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Chemical composition and antifungal activity of pennyroyal essential oil in different stages of development

Composição química e atividade antifúngica do óleo essencial de poejo em diferentes estágios de desenvolvimento

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Abstract

The aim of this study was to compare the yield, chemical composition and antifungal activity of the essential oil that was obtained from *Mentha pulegium* in different developmental stages and cultivated under controlled conditions in southern Brazil. The hydrodistillation of fresh leaves that were collected at 60, 70 and 85 days resulted in essential oil yields of 0.17 %, 0.23 % and 0.17 %, respectively. All of the essential oil samples showed antifungal activity against *Cladosporium herbarum*. The gas chromatograph (GC) and GC/MS analysis revealed eleven constituents: seven (pulegone, piperitenone, menthone, isomenthone, neoisomenthol, piperitone and 1.1-dimethoxy-2-nonyne) were common to three samples, while menthofuran and myrtenal were detected only in samples of the first and second stages. Pulegone was the main constituent of the essential oil samples from the first and second stages (26.65 %), followed by piperitenone (20.41; 12.60 %). The pulegone concentration increased to 31.05 % in the last collection, while the major constituent was piperitenone (36.32 %). In conclusion, the results demonstrated that *M. pulegium* essential oil presents potential as an antifungal agent, and its chemical composition depends on the stage of development during which it was extracted.
Key words: *Mentha pulegium,* pulegone, piperitenone, antifungal, *Cladosporium herbarum*

Resumo

O objetivo deste estudo foi comparar o rendimento, a composição química e a atividade antifúngica do óleo essencial de *Mentha pulegium* (poejo) em diferentes estágios de desenvolvimento, cultivada sob condições controladas no sul do Brasil. A hidrodestilação de folhas frescas de *M. pulegium*, coletadas aos 60, 70 e 85 dias após o transplante, apresentou rendimento de óleo essencial de 0,17 %, 0,23 % e 0,17 %, respectivamente. Todas as amostras de óleo apresentaram atividade contra *Cladosporium herbarum*. Através das análises de cromatografia gasosa (GC) e GC/MS do óleo essencial, foram identificados onze constituintes, sete (mentona, isomentona, neoisomentol, pulegona, piperitona, 1.1- dimetoxi-2-nonino e piperitenona) comuns às três amostras, enquanto dois outros (mentofurano, mirtenal) foram detectados somente nas amostras da primeira e segunda coletas. A pulegona foi o principal constituinte

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nas duas primeiras amostras (26,65 %), seguida pela piperitenona (20,41; 12,60 %). A concentração de pulegona aumentou para 31,05 % na última coleta, porém o constituinte majoritário foi a piperitenona (36,32 %). Os resultados demonstraram que o óleo essencial de *M. pulegium* apresenta potencial como agente antifúngico e sua composição química depende do estágio de desenvolvimento da planta.
Palavras-chave: *Mentha pulegium,* pulegona, piperitenona, antifúngica, *Cladosporium herbarum*

Mentha pulegium L. is known by several common names, such as pennyroyal, mint and poleo. The essential oils from this plant are indicated for the treatment of chronic catarrhal bronchitis, asthmatic bronchitis, whooping cough, leucorrhea and dysmenorrhea (LORENZI; MATOS, 2008). In addition, this plant has been used as a spice and flavoring agent (MKADDEM et al., 2007). Several literature records demonstrated the insecticidal (MIGUEL et al., 2010), antibacterial (SIVROPOULOU et al., 1995), fungicide (MUELLER-RIEBAU et al., 1995), nematicidal (CABONI et al., 2013) and antioxidant activity (KAMKAR et al., 2010) of *M. pulegium* essential oil. Although there are several studies on *M. pulegium* essential oil composition from different countries (ZWAVING; SMITH, 1971; LORENZO et al., 2002; KOKKINI et al., 2004; COOK et al., 2007; MAHBOUBI; HAGHI, 2008; MORTEZA-SEMNANI et al., 2011; AIT-OUAZZOU et al., 2012; GHAZGHAZI et al., 2013; RODRIGUES et al., 2013; OLIVEIRA et al., 2011), there are insufficient studies on the composition variation of this oil throughout the life cycle of the plant and its antifungal activity. Therefore, this study aimed to determine the chemical composition variation of the essential oil throughout the developmental cycle of *M. pulegium* at the Agronomic Institute of Paraná (IAPAR) in southern Brazil in order to determine the ideal stage for plant collection to obtain an essential oil rich in active compounds. In addition, the essential oil's activity against *Cladosporium herbarum* was evaluated in a bioautography test. This fungus is considered a saprophyte, and under stress conditions, it can become a plant pathogen (O'DONNELL; DICKINSON, 1980; BARBOSA et al., 2001); it is an important pathogen of *Passiflora edulis* (passion fruit), reducing the production yield and fruit quality (BARBOSA et al., 2001). In addition, some fungal isolates have shown the ability to penetrate into the *Phaseolus* leaves (O'DONNELL; DICKINSON, 1980).

Seedlings of *M. pulegium* were produced from plant matrices that were collected in beds at the Agronomic Institute of Paraná – IAPAR, in Londrina Municipality, using apical cuttings with a size of 5 cm. The herborization was performed according to the recommendations of the Institute of Botany (INSTITUTO DE BOTÂNICA, 1989). Vouchers were collected and deposited in the herbarium of the State University of Londrina (UEL) with the deposit number 48891. The cuttings were treated with indolebutyric acid at a concentration of 1.5 g L^{-1} by immersing the basal portion in the solution for five seconds. Then, the cuttings were rooted in wooden boxes containing rice hulls and maintained in a mist chamber. After 15 days, the rooted cuttings were transferred into plastic pots of 500 cm^3 capacity containing substrate that was composed of earth and sand (2:1, v/v) that had been sterilized for 2 hours at 120 °C. The pots containing the cuttings were kept in the greenhouse (IAPAR). The maximum and minimum temperatures in the greenhouse from 14/08/2009 to 13/10/2009 (first stage) were 26.1 °C and 15.4 °C, respectively, with an average of 20.3 °C. From 14/09/2009 to 10/23/2009 (second stage), the maximum and minimum temperatures were 25.99 °C and 15.6 °C, respectively, with an average of 20.3 °C, and from 24/10/2009 to 11/07/2009 (third stage), the maximum and minimum temperatures were 26.7 °C and 16.1°C, respectively, with an average of 21.0 °C.

Samples of fresh leaves of *M. pulegium* were obtained during three different periods. The first collection was on 10/13/2009, 60 days after planting

(60 DAT); the second on 10/23/2009, 70 days after planting (70 DAT); and the third on 11/07/2009, 85 days after planting (85 DAT) in pots. The selection of the dates was based on the recommendation that the harvesting of *M. pulegium* to be used for therapeutic purposes should begin 2-3 months after planting (RIBEIRO; DINIZ, 2008). Consequently, samples were collected at the beginning (60 days), middle (70 days) and end (85 days) of the developmental cycle of the plant in order to verify the yield and composition variation of the essential oil.

Fresh leaves of *M. pulegium* (100 g) were subjected to the process of the extraction of the essential oil by hydrodistillation for two hours using a Clevenger apparatus. The extractions of the three samples were performed in triplicate. After the extractions, the oil was separated from the aqueous phase by extraction with dichloromethane (4 times/100 mL). The organic phase was dried over sodium sulfate, filtered and concentrated on a rotary evaporator.

The gas chromatograph (GC) analysis of the essential oil was carried out on a Shimadzu GC-17 apparatus that was equipped with flame ionization detector (FID) and a fused silica capillary column (30 m × 0.25 mm), with stationary phase DB-5 (0.25 mm thick film) using nitrogen as carrier gas with a flow of 1.2 mL min^{-1}. The starting temperature was programmed at 60 °C and then increased by 7 °C/min until 320 °C, keeping a constant temperature for 5 min. The temperatures of the injector and detector were 220 °C and 300 °C, respectively. Then, 2.0 mL of diluted sample (in dichloromethane 1:10 v/v) was injected at the rate of partition of the injected volume of 1:20. CG/MS was performed using the Shimadzu equipment coupled to Shimadzu apparatus GC/MS-QP5000 using a fused silica capillary column (30 m × 0.25 mm) with stationary phase DB-1 (0.25 mM film thickness) and helium at a carrier gas flow of 1.2 mL min^{-1}. The starting temperature was programmed at 60 °C and increased by 7 °C per min until 320 °C, maintaining this temperature for 5 min. The other chromatographic conditions were the same as described for the analysis device with the flame detector. The conditions of mass spectrometry included the detector of ion capture operating on electron impact, an impact energy of 70 eV, the temperature of separator and ionic source of 250 °C and detected fragments of 40-400 Dalton. The chemical constituents of the essential oil were identified by the comparison of its mass spectra with the spectra from literature and the NIST98 library (National Institute of Standards and Technology, Gaithersburg) and by comparing their GC retention indices (Kovats index, KI) with those found by Adams (2007). The KI were determined using a calibration curve of a series of *n*-alkanes that were injected under the same chromatographic conditions as the sample. The concentrations of the constituents of the essential oil were calculated from an analysis of the gas chromatography with a flame ionization detector based on the CG peak areas.

The fungal *C. herbarum* (CCT0279) strain, from the André Tosello Foundation, was inoculated in inclined test tubes containing potato dextrose agar (PDA) medium. The material was incubated for eight days in a BOD chamber at a constant temperature of 28 °C. The microorganism was preserved at 4 °C.

The bioautography in thin-layer chromatography (TLC) testing was performed according to the method described by Homans and Funchs (1970). Samples of 500 µL of the essential oil that were obtained from the leaves of *M. pulegium* at three different stages were applied to TLC plates and developed in 3:1 dichloromethane/ethyl acetate. Then, a spore suspension of *C. herbarum* (colony of two months) in a nutrient solution (2 mL of 30 % glucose solution and 10 mL of salt solution) was sprayed over the developed TLC plates, which were incubated at 28 °C under humid conditions for 3 days. The observed inhibitory zones were correlated with the spots that were seen on the TLC plates under 254 nm UV light.

The essential oil yield was 0.17 %, 0.23 % and 0.17 % from the fresh *M. pulegium* leaf samples

in the first (60 DAT), second (70 DAT) and third (85 DAT) collections, respectively. The results showed no important variation in the amount of essential oil that was produced over the three stages of development of *M. pulegium*, with a slight increase in essential oil production in the sample of the second collection (70 days). Several studies in the literature describe the yield of *M. pulegium* essential oil from dried leaves, ranging from 0.90 % to 1.93 % (LORENZO et al., 2002; STOYANOVA et al., 2005; EL-GHORAB, 2006). However, the results that were obtained in this work agree with those obtained from the fresh leaves of this species, as reported by Aziz and Craker (2009), who yielded 0.19 % essential oil from the fresh plant. Oliveira et al. (2011) also reported yields of 0.2 % and 0.09 % of *M. pulegium* essential oil grown in Brazil in the spring and winter, respectively.

It was found from the chromatograms that were obtained from GC (FID-flame ionization detector) and GC-MS that the essential oil sample from the first stage showed the greatest diversity of chemical constituents, of which eleven were identified (Table 1). Ten of which have been identified from the first an second stages and eight from the third.

The results presented in Table 1 show that out of the total compounds, seven were common to all three samples (menthone, isomenthone, neoisomenthol, pulegone, piperitone, 1.1-dimethoxy-2-nonyne and piperitenone), while two others (menthofuran and myrtenal) were detected only in the first and second samples, indicating that they were not biosynthesized after 85 days of cultivation. However, menthol was not detected in the first stage of plant development, being present only in essential oils of samples from the second and third collections.

The GC and GC-MS results showed a similarity between the three samples that were collected in relation to major compounds, but the concentrations of these compounds varied significantly during the three stages of plant development (Table 1). The main components were piperitenone and pulegone. Pulegone had the greatest concentration in the first and second samples, maintaining the same concentration in both. However, in the last sample, pulegone had the second highest concentration. Although the concentration of pulegone increased from 26.65 % to 31.05 % in the third sample, it was overtaken by the concentration of piperitenone (36.32 %). An increase in concentration was also observed amongst the other compounds, with the exception of piperitenone and 1.1-dimethoxy-2-nonyne. The piperitenone concentration decreased from 20.41 % to 12.60 % in the second, increasing to 36.32 % in the essential oil of the third sample. The ratio of the piperitenone:pulegone content of the essential oil was 0.77 (60DAT), 0.47 (70DAT) and 1.17 (85DAT).

Pulegone is also described as the main oil constituent of *M. pulegium* in several studies as shown in the Table 2. Other constituents that are mentioned in important concentrations are menthone (CHALCHAT et al., 2000; HASSANPOURAGHDAM et al., 2011; TEIXEIRA et al., 2012; GHAZGHAZI et al., 2013) and piperitone (ZWAVING; SMITH, 1971; MAHBOUBI; HAGHI, 2008; ELHOUSSINE et al., 2010). In some studies of *M. pulegium* essential oils, however, pulegone does not occur as a major constituent (CHALCHAT et al., 2000). Zwaving and Smith (1971) studied the essential oil of *M. pulegium* from Austria and found piperitone as the main component, followed by limonene, menthone, and *neo*-menthone, while pulegone was not even detected. Mahboubi and Haghi (2008) on the other hand, described the essential oil as belonging to the piperitone/piperitenone chemotype. In this context, the two compounds appear at concentrations of 38.0 % and 33.0 %, respectively. Elhoussine et al. (2010) also reported piperitone (35.56 %) and piperitenone (21.18 %) as major the constituents of the essential oil of *M. pulegium* from Morocco. Thestudy by Derwich et al. (2010) addresses the same essential oil, and the major constituent was piperitone,

at 35.56 %. Pulegone was also a predominant constituent (6.45 %).

Recently, Oliveira et al. (2011) reported that the essential oil of *M. pulegium* in the Ilheus Municipality, northeastern Brazil, contains pulegone as the main constituent in samples that were collected in the spring (61.43 %) and in the winter (28.40 %), followed by *trans*-caryophyllene (18.68 % and 10.20 %), respectively. The results demonstrate a massive difference between the compositions of the essential oil of *M. pulegium* obtained from two different regions in Brazil, probably due to the controlled conditions of cultivation of the plant in the sub-tropical climatic region of southern Brazil, in contrast to the wild plants grown in the north-eastern region of the country with a tropical climate.

However, based on the data that were obtained in this work and published data (KOKKINI et al. 2004; AGNIHOTRI et al., 2005; EL-GHORAB, 2006; COOK et al., 2007; AZIZ; CRAKER, 2009), it appears that the essential oil composition of *M. pulegium* depends not only on the conditions of the region where the plant is grown but also on the stage of growth. Therefore, this study is of great importance because there are few reports on the variation of chemical composition of the essential oils within the life cycle of the plant; this fact is very significant in choosing the best harvest season in which to obtain an essential oil that is rich in active compounds.

Table 1. Chemical composition of *Mentha pulegium* essential oils.

Compounds	60DAT[a]	70DAT[b]	85DAT[c]	60DAT[a]	70DAT[b]	85DAT[c]	
	Concentration (% peak area)			RI (calculated)			*RI
Menthone	0.47	1.08	1.20	1149	1150	1150	1152
Isomenthone	0.72	2.33	2.51	1161	1161	1161	1162
Menthofuran	0.21	0.33	ND	1169	1170	-	1164
Menthol	ND	0.74	4.58	-	1175	1175	1171
Neoisomenthol	0.58	1.07	3.77	1175	1182	1175	1186
Myrtenal	1.20	5.49	ND	1192	1198	-	1195
Trans-dihydrocarvone	0.99	ND	ND	1202	-	-	1200
Pulegone	26.65	26.65	31.05	1237	1237	1237	1237
Piperitone	0.43	2.07	8.38	1246	1252	1249	1252
1.1-dimethoxy-2-nonyne	6.23	7.79	1.68	1309	1305	1306	1323
Piperitenone	20.41	12.60	36.32	1345	1345	1346	1343

RI=Calculated according to Kovats. *RI= Kovats index (ADAMS, 2007).
[a]60DAT = sample of the first collection (60 days after planting); [b]70DAT = sample of the second collection (70 days after planting); [c]85DAT = sample of the third collection (85 days after planting). ND = not detected.

Table 2. Previous studies of the constituents of *M. pulegium* essential oils in several countries.

Origin	Plant part	Main compound(s) (%)	Biological activities	Reference
Austria	Plant	Piperitone 70.0 Limonene 11.0 Menthone 8.0	-	ZWAVING; SMITH, 1971
Cuba	Plant	Pulegone 25.1 Neoisomenthol 20.7	-	PINO et al., 1996
Portugal	Flowers and leaves	Pulegone 78.3-80.9	-	REIS-VASCO et al., 1999
Yugoslavia	Aerial parts	Menthone 30.9 Pulegone 14.1 Neomenthol 13.8 Caryophyllene oxide 9.0	-	CHALCHAT et al., 2000
Uruguay	Flowering aerial parts	Pulegone 73.4 Isomenthone 12.9	-	LORENZO et al., 2002
Iran	Aerial parts of fully flowered	Pulegone 37.8 Menthone 20.3 Pulegone 1.3-52.0 Menthone 0-30.3	-	AGHEL et al., 2004
Greece	Aerial parts	Pulegone 0.1-90.7 Piperitone 0.0-97.2 Menthone 0.2-53.4 Isomenthone 0.14545.1 Piperitenone 0.1-39.8	-	KOKKINI et al., 2004
India	Aerial parts	Pulegone 6.5.9-83.1 Menthone 8.3-8.7 Isomenthone 3.8-4.0	-	AGNIHOTRI et al., 2005
Bulgaria	Aerial parts	Pulegone 42.9-45.4 Piperitenone 21.7-23.1 Isomenthone 1.3-12.8	-	STOYANOVA et al., 2005
Egypt	Aerial parts	Pulegone 43.5 Piperitone 12.2 *p*-menthane-1,2,3-triol 6.5	Antioxidant	EL-GHORAB, 2006
Algeria	Aerial parts	Pulegone 4.4-87.3 Piperitenone 0.1-19.2 *b*-pinene 0.5-20.9	-	BEGHIDJA et al., 2007
Greece	Inflorescence, leaf and stem	Pulegone 32.8-75.8 Piperitenone 5.1-35.0 Isomenthone 4.3-28.6 Piperitone 0.5-5.2	-	COOK et al., 2007
Spain	Leaves	Pulegone 41.1-42.3 Piperitone 5.4-5.9	-	DÍAZ-MAROTO et al., 2007
Portugal	Aerial parts	Pulegone 35.1 Piperitenone 27.4	Acetylcholinesterase inhibitory Free radical-	MATA et al., 2007
Tunisia	Aerial parts	Pulegone 41.8 Isomenthone 11.3 Carvone 6.2	-	MKADDEM et al., 2007

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Iran	Aerial parts	Piperitone 38.0 Piperitenone 33.0	Antimicrobial activity	MAHBOUBI; HAGHI, 2008
Tunisia	Leaves and Stems	Menthol 40.6-51.6 Menthone 7.3-20.1 1,8-cineole 11.1-18.5	Antimicrobial activity	MARZOUK et al., 2008
Egypt	Plant	Pulegone 88.1 Isomenthone 5.8	-	AZIZ; CRAKER, 2009
Tunisia	Aerial parts	Pulegone 61.1 Isomenthone 17.0 Piperitone 2.6	Antimicrobial and antioxidant activities	HAJLAOUI et al., 2009
Tunisia	Aerial parts	Pulegone 44.3 Isomenthone 19.1 Piperitone 10.4 Menthone 9.34	Antimicrobial and antioxidant activities	HAJLAOUI et al., 2010
Morocco	Leaves	Piperitone 35.6 Piperitenone 21.2 α-terpineol 10.9 Pulegone 6.5	Antimicrobial activity	ELHOUSSINE et al., 2010
Algeria	Aerial parts	Pulegone 38.8 Menthone 19.3 Piperitenone 16.5 Piperitone 6.3	Antimicrobial activity	BOUKHEBTI et al., 2011
Iran	Aerial parts	Menthone 38.7 Menthol 11.3 Neomenthol 10.5	-	HASSANPOURAGHDAM et al., 2011
Iran	Aerial parts	Pulegone 54.6 Menthone 5.1	Antimicrobial activity	MORTEZA-SEMNANI et al., 2011
Brazil	Leaves	Pulegone 28.4-61.4 *trans*-caryophyllene 0.2-18.7 Menthol 6.8-10.0	-	OLIVEIRA et al., 2011
Portugal	Aerial parts	Menthone 35.9 Pulegone 23.2 Neo-menthol 9.2	Antimicrobial and antioxidant activities	TEIXEIRA et al., 2012
Morocco	Aerial parts	Pulegone 69.7 Piperitenone 3.1	Antimicrobial activity	AIT-OUAZZOU et al., 2012
Turkey	Aerial parts	Pulegone 71.5 Menthone 7.7	Antioxidant activity	SARIKURKCU et al., 2012
Italy	Leaves	Pulegone 34.2 Menthone 18.8 Isomenthone 11.3	Nematicidal Activity	CABONI et al., 2013
Tunisia	Leaves	Menthone 41.7 *cis*-isopulegone 31.7 Isomenthone 15.0	Antimicrobial and antioxidant activities	GHAZGHAZI et al., 2013
Portugal	Aerial parts	Pulegone 52.0-82.0 Isomenthone 2.0-36.0 Menthone 0.1-17.0	-	RODRIGUES et al., 2013

In the bioautography test, the essential oil samples that were obtained from the three collections of *M. pulegium* showed three growth inhibition regions of *C. herbarum,* with retention values (R_f) of 0.80, 0.85 and 0.90, indicating that the active components were produced in the three stages of plant development. The *M. pulegium* essential oil activity on *C. herbarum* has also been observed by Mueller-Riebau et al. (1995), who reported the presence of one active compound, indicated to be pulegone. The activity of the essential oil of this species has also been demonstrated against the following species of fungi: *Alternaria alternata*, *Penicillium expansum* (HMIRI et al., 2011), *Botrytis cinerea* (BOUCHRA et al., 2003) and *Fusarium* sp. (DAFERERA et al., 2003).

The concentration of the essential oil was not considerably modified in the three developmental stages of *M. pulegium*. Regarding the composition of this essential oil, the most important change was that of the major compounds. Pulegone was the main compound identified in the first and second stages, while piperitenone was the main compound of the third stage. In addition, the concentration of other components present in minor concentrations, such as piperitone, increased during cultivation. The essential oil of *M. pulegium* presents potential as an antifungal agent as it demonstrated inhibition zones against *C. herbarum*.

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References

ADAMS, R. P. *Identification of essencial oil components by gas chromatography/mass spectrometry.*4th ed. Carol Stream: Allured Publishing Corporation, 2007. 804 p.

AGHEL, N.; YAMINI, Y.; HADJIAKHOONDI, A.; POURMORTAZAVI, S. M. Supercritical carbon dioxide extraction of *Mentha pulegium* L. essential oil. *Talanta,* Amsterdam, v. 62, n. 2, p. 407-411, 2004.

AGNIHOTRI, V. K.; AGARWAL, S. G.; DHAR, P. L.; THAPPA, R. K.; BALESHWAR, M.; KAPAHI, B. K.; SAXENA, R. K.; GAZI, G. N. Essential oil composition of *Mentha pulegium* L. growing wild in the north-western Himalayas India. *Flavour and Fragrance Journal*, Chichester, v. 20, n. 6, p. 607-610, 2005.

AIT-OUAZZOU, A.; LORÁN, S.; ARAKRAK, A.; LAGLAOUI, A.; ROTA, C.; HERRERA, A.; PAGÁN, R.; CONCHELLO, P. Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperuslongus* essential oils from Morocco. *Food Research International,* Washington, v. 45, n. 1, p. 313-319, 2012.

AZIZ, E. E.; CRAKER, L. E. Essential oil constituents of peppermint, pennyroyal, and apple mint grown in a desert agrosystem. *Journal of Herbs, Spices & Medicinal Plants*, Philadelphia, v. 15, n. 4, p. 361-367, 2009.

BARBOSA, M. A. G.; REHN, K. G.; MENEZES, M.; MARIANO, R. L. R. Antagonism of *Trichoderma* species on *Cladosporiumher barum* and their enzimatic characterization. *Brazilian Journal of Microbiology*, São Paulo, v. 32, n. 2, p. 98-104, 2001.

BEGHIDJA, N.; BOUSLIMANI, N.; BENAYACHE, F.; BENAYACHE, S.; CHALCHAT, J. C. Composition of the oils from *Mentha pulegium* grown in different areas of the east of Algeria. *Chemistry of Natural Compounds,* Boca Raton, v. 43, n. 4, p. 481-483, 2007.

BOUCHRA, C.; ACHOURI, M.; IDRISSI, L. M. H.; HMAMOUCHI, M. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. Fr . *Journal of Ethnopharmacology*, Yenişehir, v. 89, n. 1, p. 165-169, 2003.

BOUKHEBTI, H.; CHAKER, A. N.; BELHADJ, H.; SAHLI, F.; RAMDHANI, M.; LAOUER, H.; HARZALLAH, D. Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. *Der Pharmacia Lettre,* Gurgaon, v. 3, n. 4, p. 267-275, 2011.

CABONI, P.; SABA, M.; TOCCO, G.; CASU, L.; MURGIA, A.; MAXIA, A.; MENKISSOGLU-SPIROUDI, U.; NTALLI, N. Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incógnita*. *Journal of Agricultural and Food Chemistry*, Washington, v. 61, n. 41, p. 9784-9788, 2013.

CHALCHAT, J.; GORUNOVIC, M. S.; MAKSIMOVIC, Z. A.; PETROVIC, S. D. Essential oil of wild growing *Mentha pulegium L.* from Yugoslavia. *Journal of Essential Oil Research*, Wheaton, v. 12, n. 5, p. 598-600, 2000.

COOK, C. M.; MALOUPA, E.; KOKKINI, S.; LANARAS, T. Differences between the inflorescence, leaf and stem essential oils of wild *Mentha pulegium* plants from Zakynthos, Greece. *Journal of Essential Oil Research,* Wheaton, v. 19, n. 3, p. 239-243, 2007.

DAFERERA, D. J.; ZIOGAS, B. N.; POLISSIOU, M. G. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium*sp., and *Clavibacter michiganensis* subsp. michiganensis. *Crop Protection*, Athens, v. 22, n. 1, p. 39-44, 2003.

DERWICH, E.; BENZIANE, Z.; BOUKIR, A. Antibacterial activity and chemical composition of the leaf essential oil of *Mentha rotundifolia* from Morocco. *Electronic Journal of Environment, Agriculture and Food Chemistry*, Vigo, v. 9, n. 1, p. 19-28, 2010.

DÍAZ-MAROTO, M. C.; CASTILLO, N.; CASTRO-VÁZQUEZ, L.; GONZÁLEZ-VIÑAS, M. A.; PÉREZ-COELLO, M. S. Volatile composition and olfactory profile of pennyroyal (*Mentha pulegium* L.) plants. *Flavour and Fragrance Journal,* Chichester, v. 22, n. 2, p. 114-118, 2007.

EL-GHORAB, A. H. The chemical composition of the *Mentha pulegium* L. essential oil from Egypt and its antioxidant activity. *Journal of Essential Oil-Bearing Plants*, Dehradun, v. 9, n. 2, p. 183-195, 2006.

ELHOUSSINE, D.; ZINEB, B.; ABDELLATIF, B. GC/MS analysis and antibacterial activity of the essential oil of *Mentha pulegium* grown in Morocco. *Research Journal of Agriculture and Biological Sciences*, Faisalabad, v. 6, n. 3, p. 191-198, 2010.

GHAZGHAZI, H.; CHEDIA, A.; WESLATI, M.; TRAKHNA, F.; HOUSSINE, S.; ABDERRAZAK, M.; BRAHIM, H. Chemical composition and in vitro antimicrobial activities of *Mentha pulegium* leaves extracts against food born pathogens. *Journal of Food Safety,* New York, v. 33, n. 3, p. 239-246, 2013.

HAJLAOUI, H.; SNOUSSI, M.; NOUMI, E.; ZANETTI, S.; KSOURI, R.; BAKHROUF, A. Chemical composition, antioxidant and antibacterial activities of the essential oils of five Tunisian aromatic plants. *Italian Journal of Food Science,* Milano, v. 22, n. 3, p. 320-329, 2010.

HAJLAOUI, H.; TRABELSI, N.; NOUMI, E.; SNOUSSI, M.; FALLAH, H.; KSOURI, R.; BAKHROUF, A. Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. *World Journal of Microbiology and Biotechnology*, Oxford, v. 25, n. 12, p. 2227-2238, 2009.

HASSANPOURAGHDAM, M. B.; AKHGARI, A. B.; AAZAMI, M. A.; EMARAT-PARDAZ, J. New Menthone Type of *Mentha pulegium* L. Volatile Oil from Northwest Iran. *Czech Journal of Food Sciences*, Prague, v. 29, n. 3, p. 285-290, 2011.

HMIRI, S.; RAHOUTI, M.; HABIB, Z.; SATRANI, B.; GHANMI, M.; EL AJIJOURI, M. Evaluation of the antifungal potential of the essential oils of *Mentha pulegium* and *Eucalyptus camaldulensis* in biocentral against fungi responsible for the deterioration of apples in storage. *Bulletin de la Societe Royale des Sciences de Liege,* Liege, v. 80, n. 1, p. 824-836, 2011.

HOMANS, A. L.; FUNCHS, A. Direct bioautography on thin – layer chromatograms as a method for detecting *fungitoxic substances*. *Journal of Chromatography*, Amsterdam, v. 51, p. 327- 329, 1970.

INSTITUTO DE BOTÂNICA. Pteridófitas e fanerógamas. In: _____. *Técnicas de coleta, preservação e herborização de material botânico*. São Paulo: Governo do Estado de SP, Secretaria do Meio Ambiente Instituto de Botânica, 1989. p. 32-55.

KAMKAR, A.; JAVAN, A. J.; ASADI, F.; KAMALINEJAD, M. The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food and Chemical Toxicology,* Washington, v. 48, n. 7, p. 1796-1800, 2010.

KOKKINI, S.; HANLIDOU, E.; KAROUSOU, R.; LANARAS, T. Clinal variation of *Mentha pulegium* essential oils along the climatic gradient of Greece. *Journal of Essential Oil Research*, Wheaton, v. 16, n. 6, p. 588-593, 2004.

KOTHARI, R. The Indian essential oil industry. *Perfumer and Flavorist*, Carol Stream, v. 30, p. 46-50, 2005.

LORENZI, H.; MATOS, F. J. A. *Plantas medicinais no Brasil:* nativas e exóticas. 2. ed. Nova Odessa: Instituto Plantarum, 2008. 544 p.

LORENZO, D.; PAZ, D.; DELLACASSA, E.; DAVIES, P.; VILA, R.; CAÑIGUERAL, S. Essential oils of *Mentha pulegium* and *Menth arotundifolia* fromUruguay. *Brazilian Archives of Biology and Technology*, Curitiba, v. 45, n. 4, p. 519-524, 2002.

MAHBOUBI, M.; HAGHI, G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essencial oil. *Journal Ethnopharmacology*, Lausanne, v. 119, n. 2, p. 325-327, 2008.

MARZOUK, B.; FREDJ, M. B. H.; CHRAIEF, I.; MASTOURI, M.; BOUKEF, K.; MARZOUK, Z. Chemical composition and antimicrobial activity of essential oils from Tunisian *Mentha pulegium* L. *Journal of Food, Agriculture & Environment*, Helsinki, v. 6, n. 1, p. 78-82, 2008.

MATA, A.T.; PROENÇA, C.; FERREIRA, A. R.; SERRALHEIRO, M. L. M.; NOGUEIRA, J. M. F.; ARAÚJO, M. E. M. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chemistry*, Reading, v. 103, n. 3, p. 778-786, 2007.

MIGUEL, M. G.; ALMEIDA, M. L.; GONÇALVES, M. A.; FIGUEIREDO, A. C.; BARROSO, J. G.; PEDRO, L. M. Toxic effects of three essential oils on *Ceratitiscapitata*. *Journal of Essential Oil-Bearing Plants*, Dehradun, v. 13, n. 2, p. 191-199, 2010.

MKADDEM, M.; BOUSSAID, M.; BEN FADHEL, N. Variability of volatiles in Tunisian *Menthapulegium* L. (Lamiaceae). *Journal of Essential Oil Research*, Wheaton, v. 19, n. 3, p. 211-214, 2007

MORTEZA-SEMNANI, K.; SAEEDI, M.; AKBARZADEH, M. Chemical composition and antimicrobial activity of the essential oil of *Menta pulegium* L. *Journal of Essential Oil Research*, Wheaton, v. 14, n. 2, p. 208-213, 2011.

MUELLER-RIEBAU, F.; BERGER, B.; YEGEN, O. Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *Journal of Agricultural and Food Chemistry*, Davis, v. 43, n. 8, p. 2262-2266, 1995.

O'DONNELL, J.; DICKINSON, C. H. Pathonicity of *Alternaria* and *Cladosporium* isolates on *Phaseolus*. *Transactions of the British Mycological Society,* London*,* v. 74, n. 2, p. 335-342, 1980.

OLIVEIRA, R. A.; SA, I. C. G.; DUARTE, L. P.; OLIVEIRA, F. F. Constituintes voláteis de *Mentha pulegium* L. e *Plectranthus amboinicus* (Lour.) Spreng. *Revista Brasileira Plantas Medicinais*, Campinas, v. 13, n. 2, p. 165-169, 2011.

PINO, J. A.; ROSADO, A.; FUENTES, V. Chemical composition of the essential oil of *Mentha pulegium* L. from Cuba. *Journal of Essential Oil Research*, Wheaton, v. 8, n. 3, p. 295-296, 1996.

REIS-VASCO, E. M. C.; COELHO, J. A. P.; PALAVRA, A. M. F. Comparison of pennyroyal oils obtained by supercritical CO_2 extraction and hydrodistillation. *Flavour and Fragrance Journal,* Chichester, v. 14, n. 3, p. 156-160, 1999.

RIBEIRO, P. G. F.; DINIZ, R. C. *Plantas aromáticas e medicinais:* cultivo e utilização. Londrina: IAPAR, 2008. 218 p.

RODRIGUES, L.; POVOA, O.; TEIXEIRA, G.; FIGUEIREDO, A. C.; MOLDÃO, M.; MONTEIRO, A. Trichomes micromorphology and essential oil variation at different developmental stages of cultivated and wild growing *Mentha pulegium* L. populations from Portugal. *Industrial Crops and Products*, Amsterdam, v. 43, p. 692-700, 2013.

SARIKURKCU, C.; ERYIGIT, F.; CENGIZ, M.; TEPE, B.; CAKIR, A. Screening of the antioxidant activity of the essential oil and methanol extract of *Mentha pulegium* L. from Turkey. *Spectroscopy Letters: An International Journal for Rapid Communication*, New York, v. 45, n. 5, p. 352-358, 2012.

SIVROPOULOU, A.; KOKKINI, S.; LANARAS, T.; ARSENAKIS, M. Antimicrobial activity of mint essential oils. *Journal of Agricultural and Food Chemistry*, Davis, v. 43, n. 9, p. 2384-2388, 1995.

STOYANOVA, A.; GEORGIEV, E.; KULA, J.; MAJDA, T. Chemical composition of the essential oil of *Mentha pulegium* L. from Bulgaria. *Journal of Essential Oil Research*, Wheaton, v. 17, n. 5, p. 475-476, 2005.

TEIXEIRA, B.; MARQUESA, A.; RAMOSA, C.; BATISTA, I.; SERRANO, C.; MATOS, O.; NENG, N. R.; NOGUEIRA, J. M. F.; SARAIVA, J. A.; NUNES, M. L. European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products,* Amsterdam, v. 36, n. 1, p. 81-87, 2012.

ZWAVING, J. H.; SMITH, D. Composition of the essential oil of Austrian *Mentha pulegium*. *Phytochemistry*, Oxford, v. 10, n. 8, p. 1951-1953, 1971.