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Chemical Composition and Bioactivity of the Essential Oil of *Melissa officinalis* L., Cultivated in Southwestern, Iran

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ABSTRACT

Background & Aim: *Melissa officinalis* L. (lemon balm) a valuable medicinal plant in herbal medicine is native to the eastern Mediterranean region and western Asia. It is a traditional herbal medicine, which enjoys contemporary usage as a mild sedative, spasmolytic and antibacterial agent. The objective of this study was to assess the effects of climate on quality and quantity of the essential oil of *M. officinalis* at Shahrekord climate, southwestern (Iran) and evaluate the antioxidant activity of the oil.
Experimental: Plants were cultivated during 22-25th March 2016 in the greenhouse and transferred to the main site after three months (May), and finally harvested at the full flowering stage during August.Dried plant material (100 g) was powdered and subjected to hydro–distillation for three hours using a Clevenger–type apparatus. The hydrodistillated essential oil analyzed by GC-FID and GC/MS. The antioxidant activity was determined by using DPPH method.
Results: Results indicated that the major essential oil constituents of *M.officinalis* L. were β-caryophyllene (23.06%), *E*-citral (17.61%), *Z*-citral (13.64%), and caryophyllene oxide (10.83%). The antioxidant activityof the essential oil showed moderate antioxidant activity (IC50=749.60 µg/g), that was lower compared to butylated hydroxytoluene (BHT).
Recommended applications/industries: This potential applicability can be used as antioxidant agents for food and pharmaceutical industries.

1. Introduction

Aromatic plants have been widely used from ancient times in medicine, cosmetics and for preserving and improving the flavor of foods (Bajalan *et al.*, 2017). Herbs, spices, and essential oils are also well known for their various beneficial effects on human health. The use of herbs in phytotherapy is mostly due to the essential oils and their various biological activities, such as spasmolytic, carminative, hepatoprotective, antiviral, and anti-carcinogenic properties (Bajalan and Pirbalouti, 2014).

The family Lamiaceae commonly known Lamiaceae is famous because of its members containing chemical compositions(Ali et al., 2000). There are a large

number of studies reporting the benefits of extracts and essential oils from this family. Many biologically active essential oils have also been obtained from various Lamiaceae plants. Essential oils from them are well-known for their useful effects on diseases (Erdemoglu *et al.*, 2006; Koşar *et al.,* 2005).

Melissa officinalis L. is used in traditional medicine to prepare tea for its nerve calming effect and to treat nervous disturbance of sleep (Kennedy *et al.,* 2004; Pereira *et al.,* 2014), as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative hypnoticstrengthening the memory, and relief of stressinduced headache (Blumenthal *et al.,* 2000). It iscurrently used for the relief of stress-induced headache,as a mild sedative-hypnotic, and as an antiviral toimprove healing of herpes simplex cold sores (Blumenthal *et al.,* 2000).

M. officinalis is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are2 to 8 cm long and either heart-shaped (Zargari, 1991).The leaves emit a distinct fragrant lemon odor when bruised. The chemical composition is essential oil, polyphenolic compounds: caffeic acid derivatives in large proportions, such as RA, trimeric compounds, and also some flavonoids such as luteolin-7-0-glucoside. Some pharmacological properties have been attributed to the principal constituents. Essential oil is considered to be the therapeutic principle mainly responsible for most of the activities mentioned, spasmolytic, antimicrobial, antitumor and antioxidant, mainly (Encalada *et al.,* 2011; Sousa *et al.,* 2004).

There is a growing interest in substances exhibiting antioxidant properties that are supplied to human and animal organisms as food components or as specific preventative pharmaceuticals. The plant kingdom produces a wide range of natural antioxidants (Fecka *et al.,* 2007). However, there is still not enough knowledge about the practical usefulness of most of them. In the group of secondary plant metabolites, antioxidant phenolics and essential oils are commonly found in various fruits, vegetables and herbs and they have been shown to provide a defense against oxidative stress from oxidizing agents and free radicals (Matkowski *et al.,* 2008; Roby *et al.,* 2013). Many herbal infusions, frequently used as home medicines have antioxidative and pharmacological properties related to the presence of secondary metabolites. They are also known for their ability to prevent fatty acids from oxidative decay, and provide an additional value to plants used as food ingredients (Gülçin *et al.*, 2007; Sarikurkcu *et al*., 2009). Therefore, the objective of the present work was to study the quality and quantity of chemical constituents and oil yield of *M. officinalis* from Shahrekord climate condition and to evaluate the antioxidant activities of the essential oil.

2. Materials and Methods

2.1. Experimental site and plant material

The present study was conducted at Islamic Azad University from Shahrekord, Iran (2061 m asl, 32°20"N, 50°51"E) during 2016. The site experiences a mean annual temperature of 11.75°C and the average rainfall received is about 318.41 mm. Geographical, climate and soil properties of research farm of Islamic Azad University of Shahrekord is shown in Table 1. Plants were cultivated during 22-25th March in the greenhouse and transferred to the main site after three months (May), and finally harvested at the full flowering stage during August.

Table1. Geographical, climate and soil properties of Research Farm of Islamic Azad University of Shahrekord

Altitude (m a.s.l.)	P	T	E.C.	O.C	ᶜP (g/kg)	%N	K (g/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	pH
2061	318.41	11.75	1.35	0.8	11.20	0.1	694.0	0.48	6.51	1.11	7.23

P: annual precipitation (mm), T: average temperature (◦C); each value in the mean of 10–15 years data; E.C.: electrical conductivity (dS m−1); O.C.: organic carbon (%),.ᶜSoil characteristics are based on average of samples taken from three point in region.

2.2. Essential oil isolation

After sampling, aerial parts of plants were dried at room temperature. Two hundred grams of dried plant were submitted to hydro-distillation for four hours, using a Clevenger-type apparatus. The essential oil was separated from water, dried over anhydrous sodium sulfate and stored in dark glass bottle (sealed brown vials) at 4 °C until chemical analysis and antioxidant test.

2.3. GC-FID /MS analysis

Compositions of the essential oil were determined by GC-FID and GC-MS analyses. They were achieved on an Agilent Technologies 7890 GC equipped with FID and mass spectrometer detectors using a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses; J & W Scientific, Folsom). The carrier gas was helium at a flow of 0.8 ml/min. Initial column temperature was 60°C and programmed to increase up to 280°C at 4°C/min. The split ratio was 40:1. The injector temperature was set at 300°C. The acquisition range was 50–550 *m/z* in electron-impact (EI) mode using an ionization voltage of 70 eV. The essential oils were diluted 1:100 in *n*-hexane, then 0.1 µL were injected into the GC systems.

2.4. Compound identification

The components of the oils were identified by comparison of their spectra with those from available MS (NIST 08 and Wiley MS) libraries and by comparison of their KI (Kovats index) relative to C_5-C_{24} *n*-alkanes obtained on anapolarHP-5MS column with those reported in the literature (Adams, 2001). The percentage composition (average of three independent analyses) was computed from the GC peak areas without using any correction factors.

2.5. Evaluation of the antioxidant capacity

The antioxidant capacity of the essential oils was evaluated by the method of Hung *et al.* (2005). The essential oils at different concentrations (16 to 500 µg/ml) were mixed with the same volume of 0.2 mM methanol solution of DPPH. The disappearance of DPPH by extracts after 30 min of incubation at room temperature was determined spectrophotometrically at 515 nm. Methanol was used to zero spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e. the control was measured daily using a Perkin–Elmer Lambda UV/VIS spectrophotometer at 515 nm against a blank, i.e. without DPPH. All tests were run in triplicate and an average was used. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically and the percentage inhibition was calculated according to the follow equation; Where AC(0) is the absorbance of the control at t = 0 min and AA(t) is the absorbance of the antioxidant at t = 30 min. The food preservative butylhydroxyanisole (BHA) was used as positive control.

$$\% \text{ inhibition} = \left[\frac{AC(0) - AA(t)}{AC(0)}\right] * 100$$

3. Results and discussion

3.1. Essential oil composition of M. officinalis

Results of this study indicated that essential oil yield of *M. officinalis* was 0.2 ml/100g dry matter. A total of 23 compounds were identified from the essential oil of this plant, which represented 95.91% of the oil extracted. The most abandonment components in the essential oil were *β*-caryophyllene (23.06%), *E*-citral (17.61%), *Z*-citral (13.64%), caryophyllene oxide (10.83%), and germacrene D (9.26%) (Figure 1, Table 2). Mimica-Dukic *et al.*(2004) reported that the main components of the essential oil of *M. officinalis* from Serbia were citrals (39.9%), citronellal (13.7%), limonene (2.2%), geraniol (3.4%), *β*-caryophyllene (4.6%), *β*-caryophyllene oxide (1.7%), and germacrene D (2.4%). In addition, geranial (35.3%), neral (24.5%), and citronellal (12.9%) characterized the essential oil from Kashan (Iran) (Sadraei *et al.*, 2003). The essential oil from cultivated *M. officinalis* from Greece contains *β*-pinene (6.4 –18.2%), sabinene (6.9 –17.4%), (E)-caryophyllene (7.2 –15.3%), and caryophyllene oxide (12.6 –24.4%) (Basta *et al.*, 2005). *M. officinalis* from Morocco were rich in nerol (30.44%), citral (27.03%), isopulegol (22.02%), caryophyllene (2.29%), caryophyllene oxide (1.24%), and citronella (1.06%) (Bounihi *et al.*, 2013).Consumer demand for greater quality control and standardization of medicinal plants (Papadopoulos *et al.*, 2000). The yield of plant material, the essential oil components and quantitative composition of plants can be influenced by harvest time, climatically and ecological condition. The maximum essential oil yield depended not only on flower development, but also temperature, relative humidity and duration of sunshine, air movement and rainfall (Özguven and Tansi, 1998). Therefore, proper management of crop production is needed to achieve high quality products (Tabatabaei, 2008). Controlled growth systems make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest (Pirbalouti *et al.*, 2013).

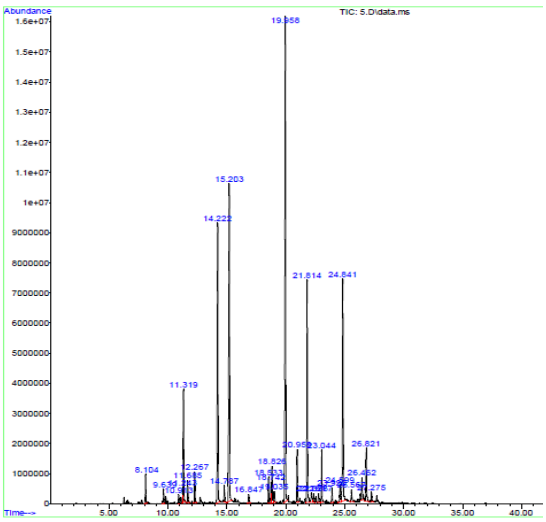

Figure 1. The chromatogram of *M. officinalis essential oil* (for peak identification see Table 2).

3.2. Antioxidant activity

The DPPH radical has been used widely to test the antioxidant activities of plant extracts and foods. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant, bringing about a color change from purple to yellow, which is measured at 515 nm (Jan *et al.*, 2013). In this work, the antioxidant activity of the *M. officinalis* essential oil was measured spectrophotometrically. Results indicated that in comparison with BHT (312.7 µg/g), the essential oil of *M. officinalis* showed moderate antioxidant capacity (749.60 µg/g essential oil) (Table 3). *M. officinalis* is also well known for its antioxidant activities. In many reports the antioxidant capacity of the extract and essential oil of *M. officinalis* (Mimica-Dukic *et al.*, 2004; Pereira *et al.*, 2014), have been studied and the results of most of them were in agreement with present study.

Table 2. Chemical components of *M. officinalis* from Iran

No.	Components	RT[a]	(%)
1	Limonene	7.60	0.57
2	*trans*-β-Ocimene	8.11	0.92
3	*trans*-Chrysanthemal	11.2	0.44
4	Citronellal	11.3	3.85
5	**Z-Citral**	14.2	**13.64**
6	Bornanone	14.8	0.61
7	***E*-Citral**	15.2	**17.61**
8	Methyl geranate	16.8	0.41
9	α-Copaene	18.5	0.95
10	Geranyl acetate	18.7	0.78
11	β-Bourbonene	18.8	1.6
12	β-Elemen	19.0	0.84
13	**β-Caryophyllene**	19.9	**23.06**
14	α-Humulene	21.0	2.29
15	**Germacrene D**	21.8	**9.26**
16	α-Farnesene	22.2	0.75
17	α-Muurolene	22.4	0.38
18	Naphthalene	22.8	0.56
19	*delta*-Cadinene	23.0	1.98
20	Fenchone	24.6	0.87
21	**Caryophyllene oxide**	24.8	**10.83**
22	*tau*-Muurolol	26.5	1.24
23	α-Cadinol	26.8	2.47
	Total (%)		95.91
	Essential oil yield (ml/100g dry matter)		0.2

[a] Retention index.

The use of antioxidant supplements at a maintenance level may provide an effective way to eliminate excessive of reactive oxygen species in human body (Koksal *et al.*, 2011). Medicinal plants are known potential source of antioxidants (Spiridon *et al.*, 2011). Antioxidants from medicinal plants have been shown positive effects on human health comparing with artificial antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and propyl gallate, which could be carcinogenic, toxic and tumorigenic (Ye *et al.*, 2015).

Tabale 2. Antioxidant activity of essential oil by DPPH

Samples	IC_{50} (µg/ml)
Essential oil of *M. officinalis*	749.604
BHT	314.23

4. Conclusions

In conclusion, the hydrodistilled oil obtained from cultivated *M. officinalis* in this study was analyzed by GC–FID/MS. A high quality of major compositions was observed in the essential oil from cultivated of *M. officinalis* L. (*β*-caryophyllene, *E*-citral, and *Z*-citral) from Iran. The herb has been used as food flavoring and preservative agent in dairy foods in Iran. The oil

also was found to be effective antioxidants in DPPH radical scavenging assays. Antioxidant activity of the essential oil from *M. officinalis* L. could be due, in part, to the presence of several compounds. The results confirm the potential applicability of natural substances from *M. officinalis* L. as antioxidants.

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6. References

Adams, R.P. 2001. Identification of essential oils by gas chromatography quadrupole mass spectroscopy. Allured: Carol Stream, IL.

Ali, M.S., Saleem, M., Ali, Z. and Ahmad, V.U. 2000. Chemistry of *Zataria multiflora* (Lamiaceae). *Phytochemistry*, 55: 933–936.

Bajalan, I. and Ghasemi Pirbalouti, A. 2014. Variation in antibacterial activity and chemical compositions of essential oil from different populations of myrtle. *Industrial Crops and Products*, 61: 303–307.

Bajalan, I., Rouzbahani, R., Ghasemi Pirbalouti, A. and Maggi, F. 2017. Chemical composition and antibacterial activity of Iranian *Lavandula* x *hybrida*. Chemistry and Biodiversity, 14: e1700064.

Basta, A., Tzakou, O. and Couladis, M. 2005. Composition of the leaves essential oil of Melissa officinalis from Greece. *Flavour and fragrance journal*, 20: 642–644.

Blumenthal, M., Goldberg, A. and Brinckmann, J. 2000. Herbal Medicine-Expanded Commission E Mongraphs. Newton, MA: *Integrative Medicine Communications,* 123: 230-232.

Bounihi, A., Hajjaj, G., Alnamer, R., Cherrah, Y. and Zellou, A. 2013. In vivo potential anti-inflammatory activity of *Melissa officinalis* L. essential oil. *Advances in pharmacological sciences*, 2013; 1–7.

Encalada, M.A., Hoyos, K.M., Rehecho, S., Berasategi, I., de Ciriano, M.G.Í., Ansorena, D., Astiasarán, I., Navarro-Blasco, Í., Cavero, R.Y. and Calvo, M.I. 2011. Anti-proliferative effect of *Melissaofficinalis* on human colon cancer cell line. *Plant foods for human nutrition*, 66: 328–334.

Erdemoglu, N., Turan, N.N., Cakõcõ, I., Sener, B. and Aydõn, A. 2006. Antioxidant activities of some Lamiaceae plant extracts. *Phytotherapyresearch*, 20: 9–13.

Fecka, I., Raj, D. and Krauze-Baranowska, M. 2007. Quantitative determination of four water-soluble compounds in herbal drugs from Lamiaceae using different chromatographic techniques. *Chromatographia*, 66:87–93.

Gülçin, I., Elmastaş, M. and AboulEnein, H.Y. 2007. Determination of antioxidant and radical scavenging activity of Basil (*Ocimumbasilicum* L. Family Lamiaceae) assayed by different methodologies. *Phytotherapy research*, 21: 354–361.

Jan, S., Khan, M.R., Rashid, U. and Bokhari, J. 2013. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of Monotheca buxifolia fruit. *Osong public health and research perspectives,* 4: 246–254.

Hung, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry,* 53:1841–56.

Kennedy, D.O., Little, W. and Scholey, A.B. 2004. Attenuation of laboratory-induced stress in humans after acute administration of *Melissaofficinalis* (Lemon Balm). *Psychosomatic medicine*, 66: 607–613.

Koksal, E., Bursal, E., Dikici, E., Tozoglu, F. and Gulcin, I. 2011. Antioxidant activity of Melissa officinalis leaves. *Journal of Medicinal Plants Research*, 5: 217–222.

Koşar, M., Dorman, H. and Hiltunen, R. 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. *Food Chemistry*, 91: 525–533.

Matkowski, A., Tasarz, P. and Szypuła, E. 2008. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae. *Journal of Medicinal Plants Research,* 2: 321–330.

Mimica-Dukic, N., Bozin, B., Sokovic, M. and Simin, N. 2004. Antimicrobial and antioxidant activities of *Melissaofficinalis* L.(Lamiaceae) essential oil. *Journal of agricultural and food chemistry,* 52: 2485–2489.

Özguven, M. and Tansi, S. 1998. Drug yield and essential oil of *Thymusvulgaris* L. as in influenced by ecological and ontogenetical variation. Turkish *Journal of Agriculture and Forestry*, 22: 537–542.

Papadopoulos, A., Luo, X., Leonhart, S., Gosselin, A., Pedneault, K., Angers, P., Gaudreau, L. and Dorais, M. 2000. Soilless greenhouse production of medicinal plants in North Eastern Canada, In: World

Congress on Soilless Culture: *Agriculture in the Coming Millennium,* 554: 297–304.

Pereira, R.P., Boligon, A.A., Appel, A.S., Fachinetto, R., Ceron, C.S., Tanus-Santos, J.E., Athayde, M.L. and Rocha, J.B.T. 2014. Chemical composition, antioxidant and anticholinesterase activity of Melissa officinalis. *Industrial Crops and Products*, 53: 34–45.

Ghasemi Pirbalouti, A., Hashemi, M. and Ghahfarokhi, F.T. 2013. Essential oil and chemical compositions of wild and cultivated *Thymusdaenensis* Celak and Thymus vulgaris L. *Industrial Crops and Products*, 48: 43–48.

Roby, M.H.H., Sarhan, M.A., Selim, K.A.H. and Khalel, K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products,* 43: 827–831.

Sadraei, H., Ghannadi, A. and Malekshahi, K. 2003. Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. *Fitoterapia*, 74: 445–452.

Sarikurkcu, C., Arisoy, K., Tepe, B., Cakir, A., Abali, G. and Mete, E. 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitexagnuscastus* L. fruits from Turkey. *Food and Chemical Toxicology,* 47: 2479–2483.

Sousa, A.C., Gattass, C.R., Alviano, D.S., Alviano, C.S., Blank, A.F. and Alves, P.B. 2004. Melissa officinalis L. essential oil: antitumoral and antioxidant activities. *Journal of pharmacy and pharmacology*, 56: 677–681.

Spiridon, I., Colceru, S., Anghel, N., Teaca, C.A., Bodirlau, R. and Armatu, A. 2011. Antioxidant capacity and total phenolic contents of oregano (*Origanumvulgare*), lavender (*Lavandula angustifolia*) and lemon balm (*Melissa officinalis*) from Romania. *Natural product research*, 25: 1657–1661.

Tabatabaei, S.J. 2008. Effects of cultivation systems on the growth, and essential oil content and composition of valerian. *Journal of herbs, spices & medicinal plants,* 14: 54–67.

Ye, F., Liang, Q., Li, H. and Zhao, G. 2015. Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthusannuus* L.). *Industrial Crops and Products*, 76: 574–581.

Zargari, A. 1991. Medicinal plants. Tehran: Tehran University Publications, pp. 77-83.