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Original Article

Evaluation of Essential Oil Composition and Rosmarinic Acid Content in Lemon Balm (*Melissa officinalis* L.) Cultivated in South of Iran

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Abstract

Melissa officinalis L. is used since ancient times in folk medicine because of its sedative, digestive, carminative, spasmolytic and analgesic effects. Its therapeutic impact is due to the essential oil content and rosmarinic acid. This study was designed to determine chemical composition of essential oil and rosmarinic acid content of *M. officinalis* cultivated in Fars province in south of Iran. The essential oil was obtained by hydro-distillation of dried aerial parts of harvested *M. officinalis* and analyzed by gas chromatography-mass spectrometry (GC/MS). Rosmarinic acid content was determined by high-performance liquid chromatography (HPLC) analysis. The results showed that essential oil content was 0.37% and its main constituents were geranial (28.9%), neral (21.5%), geranyl acetate (19.3%), caryophyllene oxide (7.0%), (E)-caryophyllene (6.8%), thymol (3.1%) and citronellal (2.9%). Rosmarinic acid content was 3% in dry weight. Our findings show south of Iran has suitable potential for production of *M. officinalis* with high quality for therapeutic usages.

Keywords: *Melissa officinalis* L., Essential oil, Rosmarinic acid, Geranial, Neral.

Introduction

Lemon balm (*Melissa officinalis* L.) is an aromatic perennial herb grown in the Mediterranean region, southwestern Siberia, western Asia, and northern Africa. The most using part of this plant is its dried leaves often with flowering tops. 'Badranjboyeh' is Persian name of *M. officinalis* L. Badranjboyeh grows widely in the provinces of Tehran, Azerbaijan, Golestan, Kurdistan, Kermanshah and Lorestan [1,2]. It is reported that the plant is mainly grown in Germany, France, Italy, Romania, Bulgaria, and North America [3]. *M. officinalis*, a member of the family Lamiaceae is a perennial herb and it commonly named as Lemon balm because of its lemon-like flavor and fragrance. According to British Pharmacopoeia, dried leaves and the top aerial part of this plant are used as medicine. *M. officinalis* has been traditionally used for multiple medical purposes such as carminative, tonic, diaphoretic, antispasmodic, sedative-hypnotic, relief of stress, strengthening the memory and reduction of headache. Ibn-Sina (Avicenna), a well-known Iranian physician, recommended *M. officinalis* in treatment of irritability and nervousness in young girls and women, boost a lack of interest and energy and depression [1,2]. Modern pharmacological studies demonstrate that *M. officinalis* is valuable in the management of mild to moderate Alzheimer, migraine and rheumatism. Also different studies mentioned antitumor, antioxidant, antimicrobial, hypolipidemic, hypoglycemic, antidepressant, anxiolytic, anti-nociceptive, spasmolytic and anti-inflammatory properties for *M. officinalis* [4,5].

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Phytochemical investigations have showed the presence of volatile compounds (monoterpens and sesquiterpens), triterpenes, phenolic acids (caffeic acid ester) and flavonoids as the main active constituents of *M. officinalis*. According to British Pharmacopoeia, rosmarinic acid (RA) content of dried leaf should not be less than 1% and the essential oil content based in Iran Herbal Pharmacopoeia is in the range of 0.02 to 0.3% [6].
Among the identified phytochemicals, rosmarinic acid showed various pharmacological mechanisms which can lead to inhibition of different serious health problem such as cardiovascular disease, stroke, cancer, dementia, Alzheimer's, Parkinson's, rheumatism and etc. [7]. Some of these pharmacological activities are including inhibition of proliferation, migration, adhesion and tube formation of human umbilical vein endothelial cells in cancer angiogenesis [8]. Rosmarinic acid also reduced intracellular reactive oxygen species level, reduced inflammatory cytokines (IL-6, IL-8, TNF-α, and CRP), up-regulated PPARγ expression and down-regulated NF-κB expression in myocardial tissue during Myocardial Ischemia [9]. Rosmarinic acid showed protection against the apoptotic cells death by oxidative stress. It exerted neuroprotective and anti-oxidant effect against neurotoxins and significantly protected neurons in neurodegenerative disease such as Alzheimer's. Also Rosmarinic acid inhibits fibrillation and diminishes vibrational modes associated to β sheet in tau protein linked to Alzheimer's disease [10, 11]. As a result of various scientific reports and also according to WHO and British pharmacopeia monographs on selected medicinal plants, RA is a biomarker for quality control of *M. officinalis* L. [12,13]. Essential oil content and essential oil composition also is an important parameter in quality control of *M. officinalis*. The aim of this study was the investigation of the quality of *M. officinalis* cultivated in Fars province in south of Iran.

Material and Method

Chemical and Reagents

RA standard was purchased from Sigma-Aldrich (Germany). Methanol (HPLC grade), ethanol (analytical grade) and formic acid (analytical grade) were purchased from Merck (Germany).

Plant material

This research was conducted during 2016 in a research field located in Kamfirooz city (Mozafari's field), Fars Province, Iran (1804 m above sea level and 30° 19' N; 52° 11'E). The mean annual temperature was 15.6 °C and the average rainfall was about 261 mm. The seeds of *M. officinalis* were purchased from Pakan Bazr Company (Isfahan, Iran). Seedlings were cultivated during March in the greenhouse and transferred to the main field after three months (May), and finally harvested at the full flowering stage during August. Aerial parts of *M. officinalis* were collected during flowering stage and dried under shadow at room temperature.

Essential Oil Isolation

For determination of the essential oil, three samples were analyzed and the mean value was reported. 50 g of dried leaves were hydro distillated in Clevenger apparatus in 500 mL water at 100 °C for 3 hours. The essential oil was dried with anhydrous Na_2SO_4 and the percentage of essential oil was measured volumetrically (v/w).

Determination of Essential Oil Composition

The composition of the essential oil was analyzed by Gas Chromatography-Mass Spectrometry. For this work analysis was performed on an Agilent GC-MS system (Agilent Technologies-5975C-MS, 7890A-GC) equipped with an HP-5MS capillary column (0. 25 mm×30 m, 0. 25-µm film thickness, Agilent). The operating conditions were as follows: the oven temperature was programmed from 60 to 210 ºC at the rate of 3 ºC/min then increased to 240 ºC at the rate of 20 ºC/min and the final temperature kept for 8.5 min; run time was 60 min; ion-source temperature was 230 ºC; interface line and Injector temperature was 280 ºC; Split ratio was 1:50; injection volume was1 µL; carrier gas was helium at flow rate of 1ml/min; mass range was 50-480 and the electron ionization energy was 70 eV. The identification of the essential oil components of *M. officinalis* was confirmed by comparison of their relative retention times and mass spectra with Adams, NIST and Wiley libraries.

Determination of RA

Air-dried and crushed balm leaves (approx. 2 g) were grounded to powder form using a mixer mill (IKA, Germany). 90 ml 50% (v/v) aqueous ethanol was added to 100 mg ground leaf material in a reflex apparatus and boiled in water bath for 30

min. Obtained extract was centrifuged and the supernatant was transferred to a 100 mL volumetric flask and its volume adjusted to 100 mL using 50% (v/v) aqueous ethanol. An aliquot of the resulting solution was filtered into a vial using a 0.45 µm pore size syringe filter and used for analysis. RA analysis was performed on an Agilent Technologies 1200 series HPLC system with diode array detector (DAD). Extracts (injection volume 20µL) were separated on a Zorbax eclipse (XDB) C18 Column (5µm (FT), 4.6 (ID) × 150 mm). Oven temperature was 30 °C. Run time was 40 min, flow rate was 1 mL/min and detection wavelength was 280nm. As mobile phase Eluent A was methanol and eluent B was 0.1% (v/v) aqueous formic acid. A gradient elution program according to table 1 was used. Chemstation software (version B.03.02) was applied for controlling the instrument and quantitative analysis. RA was quantified using an external standard calibration (calibration range was 25–400 mg/L). A linear calibration model was used resulting in R^2>0.99. Fig. 1 shows the HPLC diagram of RA standard.

Table 1 Gradient time program for analysis of rosmarinic acid with HPLC method.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0-10	10-25	90-75
10-20	25-60	75-40
20-30	60-70	40-30
30-40	60-70	40-30

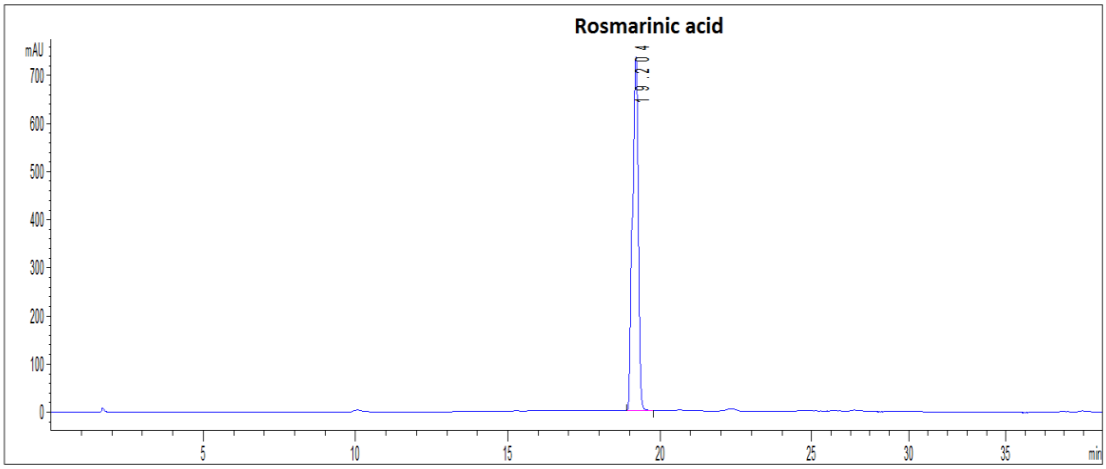

Fig. 1 Chromatographic diagram of rosmarinic acid standard injection.

Results and Discussion

Essential oil Content and its Composition

The hydro-distillation of the dried aerial parts of *M .officinalis* gave yellow-pale color oil, in total yield of 0.37% (v/w). GC-MS analysis of essential oil identified 33 compounds which represented about 98.39% of total oil. Table 2 shows the chemical components of essential oil with retention time as a result of GC-MS analysis. As given in table 2 the major components in the essential oil were geranial (28.9%), neral (21.5%), geranyl acetate (19.3%), caryophyllene oxide (7.0%), (E)-caryophyllene (6.79%), thymol (3.1%) and citronellal (2.9%) (Fig. 2, Table 2).

Previously Kittler *et al* [14] evaluated 68lemon balm genotypes for content and composition of essential oil. In their study, the content of essential oil varied in ranges from 0.01 to 0.35% and caryophyllene, germacrene D, and β-caryophyllene oxide were characterized as main components of essential oil. The essential oils were classified in two chemotypes; citral chemotype and β-caryophyllene oxide chemotype. In the group with citral chemotype, concentrations of essential oil components were 3.58–59.95% for citronellal, 10.9–45.6% for (E)-citral (neral), 6.8–34.3% for (Z)-citral (geranial), 1.2–8.9% for β-caryophyllene, 0–1.9% for germacrene D, and 0.4–6.2% for β-caryophyllene oxide. In β-caryophyllene oxide

chemotype, the essential oil components were 4.7–18.6% for β-caryophyllene, 1.8–13.6% for germacrene D and 18.4–54.1% for β-caryophyllene oxide. 62 genotypes of 68 genotypes were classified as citral chemotype and 6 genotypes was β-caryophyllene oxide.

According to the analysis results of our study and different other origins *M. officinalis* in table 3, almost all of samples were classified in citral chemotype as the most common chemotype of this plant [14].

In this study essential oil content and the composition of lemon balm harvested qualitatively and quantitatively were comparable with the oils from Iran Kurdistan [15], Iran Shahrekord [16], Iran Qom [17], Serbia [18], Tajikistan [19], Poland [20], Algeria [21], Egypt [22], France [23], Brazil [24] and Turkey [25] as seen in Table 3.

Differences in the essential oil content and composition in various *M. officinalis* samples can be affected by harvesting time and ecological condition including relative humidity, rainfall, duration of sunlight and temperature. Naturally a lot of parameters can affect the essential oil content and composition of plants, however controlling all these parameters is impossible, proper management of crop production, appropriate selection of plant genotype and also ecological condition of field is needed to achieve high quality products based on the consumer demand [16].

Rosmarinic Acid

Beside of essential oil for the pharmaceutical use, RA is the substance of interest, because of its proven pharmaceutical effects such as anti-microbial, anti-inflammatory, anti-cancer, antioxidant, anti-diabetic neuro protective and anti-Alzheimer effects [26].

It is important in preventing oxidative stress and associated diseases, herbs with high RA content should be considered a part of regular diet. Plants of Lamiaceae family especially *Rosmarinus officinalis* and *M. officinalis* are rich source of RA and can be used in food, cosmetic and pharmaceutical industries. Previously RA was determined by a spectrophotometric method as the sum of all hydroxyl cinnamic acid derivatives.

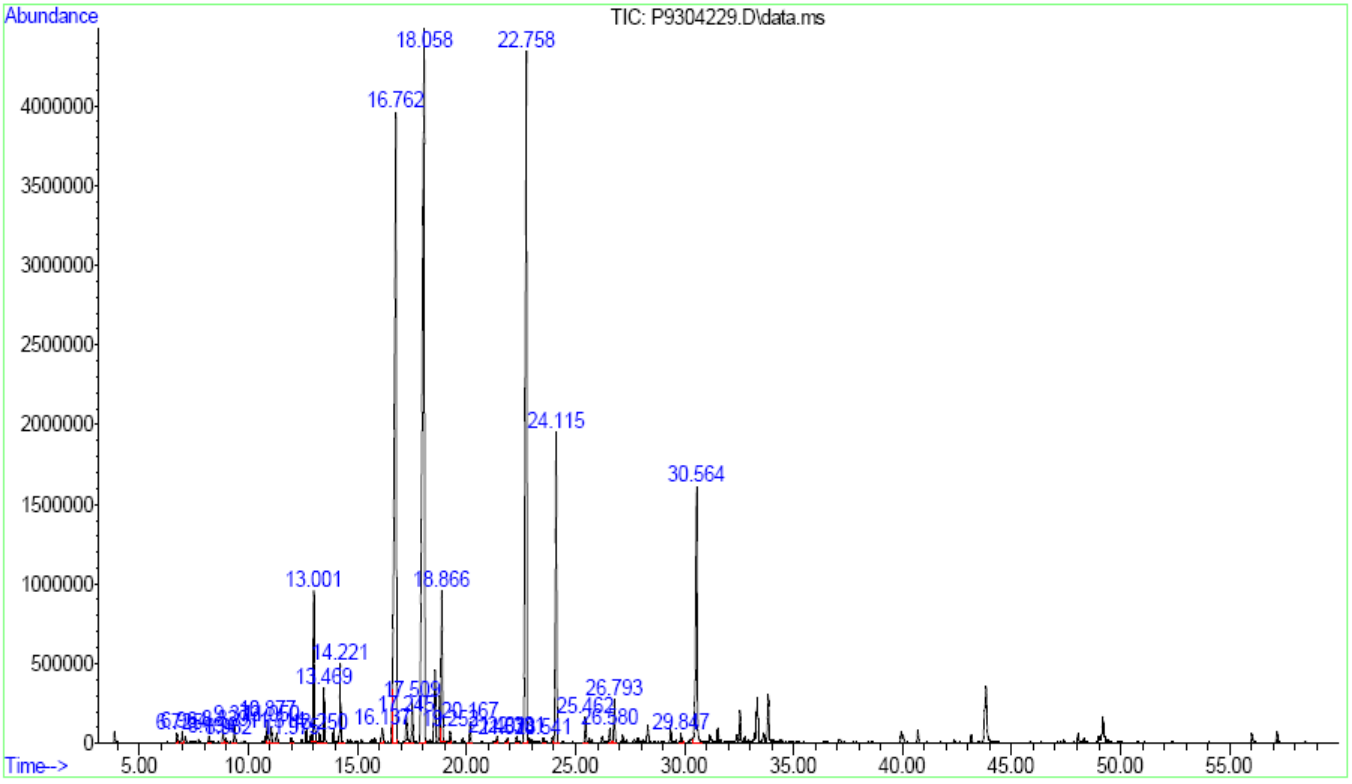

Fig. 2 The chromatogram of *Melissa officinalis* essential oil

Table 2 Chemical components of *Melissa officinalis* from south of Iran

No	Compound name	RI	(%)
1	1-Octen-3-ol	974	0.2
2	6-Methyl-5-hepten-2-ol	983	0.2
3	p-Cymene	1022	0.1
4	Benzene acetaldehyde	1040	0.3
5	(E)-β-Ocimene	1044	0.1
6	γ-Terpinene	1055	0.3
7	Linalool	1097	0.5
8	n-Nonanal	1102	0.4
9	*Cis*-Rose oxide	1108	0.2
10	*Trans*-Rose oxide	1124	0.1
11	Citronellal	1149	2.9
12	(2E)-Nonen-1-al	1156	0.2
13	*Cis*-Chrysanthenol	1161	1.1
14	Unknown	1179	1.6
15	Citronellol	1225	0.4
16	Neral	1238	21.5
17	Geraniol	1251	0.7
18	Methyl citronellate	1258	0.8
19	Geranial	1267	28.9
20	Thymol	1290	3.1
21	Carvacrol	1299	0.3
22	Methyl geranate	1321	0.4
23	Citronellyl acetate	1350	0.1
24	Neryl acetate	1362	0.1
25	α-Copaene	1372	0.1
26	Geranyl acetate	1383	19.3
27	Methyl eugenol	1402	0.1
28	(E)-Caryophyllene	1416	6.8
29	α-Humulene	1449	0.6
30	Germacrene D	1477	0.3
31	(E)-β-Ionone	1482	0. 9
32	(E)-Nerolidol	1560	0.2
33	Caryophyllene oxide	1579	7.0
Known			98.4
Unknown			1.6

Table 3 Main chemical constituents of *Melissa officinalis* essential oil from various origins.

Constituents	**This work, South of Iran (2018)**	**Iran, Kurdistan (Taherpour *et al.*, 2012)**	**Iran, Shahrekord (Mohamadpoor *et al.*, 2018)**	**Iran, Qom (Arzhang *et al.*, 2015)**	**Serbia (Mimica-Dukic *et al.*, 2004)**	**Tajikestan (Sharopov *et al.*, 2013)**	**Poland (Seidler-Łożykowska *et al.*, 2017)**	**Algeria (Abdellatif *et al.*, 2014)**	**Egypt (Shabby *et al.*, 1995)**	**France (Carnat *et al.*, 1998)**	**Brazil (Silva *et al.*, 2005)**	**Turkey (Turgut*et al.*, 2016)**
Essential oil content%	0.37	0.28	0.2	0.36	0.2	0.3	0.15	0.34	Nr	0.32	Nr	<0.5
Citronellal	2.9	20.3	3.8	-	13.7	2.8	4.8	6.3	13.3	39.5	26.7	-
Neral	21.4	23.9	17.6	2.7	16.5	31.5	19.4	30.2	19.7	20.4	-	-
Geranial	28.9	-	13.6	3.8	23.4	43.2	13.4	44.2	26.8	27.8	38.1	-
Geranyl acetate	19.3	2.8	-		-	1.2	-	-	1.7		-	-
Germacrene D	-	-	9.3	8.1	2.4	-	-	-	-	-	-	-
β-Caryophyllene	6.8	1.8	23.1	17.3	4.6	4.0	18.5	1.3	4.9	2.4	-	7.9
Caryophyllene oxide	7.0	-	10.8	21.1	1.7	-	28.9	1.3	10.0	-	-	38.6

Origin of the samples; Iran Kurdistan (wild), Iran Sharekord (cultivated), Iran Qom (cultivated), Serbia (cultivated), Tajikistan (wild), Poland (cultivated), Algeria (cultivated), Egypt (wild), France (cultivated), Brazil (greenhouse cultivated) and Turkey (cultivated).
Nr: not reported.
-: not detected or less than 1%.

Since 2009 the European Pharmacopoeia changed the method to high performance liquid chromatography (HPLC) and now the acceptable method for RA content is HPLC [27]. RA content is an important quality requirement and a raised content is desired in planting programs. HPLC analysis of the samples was done for cultivated sample. Fig.3 shows the chromatogram of sample. RA content of *M. officinalis* in this study was 3% which is greater than 1% that mentioned in BP. Kittler *et al.* reported that the RA content of *M. officinalis* is ranged from 2.45 to 8.78% in different genotype [28]. RA content of *M. officinalis* from Slovakia reported in the range of 1.1 to 1.7% and cultivated *M. officinalis* in Mazandaran, Iran was contained 3.6% RA [29, 30]. The RA content in *M. officinalis* samples collected in Bosnia and Herzegovina was 0.5% and for Turkish sample was 0.02% [31].

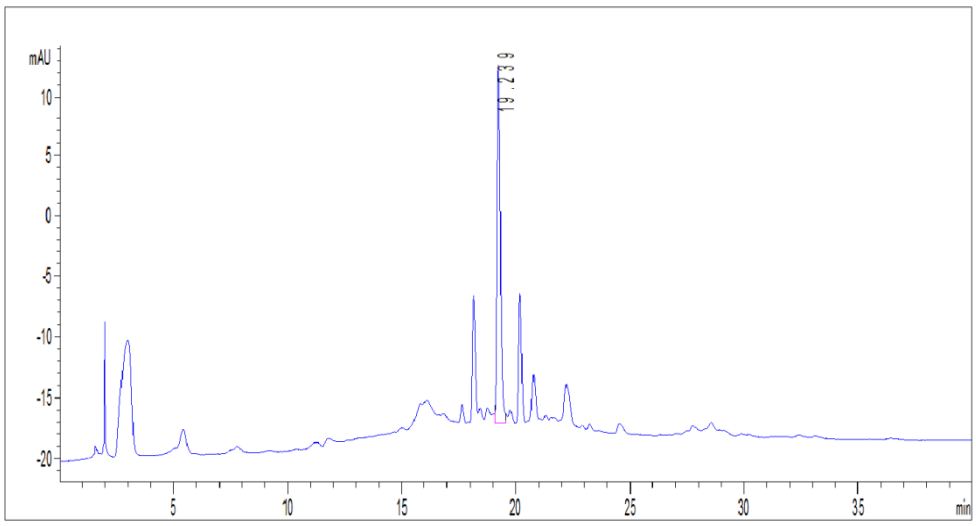

Fig. 3 Chromatographic diagram of sample injection.

As a result, in different regions, *M. officinalis* varies in its RA content. RA in *R. officinalis* reported 0.6-0.7% [5, 30]. It is too low in comparison with 3% in cultivated *M. officinalis.* The result of our analysis showed that the cultivated *M. officinalis* in this study produce high content of RA and can use as a source of RA.

Conclusion

The essential oil of *M. officinalis* harvested in south of Iran was found to be rich in geranial and neral, same as several other samples origin reported previously, and represents the most common chemotype of this plant. Also high content of RA as the main pharmacologically active phenolic compound in this sample was determined. In the other hand, cultivated *M. officinalis* is a rich source of rosmarinic acid and it can be used instead of other source of rosmarinic acid like rosemary (*R. officinalis*) and etc. The chemical composition of this plant was done and in conclusion we can introduce the Kamfirooz region in south of Iran as a new place for cultivation of *M. officinalis* however it needs more investigation on larger farm cultivation and also economic and ecological investigation is necessary.

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