress that can cause various biochemical alterations, and consequently serious disorders in the human body. Oxidative stress can result in damage to the basic biomolecules such as lipids, proteins and DNA and can promote cytotoxic and genotoxic effects in cells [5].

Many compounds produced by plants have been evaluated to obtain substances active against oxidative stress, that is, compounds with antioxidant properties. The essential oils are among the natural products that have been studied to evaluate their antioxidant potentials [6] [7]. In this study we sought to characterize the essential oils extracted from *Mentha viridis* L. and *Mentha pulegium* L. (L), as well as to evaluate their antibacterial and antioxidant activities.

2. Material and Methods

2.1. Collection, Identification and Extraction of the Essential Oils

Leaves and flowers from *M. viridis* and *M. pulegium* were collected at 07:00 a.m. in the month of November 2012 in the Garden of Medicinal Plants of the Federal University of Lavras on a mild day with no precipitation. The city of Lavras is located in the southern part of the state of Minas Gerais (Brazil) at 21˚14'S, longitude 45˚00'W Gr. and at 918 meters in altitude. The plants were identified in the Herbarium of the Department of Biology (DBI) of the Federal University of Lavras (UFLA). After being identified, they were incorporated into the collection of the ESAL Herbarium under registration numbers 27,122 (*Mentha pulegium* L. (L)) and 27,123 (*Mentha viridis* L.).

The leaves of the two *Mentha* species were weighed and extracted by hydrodistillation for two hours using a modified Clevenger apparatus adapted to a six-liter round bottom flask. The distillate was centrifuged in a centrifuge with a horizontal crosspiece (Model R 206 T FANEM BL) at 965.36 g for 10 minutes. The essential oil was collected with the aid of a Pasteur pipette, transferred to a vial and placed in a drying pistol to remove any trace of moisture. Subsequently, the flask was kept refrigerated until use for biological analyses [8]. The extraction of the essential oils was performed in triplicate. To determine the moisture content for subsequent calculation of the yield of the oil present in the fresh plant, 5 g of the chopped plant material was extracted with 80 mL of cyclohexane, employing a Dean-Stark extractor. The moisture content was determined in triplicate according to the method described by Pimentel *et al.* [9].

2.2. Identification and Quantification of Essential Oil Components

Quantitative analysis and the identification of the essential oil constituents were performed at the Federal University of Lavras (UFLA). Quantitative and qualitative analyses of the essential oils were performed on a Shimadzu GC-2010 gas chromatograph equipped with a GCMS-QP 2010 Plus mass spectrometer under the following operating conditions: an RTX-5MS (5% dimethylpolysiloxane) fused silica capillary column (30 m × 0.25 mm i.d. × 0:25 μm film), using helium as the carrier gas with a flow rate of 1.18 mL·min^{-1}. The initial column temperature was 60˚C. The temperature was programmed at 3˚C·min^{-1} to 240˚C, followed by an in-

crease of 10˚C·min^{-1} to 300˚C, where the temperature was maintained constant for 7 min. The injector temperature was 200˚C and the detector (or interface) temperature was 240˚C. A one µL volume of the essential oil dissolved in dichloromethane was injected into the chromatograph. The ion capture detector was operated in electron impact mode with an impact energy of 70 eV. The scan speed was 1000, the scan interval was 0.50 fragments, and the fragments were detected in the range of 45 to 500 Da.

2.3. Antibacterial Activity

The evaluation of the antibacterial activity of the essential oils was accomplished at the Food Microbiology Laboratory of the Department of Food Science at the Federal University of Lavras. The microorganisms employed were: *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538, *Salmonella choleraesuis* ATCC 6539 and *Listeria monocytogenes* ATCC 7644. During the experiment, the microorganisms were kept in tubes containing freezing medium under refrigeration (4˚C). For activation of the bacterial cultures, strains were transplanted to brain-heart-infusion (BHI) broth and were incubated at 37˚C for 24 hours to obtain the inoculum.

Upon activation of the bacterial suspension, 300 µL of the medium was transferred to a tube containing 5 mL of peptone, casein and soy broth (TSB). The tubes were again incubated at 37˚C until they reached the turbidity of a 0.5 McFarland standard reference solution, resulting in a suspension containing approximately 1 to 2 × 10^8 CFU/mL. The correct density of the turbidity control was verified using a spectrophotometer (Shimadzu UV-160 PC 1) at a wavelength of 625 nm, wherein the absorbance occurred between 0.10 and 0.8, a value recommended by the National Committee for Clinical Laboratory Standards [10].

The antibacterial activity of the essential oils was determined by diffusion in an agar cavity employing TSA agar (Triptic Soy Agar) for *Listeria monocytogenes* ATCC 7644 and Mueller-Hinton agar Pereira *et al.* [11] for the other species (*Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538, and *Salmonella choleraesuis* ATCC 6539). Initially, a thin layer of agar was added to Petri dishes (140 mm diameter). After solidification, 4-mm sterile glass beads were placed on the solid medium. Aliquots of standardized cultures of *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538, *Salmonella choleraesuis* ATCC 6539 and *Listeria monocytogenes* ATCC 7644 were transferred to glass flasks containing 90 mL of Mueller-Hinton agar or TSA, respectively, to furnish concentrations of 10^6 CFU mL^{-1} in the developing culture. The liquid medium was poured onto the previous layer.

After solidification of the agar or TSA, the glass beads were removed with the aid of sterile forceps, and the essential oil was deposited in the pits. Dilutions were made in dimethylsulfoxide solution (DMSO) in the proportions 1:1; 1:2; 1:4; 1:8; 1:16; 1:32; 1:64 and 1:128 parts of essential oil to parts of diluent to give concentrations of 500; 250; 125; 62.5; 31.3; 15.62; 7.81 and 3.90 µL·mL^{-1}. Ten microliters of diluted essential oil was applied to the cavities. The plates were incubated in BOD at 37˚C for 24 hours, and the diameters of the inhibition zones were measured. Three replicates were performed for each treatment. For the negative control, 10 µL of DMSO was applied. For the positive standard, a solution of 1000 mg·L^{-1} of chloramphenicol (CL) was used [12]. The sensitivity of the bacteria to different concentrations of essential oils was determined from the diameters of the inhibition zones. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of essential oil for which an inhibition halo was observed.

2.4. Antioxidant Activities

2.4.1. 2-Deoxyribose Assay

The antioxidant activity of sequestering the hydroxyl radical was determined from the decrease in the formation of substances reactive to TBA (thiobarbituric acid) from deoxyribose caused by degradation by the hydroxyl radical [13]. Solutions of essential oils were prepared at concentrations of 0.78; 1.56; 3.12; 6.25; 12.5; 25; 50; 100; 150; 200; 250; 500 and 750, and 1000 µL·mL^{-1} in water. The reaction mixtures were prepared in tubes containing 50 µL of the sample; 100 µL of Fe-EDTA Mix, 100 µL of deoxyribose, 680 µL of 0.1 M phosphate buffer, pH 7.4, and 100 µL of H_2O_2 were incubated for 2 hours in a water bath at 37˚C. To terminate the reaction, 500 µL of trichloroacetic acid and 500 µL of TBA were added. The tubes were placed in a water bath for 10 minutes. For the blank, all the reagents were added except deoxyribose, and for the control, all the reagents were added except the sample. Tests for comparison were performed with mannitol, which is used to reduce brain edema, hypertension and other diseases. Absorbance readings were performed on a Shimadzu UV-1601PC spectrophotometer at 527 nm.

2.4.2. Reducing Power Assay

The test was performed by colorimetric measurements at 700 nm in a Shimadizu UV-1601PC spectrophotometer. To measure the activity, 50 µL of different concentrations of essential oils (0.78; 1.56; 3.12; 6.25; 12.5; 25; 50; 100; 150; 200; 250; 500 and 750 and 1000 $\mu L \cdot mL^{-1}$) in ethanol were added to 500 µL of 200 mM phosphate buffer, pH 6, and 500 µL of 1% potassium hexacyanoferrate (III). The mixture was stirred and incubated for 20 minutes at 50˚C. To the mixture, were added 500 µL of TCA, 1500 µl H_2O and 300 µL of $FeCl_3$. The procedure was repeated without the sample for the negative control. The blank only contained ethanol.

A curve of the absorbance versus the concentration of the sample was constructed for the determination of the antioxidant activity. BHT was used as a positive control, and the analyses were performed in triplicate [14].

2.4.3. Reduction of the Phosphomolybdenum Complex

Solutions (50 µL) of the essential oils in different concentrations (0.78; 1.56; 3.12; 6.25; 12.5; 25; 50; 100; 150; 200; 250; 500; 750 and 1000 $\mu L \cdot mL^{-1}$ ethanol) was added to 2 mL of the phosphomolybdenum reagent (10% sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated for 60 min at 95˚C. After cooling, the tubes were read at 695 nm on a Shimadizu UV-1601PC spectrophotometer at 527 nm. Ethanol was used as a blank, and the sample was replaced by ethanol in the negative control. The antioxidant capacity of the essential oils was expressed relative to that of ascorbic acid [15]. Analytical curves of thirteen points with ascorbic acid (0.78 to 1000 $mg \cdot L^{-1}$) were constructed. BHT was also tested for comparison.

Calculations were performed using the absorbances obtained from the different dilutions (thirteen concentrations) of the essential oils. The graph of the the essential oils and BHT with the absorbance on the y-axis and the corresponding concentration for each dilution ($\mu g \cdot L^{-1}$) on the X axis was constructed, and the equation of the line was determined. The absorbance corresponding to the ascorbic acid standard was substituted into this equation to furnish the concentration of the sample that corresponded to 1.000 $\mu g \cdot L^{-1}$ of ascorbic acid. The final result was calculated by dividing 1000 by the value of × (g) and multiplying by 1 g and was expressed in $\mu g \cdot L^{-1}$ g^{-1} ($\mu g \cdot L^{-1}$ essential oil per gram BHT) [16].

2.5. Statistical Analysis

The data resulting from the antibacterial activity experiments were subjected to analysis of variance by the statistical program Variance Analysis System for Balanced data (SISVAR) in a factorial 2 × 8 system (2 essential oils and 8 concentrations) according to the method of Ferreira [17], and the means were compared by the Tukey test at 5% probability. Quantitative variables (antioxidant activity) were subjected to regression analysis for each method tested. To obtain the RI_{50} (relative inhibition) for 2-deoxyribose antioxidant activity, graphs of the AA% values (percentage of antioxidant activity) versus the concentrations analyzed or A (absorbance) versus the concentrations were constructed.

3. Results and Discussion

The yields of the essential oils of *M. pulegium* and *M. viridis* were 2.54% and 1.52%, respectively, on a moisture-free basis. Seven components were identified in the essential oil from *M. pulegium* (**Table 1**). The major constituents were pulegone (50.01%), menthol (31.9%) and menthone (16.56%).

Table 1. Composition of the essential oil from *Mentha pulegium* L.

Peak No.	RT*	Compound	% Área
1	5.75	*α*-Phelandrene	0.2
2	6.96	*β*-Pinene	0.17
3	8.64	Cyclohexene	0.41
4	13.84	Menthone	16.56
5	14.40	*cis*-Isopulegone	0.75
6	14.63	Menthol	31.90
7	17.03	Pulegone	50.01
Total			100

*RT = Retention time.

Oliveira *et al.* [18] studied the essential oil from *Metha pulegium* collected in the spring and winter. They detected 6.62% borneol, 61.43% pulegone in the spring and 8.87% borneol and 28.40% pulegone in the winter. The authors demonstrated the influence of seasonality with a 54% reduction in the concentration of pulegone in the winter. In the essential oil from *M. pulegium*, pulegone is the precursor for the formation of the stereoisomers of menthone, and this transformation leads to a reduction in the pulegone content of the essential oils. These stereoisomers form the acetylated menthyl, neoisomenthyl and isomenthyl derivatives. The concentration of pulegone (61.43%) in the spring is similar to that observed in the present study. The concentration of borneol (8.87%), however, is different.

Ait-Ouazzou *et al.* [19] also observed that pulegone was the principal constituent (69.8%) in the essential oil from *M. pulegium*, followed by piperitenone (3.1%), isopulegone (1.8%), and cis piperitone epoxide (1.7%). These authors found pulegone in all their studies on the essential oils from *M. pulegium*. Variations in the compositions of essential oils are explained by the variation in location, time of collection, and the stress to which the plant was exposed, among other factors. Therefore, differences in the concentrations of constituents are very common when work is performed with essential oils [20].

The 32 constituents encountered in the essential oil from *M. viridis* are presented in (**Table 2**). The principal

Table 2. Composition of the essential oil from *Mentha viridis* L.

Peak No.	RT*	Compound	% Área
1	5.544	*α*-Tujene	0.2
2	5.74	*α*-Pinene	0.69
3	6.818	Sabinene	1.04
4	6.936	*β*-Pinene	1.01
5	7.164	3-Octanone	3.07
6	7.284	Myrcene	2.21
7	7.429	*α*-Terpinene	8.56
8	8.155	Ethylhexanol	0.28
9	8.425	p-Cymene	2.81
10	8.563	Limonene	3.53
11	8.667	1,8-Cineol	2.78
12	8.848	*β*-Ocimene	0.48
13	9.633	*ɤ*-Terpinene	0.93
14	9.955	*trans*-Sabinene	5.24
15	10.164	*trans*-Linalool	0.03
16	10.757	Terpinolene	0.47
17	11.266	Linalool	40.7
18	12.086	Menth-2-en-1-ol	0.54
19	14.359	Terpinen-4-ol	5.82
20	14.933	*α*-Terpineol	0.84
21	15.105	Cyclohexanol	0.21
22	16.192	*trans*-Carveol	0.09
23	16.414	Isoascaridol	0.07
24	16.793	Isovalerate	1.07
25	17.209	Carvone	13.52
26	24.119	*β* –Farnesene	0.01
27	24.517	Bicyclo[7.2.0]undec-4-ene	2.01
28	26.289	*α*-Humulene	0.09
29	27.024	*ɤ*-Cadineno	0.32
30	27.434	Germancrene D	0.07
31	28.704	Propanoic acid	0.05
32	31.051	Caryophylene oxide	0.25
Total			99.7

constituents were linalool (40.7%), carvone (13.52%) and α-terpinene (8.56%). Mkaddem *et al.* [21] identified carvone (50.47%) as the principal constituent in the essential oil from *M. viridis*, followed by 1,8-cineole (9.14%), limonene (4.87%), camphor (3.68%) and β-caryophyllene (3%). All the major compounds found by those authors, except camphor, were also found in the present study. However, only carvone was found to be the principal constituent in both studies. Ashnagar *et al.* [22] identified carvone as the principal constituent in the essential oil from *M. viridis*. Similarly, linalool and carvone were observed to be the principal components in the current study.

The chemical composition of the essential oils is influenced by factors such as leaf development and the emergence of new organs, which may lead to lower concentrations of these metabolites caused by translocation, as well as by effects such as seasonality, rain levels, and the stress to which the plant is exposed. These effects can directly influence the quantity and quality of the constituents in the essential oil [20].

3.1. The Antibacterial Activities of the Essential Oils

The antibacterial activities of the essential oils from *M. viridis* and *M. pulegium* against the Gram-negative bacteria *E. coli* and *S. choleraesuis* and against the Gram-positive bacteria *S. aureus* and *L. monocytogenes* are described in (**Table 3**).

The essential oils from *M. viridis* and *M. pulegium* presented the same minimal inhibitory concentration (MIC) of 62.5 $\mu L \cdot mL^{-1}$ for the Gram-negative bacterium *E. coli*, forming 6-mm inhibition halos at this concentration. Unlike *E. coli*, *S. choleraesuis* was more sensitive to the essential oils. The minimum inhibitory concentration was 31.3 $\mu L \cdot mL^{-1}$ for both essential oils. The diameters of the inhibition zones were 8 mm.

Differences in the minimum inhibitory concentrations of the essential oils were observed for Gram-positive bacteria. The essential oil from *M. pulegium* was more effective against *S. aureus*, but the minimum inhibitory concentration was 15.6 $\mu L \cdot mL^{-1}$, and the inhibition halo formed was 5 mm. The minimum inhibitory concentration was 62.50 $\mu L \cdot mL^{-1}$ for the essential oil from *M. viridis*, and an 8-mm inhibition zone was formed. The minimum inhibitory concentrations and inhibition halos for the Gram-positive bacteria *L. monocytogenes* were 62.50 $\mu L \cdot mL^{-1}$ (6 mm) and 125 $\mu L \cdot mL^{-1}$ (7 mm) for oils from *M. viridis* and *M. pulegium*, respectively. With respect to the microorganisms *S.* choleraesuis and *E. coli*, the MIC for the essential oil from *M. pulegium* was similar to that for the essential oil from *M. viridis* (31.3 $\mu L \cdot mL^{-1}$ and 62.5 $\mu L \cdot mL^{-1}$, respectively, for the two microorganisms). This fact was probably due to both the difference between the bacteria and the difference in the chemical constituents present in essential oils. The antibiotic chloramphenicol inhibit all the bacteria, forming a 25-mm inhibition zone for all the microorganisms tested at the concentration of 1000 $mg \cdot L^{-1}$.

Boukhebti *et al.* [23] studied the essential oil from *M. pulegium* and observed that 10-mm and 7-mm inhibition zones were formed with the microorganism *E. coli* ATCC 25922 only when treated with 1:1 and 1:2 dilutions of the essential oil, respectively. Similarly, 12.5-mm and 7-mm inhibition zones, respectively, were formed with *S. aureus* ATCC 25923 when the same concentrations were tested. These results are different from those found in this study in which the essential oil from *M. pulegium* presented activity in a 1:4 dilution for *E. coli* and

Table 3. Antibacterial activities of the essential oils from *M. viridis* and *M. pulegium*.

Mean diameters of the inhibition halos								
		Concentration ($\mu L \cdot mL^{-1}$)						
Bacteria	**Sample**	**500**	**250**	**125**	**62.5**	**31.3**	**15.6**	**7.8**
E. coli	*M. pulegium*	11.0Aa	10.0Aa	7.0Bb	6.0Ac	NIAd	NIAd	NIAd
	M. viridis	8.0Ba	8.5Ba	8.5Ba	6.0Ab	NIAc	NIAc	NIAc
S. choleraesuis	*M. pulegium*	14.0Aa	11.0Bb	10.0Bc	10.0Ac	8.0Ad	NIAe	NIAe
	M. viridis	13.0Ba	13.0Ab	12.0Ab	11.5Ac	8.0Ac	NIAd	NIAd
S. aureus	*M. pulegium*	9.5Ba	8.5Ab	5.5Bc	5.5Bc	5.0Ac	5.0Ac	NIAd
	M. viridis	11.0Aa	8.0Ab	8.0Ab	8.0Ab	NIBc	NIBc	NIAc
L. monocytogenes	*M. pulegium*	10.5Aa	9.5Ab	6.5Bc	6.0Ad	NIAe	NIAe	NIAe
	M. viridis	9.5Ba	9.5Aa	7.0Ab	NIBc	NIAc	NIAc	NIAc

NI: No inhibition. The capital letters represent the statistical analysis of essential oils (*M. viridis* and *M. pulegium*) within only one concentration, where the antibacterial activity in the column can be evaluated separately for each bacterium. Lowercase letters represent statistical analysis of only one essential oil at all the concentrations tested. Means followed by the same letter do not differ in the level of 5% probability by the Tukey test.

a 1:6 dilution for *S. aureus*. This difference may be associated with variations in the type and quantities of the constituents present in the essential oil, because Boukhebti *et al.* [23] found pulegone (38.8%), menthone (19.2%) and *β*-bourbonene (16.4%) and pulegone (50.01%), menthone (31.90%) and menthol (16.56%) were observed to be the principal constituents in the present study.

3.2. Antioxidant Activities of the Essential Oils

The determination of antioxidant activity by the 2-deoxyribose method is summarized in (**Figure 1**).The essential oil from *M. pulegium* presented the highest antioxidant activity, followed by the essential oil from *M. viridis*. The lowest antioxidant activity was that of mannitol. The RI_{50} activities were 196.37; 340.36; and 5115.34 $\mu g \cdot mL^{-1}$, respectively.

Mimica-Dukic *et al.* [24] studied the essencial oils from *Mentha piperita*, *Mentha longifolia* and *Mentha aquatica* neat and in solution (20% and 50% hexane). They observed that the essential oil from *M. aquatica* contained 1,8-cineol (14.15%), menthofuran (16.94%) and E-caryophyllene (9.36%) as the principal constituents. It exhibited the lowest activity of the samples studied (8.6% in 20% hexane solution), much lower than those observed for *M. viridis* and *M. pulegium* in the present study. The essential oil from *M. longifolia* was shown to exhibit antioxidant activity (18.2%) when tested neat in the Fenton reaction, in which the OH radical is reduced. The authors identified piperitone (38.79%), menthone (11.20%) and pulegone (4.88%) as the principal components of this oil, compounds which are also present in the essential oil from *M. pulegium*. The essential oil from *M. piperita* exhibited the highest activity (24%) by this method. Menthol (39.63%), methylacetate (10.44%) and menthone (8.93%) were among the constituents present in this oil. In the current study, the essential oils from *M. pulegium* and *M. viridis* exhibited higher antioxidant activities than was observed in their study.

The test of antioxidant activity by the reducing power method is commonly used to study the antioxidant capacity of plant materials. The determination of antioxidant activity by the power to reduce the ferric ion (**Figure 2**) showed that the antioxidant activity increased with increasing concentration of the samples. This fact is demonstrated by the BHT standard because its antioxidant action also increased with increasing concentration.

Salarbashi *et al.* [25] studied the effect of packaging films produced from soluble soybean incorporated with the essential oils from *Zataria multiflora* Boiss and *M. pulegium*. They observed that the reduction of ferric ion was dependent on the concentration of the essential oils. The authors suggested that the reducing power of these films was higher for films containing the essential oil from *Z. multiflora* Boiss than those containing the essential oil from *M. pulegium* in all the concentrations tested. This difference in activity was attributed to phenylpropanoids and terpenes present in the essential oil from *Z. multiflora* Boiss (thymol, carvacrol and *γ*-terpinene), which were different from those found in the essential oil from *M. pulegium* (pulegone, menthone and piperitone). In the present study, the essential oil from *M. viridis* exhibited very limited antioxidant activity in reducing the ferric ion. On the other hand, the antioxidant activity of the essential oil from *M. pulegium* exceeded that of the antioxidant standard BHT in concentrations from 0.78 to 50 $\mu L \cdot mL^{-1}$, but the control showed higher antioxidant activity at concentrations of 100 $\mu L \cdot mL^{-1}$ and above. BHT (the standard widely used in industry) was the most efficient antioxidant at the concentration of 250 $\mu L \cdot mL^{-1}$ in the phosphomolybdate method. At lower concentrations, the essential oil from *M. pulegium* presented an antioxidant activity higher than that of the standard, followed by the essential oil from *M. viridis*, as is shown in (**Figure 3**).

In evaluating the bioprospection of the aerial parts and the essential oil from *Liquidambar styraciflua* L., Franco [26] noted that the total antioxidant capacity was dose-dependent both for the standard and the sample because the antioxidant capacity increased as the concentration increased. The highest value found for the total antioxidant capacity of the stem and bark samples from *L. styraciflua* may be related to the presence of compounds that act by both reducing mechanisms, redox and radical, causing a greater reduction in the complex in a similar manner. The effects of the essential oils in this study were dose-dependent because the increase in concentration of the essential oils resulted in an increased activity.

There are several in vitro methods for the evaluation of the antioxidant activity of a biologically active compound, and there is no universal method by which the antioxidant activity can be measured precisely and quantitatively. Different methods lead to different values for the antioxidant activities for the removal of distinct radicals. Numerous reactive species are used to assess antioxidant activities because of their different forms of acting in living organisms. Therefore, the greater the variety of methods used, the better, because each technique has a principle that depends on the reaction matrix. In addition, the bioactive compounds may react differently depending on the radical or oxidizing agent used in the method [27].

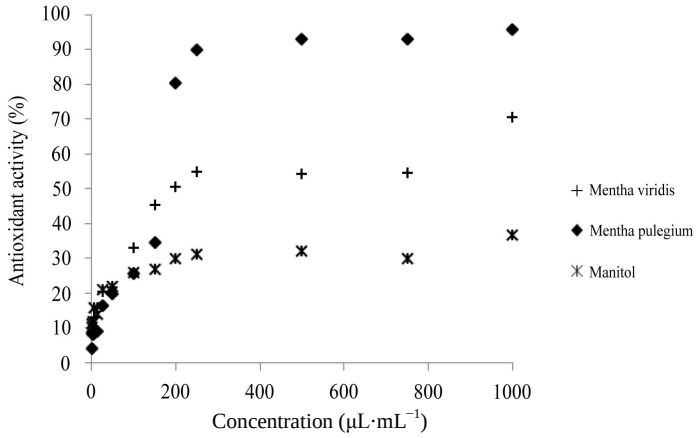

Figure 1. Antioxidante activity determined by the 2-deoxyribose method.

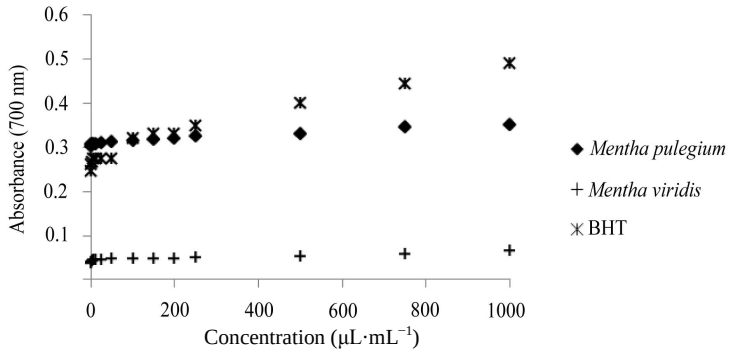

Figure 2. Antioxidant activity determined by the reducing power method.

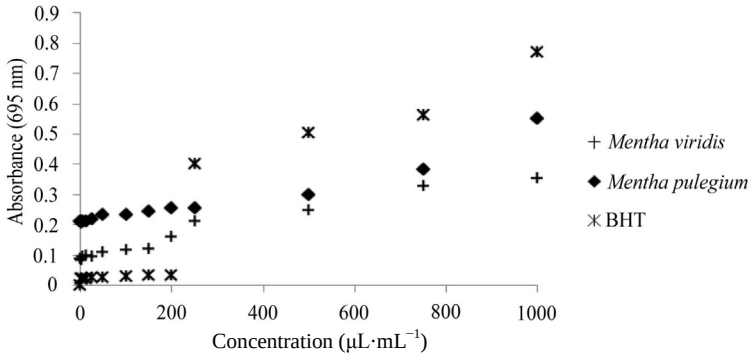

Figure 3. Antioxidant activity determined by the phosphomolybdate reduction method.

4. Conclusion

The yield of the essential oil from *M. pulegium* (2.54%) was higher than the yield of the essential oil from *M. viridis* (1.52%). The major constituents of the essential oil from *M. pulegium* were pulegone (50.01%), menthol (31.90%) and menthone (16.56%), and those in the essential oil from *M. viridis* were linalool (40.70%), carvone (13.52%) and *α*-terpinene (8.56%). Both the oils exhibited inhibitory effects on the growth of the bacteria tested, being more effective against strains of *S. aureus* and *S. choleraesuis*. The species of bacteria that was the most resistant to the essential oils was *E. coli*. The essential oil from *M. pulegium* exhibited the highest antioxidant activity by the 2-deoxyribose method, followed by the essential oil from *M. viridis* and mannitol. By the iron reducing power method, only the BHT and the essential oil from *M. pulegium* possessed antioxidant activity.

Both essential oils exhibited an antioxidant activity that was a third of the activity observed for the BHT using the phosphomolybdenum method. Data from bactericidal and antioxidant tests suggest that there is a high potential for application of the essential oils tested as natural preservatives, and they may have wide applications in food and cosmetics industries, among others.

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