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Phytochemical investigation of *Melissa officinalis* L. flowers from Northern part of Iran (Kelardasht)

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Abstract
Melissa officinalis L. (Lamiaceae/Labiatae) is a medicinal herb having used in traditional medicine all over the world. Analyzing the volatile oils by hydrodistillation method along with characterizing major flavonoids of *M. officinalis* flowers growing wild in the north of Iran was the aim of this study. Hence, the study led to the identification of 37 oil compositions by a combination HP-5 GC-FID and GC-MS analytical techniques. The results revealed that the major oil components for *M. officinalis* was β-caryophyllene (24.4%), followed by geranial (8.6%), 1,8-cineole (6.9%), neral (6.7%), dehydroaromedendrene (5.8%) and thymol (4.8%), accounting for 91.34% of the total components. The essential oils of *M. officinalis* inhibited mildly the DPPH radicals were identified by approximately equal amount of monoterpenes (42.22%) and sesquiterpenes (43.72%). Furthermore, based on high amount of total flavonoid content in EtOAc extract of flowers (49.25 ± 1.03 mg/g) and consequently the potent antioxidant activity (inhibition of the DPPH radicals, IC_{50}= 25.26 ± 0.61 µg/ml), two major flavonoids (apigenin 1 and quercetin 2) have been isolated from flowers of *M. officinalis* by using Column Chromatography method. The structures of 1 and 2 were established by analysis of NMR spectroscopic data and comparison with their literature data.

Keywords: Apigenin; β-Caryophyllene; DPPH; Lamiaceae; *Melissa officinalis*

1. Introduction
Nowadays, plants are considered as the main resource of natural medicines. Then, natural products chemists are aware of the potential effects on natural compounds which are responsible for an array of vital biological activities [1]. *Melissa officinalis* L. is an aromatic perennial plant belonging to Lamiaceae (Labiatae) family, which are distributed in Southern Europe and Mediterranean region. It is commonly known as lemon-balm with various traditional uses especially in pharmaceutical aims and cooking because of its fragrant leaves [2]. The plant has been used in folk medicine as the main medicinal herb in order to memory improvement [3], cardiotonic [4], antidepressant and anxiolytic agent [5], and also for the treatment of cardiovascular and respiratory problems [6, 7], mental and central nervous system (CNS) diseases [8], several cancers [9]. There are different investigations with regard to isolation and characterization of various natural constituents in *Melissa officinalis* by several techniques such as volatile oils [10-13], triterpenes/triterpenoids [14], flavonoids [15,16], and phenolic acids [17,18]. Therefore, according to recent biological studies, extracts and volatile oils of the plant are clearly known for their antiviral [19], antiepileptic [20], anti-inflammatory [21], antibacterial, antiproliferative, antioxidant [14, 22-27], antidepressant [28], neuroprotective [29], antihyperglycemic and antihyperlipidemic [30] activities. The aim of the present study was phytochemical investigation of *M. officinalis* flowers collected from north of Iran because of possessing applicable uses for ethnopharmacologists and specialists. Thus, in the course of our research the volatile oils were extracted by hydrodistillation method and consequently analyzed by gas chromatography (GC) as well as gas chromatography coupled with mass spectrometry (GC-MS). Major flavonoids elicited by column chromatography (CC) technique were characterized by Nuclear Magnetic Resonance (NMR) method.

2. Material and Methods
2.1 General experimental procedures and chemicals
All reagents and solvents for analysis were commercially available and used as received without further purification. IR spectra were recorded using KBr disks on a FT-IR Perkin-Elmer spectrometer. ^{1}H and ^{13}C-NMR spectra (in $CDCl_3$) are measured on a Bruker Avance

TM 500 DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with TMS as an internal standard. Silica gel (230–400 mesh, Merck) were used for Column Chromatography. TLC was performed on Merck F_{254} silica gel plates (10 × 10 cm). Reddish-orange spots of flavonoids were detected by spraying anisaldehyde-sulfuric acid reagent followed by heating. DPPH was obtained from E. Merck (Germany).

2.2 Plant Materials

The fresh flowers of *Melissa officinalis* L. were collected during the flowering stage from Roudbarak village, Kelardasht, Gilan province, Iran, in August 2015. The taxonomic identification of the harvested plant was confirmed at Herbarium of the Faculty of Sciences, Golestan University, Gorgan, Iran and a sample was deposited in the Herbarium of the same institute.

2.3 Hydrodistillation of volatile oils

The air-dried flowers (200 g) of *M. officinalis* were extracted by hydrodistillation method using Clevenger-type apparatus for 4-5 hours to give the following yield (w/w): 1.05%. Anhydrous sodium sulfate was used to get rid of water after extraction and the volatile oils stored in refrigerator at 4 °C for further analyses.

2.4 Analysis of the volatile oils

FID-GC analysis was measured using an Agilent 7890A gas chromatograph equipped with a split injector (250 °C with ratio, 1:50). A silica HP-5 capillary column (30m × 0.32mm × 0.25µm film thickness) was used with helium as carrier gas (1.0 mL/min). The GC oven temperature was held at 50 °C for 5 min and programmed to 250 °C at the rate of 4 °C/min, and kept constant at 250 °C for 5 and then programmed to 280 °C at a rate of 3 °C/min. The FID detector was at 280 °C. GC-MS was carried out on an Agilent 5975C GC-MS system with a HP-5 fused silica column using the aforementioned chromatographic conditions. The retention indices (RI) for *M. officinalis* were individually determined by conjunction of the sample with containing the homologous series of C_8 to C_{26} n-alkanes [31]. The constituents of *M. officinalis* oil were identified by their retention indices compared with published data in the literature [32], computer library (NIST/NIH libraries) and Wiley library [33]. Finally, the percentage of oil components were acquired by peak area normalization.

2.5 Extraction and Estimation of Antioxidant activity and Total Flavonoid

The air-dried powdered flowers (400 g) of *M. officinalis* were extracted at room temperature with 80% methanol by maceration method (3×1 L, rt for 24 h) and the combined extracts evaporated to give a bright gummy residue (10 g). Having suspended the methanol extract in water, five sequential solvents (with analytical grade) – n-hexane, chloroform, ethyl acetate, n-butanol and residual methanol/water – were used to give fractions with different attitudes and phytochemicals. The dried fractions were 1.25 g, 2.10 g, 3.66 g, 1.02 g and 1.97 g, respectively. Therefore, based on determination of total flavonoid content determined by colorimetric method and calculated as quercetin standard [34], the antioxidant activity – free radical scavenging [35] (DPPH: 2,2-Diphenyl-1-Picrylhydrazyl) – were precisely investigated for obtained volatile oils along with five kind of extracts of *M. officinalis* flowers. To measure the spectrophotometric DPPH assay, 100 µl of each extract was added to 3 ml MeOH. From the eluted extracts the amount of 0, 10, 20, 30, etc., µl concentrations were mixed with 2 ml MeOH and 2 ml DPPH/EtOH equivalent to 0.004%. The control tube was consist of eluted DPPH and MeOH (2 ml for each of them). In continue, the samples were carefully stored at room temperature and darkness condition for 30 minutes. The DPPH had an absorption band at 517 nm in its radical form that vanishes upon reduction by an antiradical compound. The percentage of inhibition of the DPPH radical by samples was calculated according to the formula of Yen and Duh [36]. All measurements were carried out in triplicate and the results were reported by their meaning.

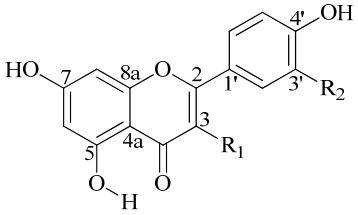
$$\%\text{Inhibition} = 100 \times (A_{control} - A_{sample})/A_{control}$$

$A_{control}$: Absorbance of the control (t = 0 min)

A_{sample}: Absorbance of the tasted sample (t = 30 min)

2.6 Extraction and Isolation of Flavonoids

Three g of the obtained EtOAc extract was subjected by utilizing a normal column chromatography (CC) and gradient eluting with n-hexane: EtOAc: MeOH system [100:0:0, 90:10:0, 80:20:0, 70:30:0, 60:40:0, 50:50:0, 40:60:0, 20:80:0, 0:100:0 and 0:50:50 (v/v), respectively] to yield 10 fractions 1-10. Fraction 4 (350 mg) was re-chromatographed by n-hexane: EtOAc [90:10, 80:20 and 70:30 (v/v), respectively] to yield four fractions A-D. Fraction B (135 mg) was also eluted by Et_2O: EtOAc [99:1, 97:3, 95:5 and 93:7 (v/v), respectively] to afford yellowish compound 1 (54 mg, with R_f = 0.6 for Et_2O: EtOAc, 8:2). Compound 2 (47 mg, with R_f = 0.7 for n-hexane: EtOAc, 5:5) was purified from fraction 7 (296 mg) by a silica gel column (stepwise gradient eluting with n-hexane: EtOAc, 10:0-8:2). The ^{1}H-^{13}C NMR data for Apigenin (1) and Quercetin (2) have previously been reported (see Figure 1) [37, 38].



Apigenin (**1**): R_1=H, R_2=H

Quercetin (**2**): R_1=OH, R_2=OH

Fig 1: Chemical structures of two major Flavonoids isolated from *Melissa officinalis* L flowers.

3. Results and Discussion

The volatile oils – colorless – were obtained from fresh flowers of *M. officinalis* by the use of hydrodistillation method. The samples acquired were similarly analyzed by GC-FID and GC-MS apparatuses. The flowers essential oil extraction yield was 1.05% (w/w). 37 compositions representing approximately 91.34 % of the flowers of *M. officinalis* were identified by comparing their retention indices (RI) and their MS in the literature (see Table 1 and Figure 2) as volatile components. The volatile oils were complex mixture of monoterpene hydrocarbons (11.63%); sesquiterpene hydrocarbons (41.82%); oxygenated monoterpenes (30.59%); oxygenated sesquiterpenes (1.9%) and other compounds (5.40 %). A chromatogram of volatile oil is displayed in Figure 1. The most abundant compound was β-caryophyllene with 24.36%, followed by geranial (8.56%), 1,8-Cineole (6.93%), neral (6.69%), dehydroaromedendrene (5.84%), thymol (4.82%) and α-

pinene (3.85%). It has been reported by Kazemi and Esmaili that the major compositions of the essential oils of Iranian *M. officinalis* were β-caryophyllene (16%), β-cubebene (10.21%), α-cadinol (9.05%) and geranial (6.30%) [12]. Moreover, the most abundant components of the volatile oils of *M. officinalis* collected from central part of Iran (Qom province) were Caryophyllene oxide (21.11%), β-caryophyllene (17.33%) and germacrene D (8.14%) [13]. On the other hand, Algerian *M. officinalis* oils were identified by high percentage of monoterpene aldehyde, such as geranial (44.20%). The data indicated that monoterpene aldehyde (geranial, neral and citronellal) are mainly chemo-type for *M. officinalis* in Algeria [11], while *M. officinalis* oils gathered from three different regions of Greece were mostly β-pinene, sabinene, β-caryophyllene, caryophyllene oxide as well as germacrene D by the absence of citral (neral/geranial) and citronellal [10]. Interestingly, Mimica-Dukic *et al* have explicitly identified the potent inhibitory of DPPH radical formation (IC_{50}= 7.58 µg/ml) because of the presence of geranial (23.4%), neral (16.5%), citronellal (13.7%) and β-caryophyllene (4.6%) as the main constituents of the *M. officinalis* oils cultivated in Serbia and Montenegro [22].

The consequences of total flavonoid contents (TFCs) and bleaching the DPPH radicals of the volatile oils and the five extracts of *M. officinalis* is given in Table 2. The TFCs data varied from 2.68 to 49.25 mg quercetin/g weight. As assumed, the antioxidant activity of the extracts was directly related to the amount of TFCs. The estimated capabilities of individual extracts to trap the DPPH radicals displayed that the most active extracts were ethyl acetate (25.3 µg/ml), residual methanol/water (50.2 µg/ml) and chloroform (68.8 µg/ml). Hence, the EtOAc extract was knowingly selected for further isolation and characterization of flavonoids. According to Mencherini *et al* on leaves of *M. officinalis* cultivated in Italy, the reducing of the DPPH radicals by n-BuOH soluble portion with high concentration of total phenol content – 356.0 ± 4.0 µg/mg – was more than the EtOH-H_2O extract (EC_{50}= 15.2 µg/ml and 18.5 µg/ml, respectively) [14], whereas the presence study revealed that the antiradical activity of EtOAc extract (IC_{50}= 25.26 ± 0.61 µg/ml) was more than other obtained extracts. In Bulgarian *M. officinalis* leaves the potent inhibition of DPPH was found in ethanol extract, 31.17 ± 0.13 µM TE/g fw [27]. It was shown earlier that the water extract of Turkish *M. officinalis* bleached the DPPH radicals by IC_{50}= 31.4 µg/ml [25].

Additionally, elucidating the chemical structures of 1 and 2 were carried out by NMR data commentaries. The ^{1}H-NMR spectroscopy of compounds 1 and 2 represents the presence of a 5-OH functional group (δ_{OH} > 12 ppm). Moreover, 15 carbon peaks depicted in the ^{13}C-NMR spectroscopy indicated that a sample flavonoidal structure is expected for both of them. Briefly, the ^{1}H and ^{13}C-NMR spectral data of compounds 1 and 2 are identical with those previously delineated [37, 38]. All NMR assignments for obtained compounds 1 and 2 have been noted in the followings:

Apigenin (1); IR (KBr) = 3399.42 (OH), >3000 (=C–H), 1639.49 (C=O), 1493.30 (aromatic C=C), 1171.50 cm^{-1} (etheric C–O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 12.50 (1H, s, OH-5), 7.68 (each 1H, d, *J*= 8.5 Hz, H-2′ and H-6′), 7.54 (each 1H, d, *J*= 9 Hz, H-3′ and H-5′), 6.88 (1H, s, H-3), 6.42 (1H, s, H-8), 6.20 (1H, s, H-6); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 146.79 (C-2), 102.96 (C-3), 175.24 (C-4), 119.93 (C-4a), 160.67 (C-5), 98.10 (C-6), 163.78 (C-7), 93.34 (C-8), 155.94 (C-8a), 121.90 (C-1′), 146.79* (C-2′), 116.63** (C-3′), 160.67 (C-4′), 114.97** (C-5′), 144.94* (C-6′). ^{1}H and ^{13}C-NMR data were identical to those recorded in reference [38].

*: the data are exchangeable

**: the data are exchangeable

Quercetin (2); IR (KBr) = 3403.05 (OH), >3000 (=C–H), 1639.86 (C=O), 1494.36 (aromatic C=C), 1171.79 cm^{-1} (etheric C–O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 12.55 (1H, s, OH-5), 7.73 (1H, d, *J*= 2.1 Hz, H-2′), 7.69 (1H, dd, *J*= 2.1,8 Hz, H-6′), 6.94 (1H, d, *J*= 8 Hz, H-5′), 6.48 (1H, d, *J*= 2.5 Hz, H-8), 6.25 (1H, d, *J*= 2.5 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 146.76 (C-2), 135.66 (C-3), 175.77 (C-4), 102.95 (C-4a), 160.64 (C-5), 98.13 (C-6), 163.79 (C-7), 93.33 (C-8), 156.09 (C-8a), 121.90 (C-1′), 114.96 (C-2′), 144.98 (C-3′), 147.62 (C-4′), 115.54 (C-5′), 119.98 (C-6′). ^{1}H and ^{13}C-NMR data were identical to those recorded in reference [38].

Table 1: Volatile oil components of *Melissa officinalis* flowers from north of Iran (Kelardasht).

Volatile Compounds [a]	RI [b]	LRI [c]	*M. officinalis*	Method of identification [d]
α-Pinene	936	939	3.85	MS, RI
β-Pinene	980	979	1.35	MS, RI
6-Methyl-5-hepten-2-one	983	981	0.96	MS, RI
n-Decane	999	1000	0.37	MS, RI
δ-3-Carene	1006	1010	2.72	MS, RI
o-Cymene	1021	1022	0.91	MS, RI
Sylvestrene	1023	1025	0.59	MS, RI
1,8-Cineole	1029	1026	6.93	MS, RI
γ-Terpinene	1052	1054	0.58	MS, RI
n-Nonanal	1101	1100	1.72	MS, RI
1(7),3,8-p-Menthatriene	1110	1108	1.63	MS, RI
Citronellal	1150	1148	0.79	MS, RI
Pinocarvone	1164	1160	0.37	MS, RI
Isothujol	1165	1166	0.46	MS, RI
p-Cymen-8-ol	1175	1179	0.55	MS, RI
Myrtenal	1196	1195	0.79	MS, RI
Pulegone	1235	1233	0.63	MS, RI
Neral	1240	1240	6.69	MS, RI
Geranial	1263	1264	8.56	MS, RI
Thymol	1293	1289	4.82	MS, RI
α-Copaene	1372	1374	0.40	MS, RI
β-Bourbonene	1388	1387	0.50	MS, RI
β-Caryophyllene	1417	1417	24.36	MS, RI
Aromadendrene	1443	1439	0.94	MS, RI
α-Humulene	1455	1452	1.15	MS, RI
Dehydroaromadendrene	1461	1460	5.84	MS, RI
γ-Gurjunene	1479	1475	0.38	MS, RI
α-Amorphene	1486	1483	2.23	MS, RI
Germacrene D	1488	1484	2.59	MS, RI
β-Ionone	1489	1487	1.04	MS, RI
β-Selinene	1491	1489	1.27	MS, RI
α-Muurolene	1505	1500	0.37	MS, RI
cis-Calamenene	1531	1528	1.79	MS, RI
3-Hexenyl benzoate	1566	1565	0.40	MS, RI
Valeranone	1680	1674	1.90	MS, RI
Dibutyl phthalate	1963	1960	0.53	MS, RI
n-Eicosane	2002	2000	0.38	MS, RI
Monoterpene hydrocarbons			11.63	
Oxygenated Monoterpenes			30.59	
Sesquiterpene hydrocarbons			41.82	
Oxygenated Sesquiterpene			1.90	
Other compounds			5.40	
Total identified			91.34 %	

[a] Compounds are listed in order of their elution from a HP-5MS column using homologous series of C_8 to C_{26} *n*-alkanes. [b] RI, calculated retention indices. [c] LRI, literature on HP-5MS column. [d] Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature.

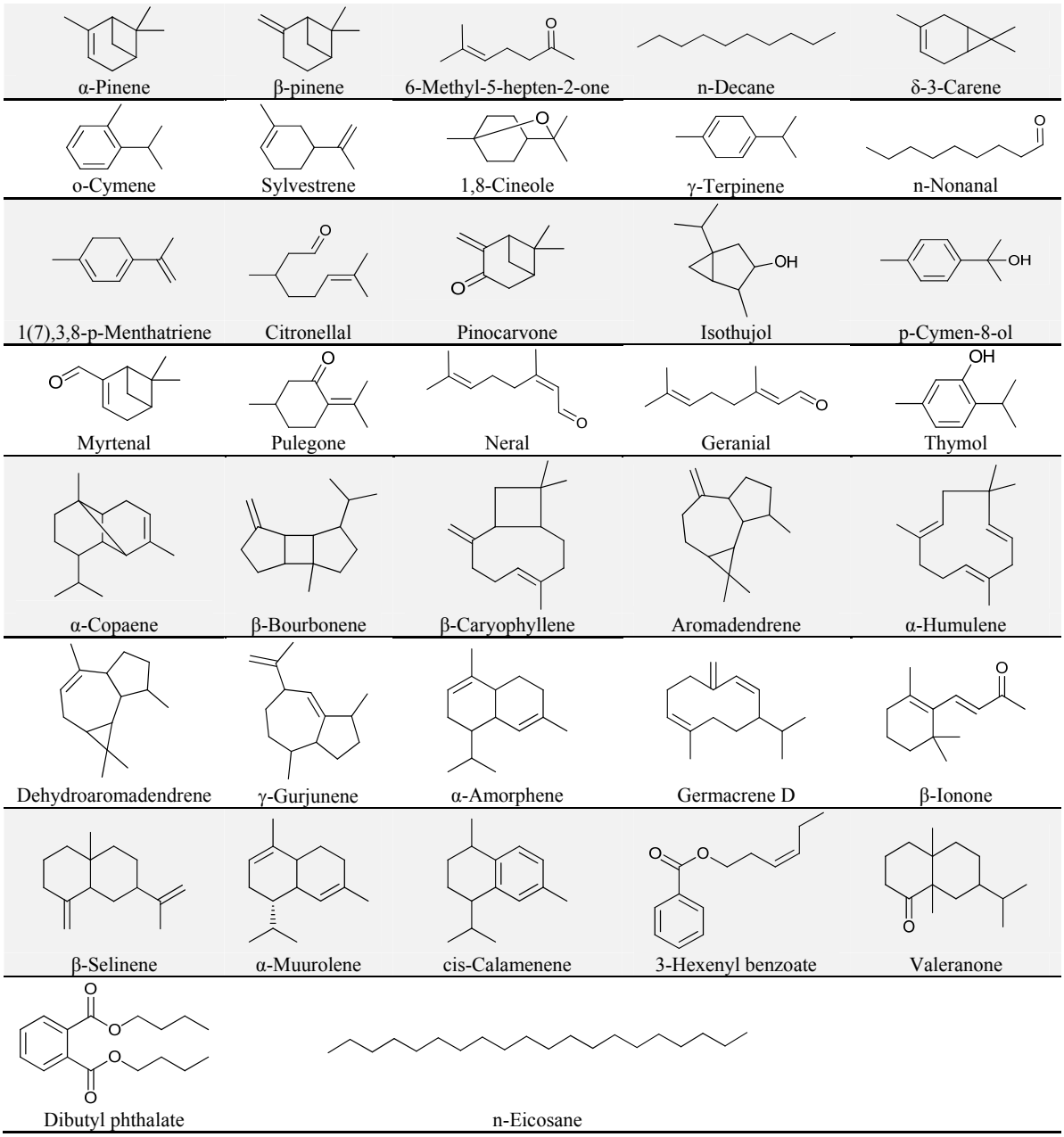


Fig 2: Structures of Chemical components of the Volatile oils of *Melissa officinalis* L. from northern of Iran (Gilan province, Kelardasht).

Table 2: The total flavonoid contents (TFCs), *In vitro* antiradical activity of the varied sequential extracts along with the purified compounds 1 and 2.

Extract	TFCs (mg/g)	DPPH radical scavenging (IC_{50} µg/ml)*
Volatile oils	–	61.33 ± 0.52
n-Hexane	2.68 ± 0.94	156.85 ± 0.39
Chloroform	23.51 ± 0.21	68.76 ± 0.44
Ethyl acetate	49.25 ± 1.03	25.26 ± 0.61
n-Butanol	9.32 ± 1.78	138.21 ± 0.74
residual Methanol/Water	35.87 ± 1.2	50.18 ± 1.07
Apigenin **1**	–	17.51 ± 0.93
Quercetin **2**	–	12.64 ± 0.47

– The values are means ± SD of three dimensions.
* The IC_{50} values were calculated by linear regression analysis.

4. Conclusion

To the best of our knowledge, a comprehensive survey was carried out on *Melissa officinalis* L. flowers. Two major flavonoids were isolated and identified using chromatographic separation from the ethyl acetate extract. It is noteworthy to note that Apigenin 1 and Quercetin 2 were purified from *M. officinalis* flowers for the first time. Their structure elucidation was established on the basis of their physiochemical and spectroscopic data (^{1}H NMR and ^{13}C NMR). The free radical scavenging of various extracts were evaluated through DPPH assay. Among them, EtOAc extract because of consisting the high amount of total flavonoid content was responsible for the strong activity for trapping DPPH radicals with IC_{50} values 25.26 ± 0.61 µg/ml and therefore it may be promising natural antioxidant plant. In contrary to an array of researches on *M. officinalis* oils, the present study is the first report of possessing β-caryophyllene chemo-type *M. officinalis* volatile oils collected from Kelardasht, Gilan province, Iran. Last but not least, the acceptable antioxidant activity (DPPH radicals scavenging) of the volatile oils of *M. officinalis* reaffirmed the recent studies because of its phytochemicals such as β-caryophyllene,

geranial, neral, caryophyllene oxide, citronellal and 1,8-cineole. This is another research on *M. officinalis* extracts accompanied by the major antiradical compounds to approve its nature as a potential plant to protect against various damages originated from free radicals.

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6. References

1. WHO (World Health Organization). Foodborne disease outbreaks: Guidelines for investigation and control. France: WHO Library Cataloguing-in-Publication Data, 2008.
2. Mozaffarian V. Dictionary of the Names of Iranian Plants. Frahang Mo'aser Pub, Tehran. 1997, 344.
3. Dastmalchi K, Ollilainen V, Lackman P, Boije af Gennas G, Dorman HJ, Jarvinen PP, *et al.* Acetylcholinesterase inhibitory guided fractionation of *Melissa officinalis* L. Bioorg Med Chem. 2009; 15:867-871.
4. Kultur S. Medicinal plants used in Kirklareli Province (Turkey). J Ethnopharmacol. 2007; 111:341-364.
5. Taiwo AE, Leite FB, Lucena GM, Barros M, Silveira D, Silva MV, *et al.* Anxiolytic and antidepressant-like effects of *Melissa officinalis* (lemon balm) extract in rats: Influence of administration and gender. Indian J Pharmacol. 2012; 44:189-192.
6. Joukar S, Asadipour H. Evolution of *Melissa officinalis* (lemon balm) effects on heart electrical system. Res Cardiovasc Med. 2015a; 4:e27013.
7. Akhondali Z, Dianat M, Radan M. Negative chronotropic and antidysrhytmic effects of hydroalcoholic extract of lemon balm (*Melissa officinalis* L.) on CaCL2-induced arrhythmias in rats. Electron Physician. 2015; 7:971-976.
8. Lopez V, Martin S, Gomez-Serranillos MP, Carretero ME, Jager AK, *et al.* Neuroprotective and neurological properties of *Melissa officinalis*. Neurochem Res. 2009; 34:1955-1961.
9. Zargari A. Medicinal Plants. 5th Edition, Tehran University Press, Tehran. 1995, 77-81.
10. Basta A, Tzakou O, Couladis M. Composition of the leaves essential oil of *Melissa officinalis* s. l. from Greece. Flavour Fragr J. 2005; 20:642-644.
11. Abdellatif F, Boudjella H, Zitouni A, Hassani A. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinals* L. Excli J. 2014; 13:772-781.
12. Kazemi M, Esmaili F. Volatile constituents of essential oils isolated from flowers and leaves of *Melissa officinalis*. J Biol Environ Sci. 2014; 8:111-113.
13. Arzhang M, Dakhili M, Farahani F. Investigation of chemical compounds and antimicrobial activity of essential oil of *Melissa officinalis* L. Qom Univ Med Sci J. 2015; 9:7-13.
14. Mencherini T, Picerno P, Scesa C, Aguino R. Triterpene, antioxidant, and antimicrobial compounds from *Melissa officinalis*. J Nat Prod. 2007; 70:1889-1894.
15. Heitz A, Carnat A, Fraisse D, Carnat AP, Lamaison JL. Luteolin 3'-glucuronide, the major flavonoid from *Melissa officinalis* subsp. *officinalis*. Fitoterapia. 2000; 71:201-202.
16. Patora J, Kilmek B. Flavonoids from *Lemon balm* (*Melissa officinalis* L., Lamiaceae). Acta Pol Pharm. 2002; 59:139-143.
17. Caniova A, Brandsteterova E. HPLC analysis of phenolic acids in *Melissa officinalis*. J Liq Chrom Rel Technol. 2001; 24:2647-2659.
18. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15:7313-7352.
19. Allahverdiyev A, Duran N, Ozguven M, Koltas S. Antiviral activity of the volatile oils of *Melissa officinalis* L. against Herpes simplex virus type-2. Phytomedicine. 2004; 11:657-661.
20. Bhat JU, Nizami Q, Aslam M, Asief A, Ahmad Sh-T, Parray Sh-A. Antiepileptic activity of the whole plant extract of *Melissa officinalis* in Swiss albino mice. Int J Pharm Sci Res. 2012; 3:886-889.
21. Birdane YO, Buyokokuroglu ME, Birdane FM, Cemek M, Yavuz H. Anti-inflammatory and Antinociceptive effects of *Melissa officinalis* L. in rodents. Rev Med Vet. 2007; 158:75-81.
22. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J Agric Food Chem. 2004; 52:2485-2489.
23. Canadanovic-Brunet J, Cetkovic G, Djilas S, Tumbas V, Bogdanovic G, Mandic A *et al*. Radical scavenging, antibacterial, and antiproliferative activities of *Melissa officinalis* L. extracts. J Med Food. 2008; 11:133-143.
24. Encalada MA, Hoyos KM, Rehecho S, Berasategi I, de Ciriano MG, Ansorena D *et al*. Antiproliferative effect of *Melissa officinalis* on human colon cancer cell line. Plant Foods Hum Nutr. 2011; 66:328-334.
25. Koksal E, Bursal E, Dikici E, Tozoglu F, Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. J Med Plants Res. 2011; 5:217-222.
26. Luno V, Gil L, Olaciregui M, Jerez RA, de Blas I, Hozbor F. Antioxidant effect of lemon balm (*Melissa officinalis*) and mate tea (*Ilex paraguensys*) on quality, lipid peroxidation and DNA oxidation of cryopreserved boar epididymal spermatozoa. Andrologia. 2014; 47:1004-1011.
27. Popova AT, Mihaylova DS, Alexieva IN. Comparative study on the antioxidant activity of selected culinary plants growing in Bulgaria. Int J Curr Microbiol App Sci. 2014; 3:436-444.
28. Emamghoreishi M, Talebianpour M. Antidepressant effect of *Melissa officinalis* in the forced swimming test. DARU J Pharm Sci. 2009; 17:42-47.
29. Hassanzadeh G, Pasbakhsh P, Akbari M, Shokri S, Ghahremani M, Amin G *et al*. Neuroprotective properties of *Melissa officinalis* L. extract against ecstasy-induced neurotoxicity. Cell J. 2011; 13:25-30.
30. Weidner C, Wowro SJ, Freiwald A, Kodelja V, Abdel-Aziz H, Kelber O *et al*. Lemon balm extract causes potent antihyperglycemic and antihyperlipidemic effects in insulin-resistant obese mice. Mol Nutr Food Res. 2014; 58:903-907.
31. Van Del Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr A. 1963; 11:463-471.
32. Adams RP. Identification of essential oil components by gas chromatography/quadruple mass spectroscopy. Carol Stream (IL): Allured Publishing Corporation, 2001.
33. Massada Y. In analysis of essential oil by gas chromatography and spectroscopy. Wiley, New York, 1976.

34. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Food Drug Analysis. 2002; 10:178-182.
35. Imai K, Takagi Y, Iwazaki A, Nakanishi K. Radical scavenging ability of glycyrrhizin. Free radicals and antioxidants. 2013; 3:40-42.
36. Yen GC, Duh PD. Scavenging effect on methanolic extracts of peanut hulls on free radical and active oxygen species. J Agric Food Chem. 1994; 42:629-632.
37. Markham KR and Chari VN. Carbon-13 NMR spectroscopy of flavonoids In: Harboren JB, Mabry T, Editors. The Flavonoids: advances in research. London, Chapman and Hall Ltd, 1982.
38. Ewais EA, Abd El-Maboud MM, Elhaw MH, Haggag MI. Phytochemical studies on *Lycium schweinfurthii var. schweinfurthii* (Solanaceae) and Isolation of five Flavonoids from leaves. Journal of Medicinal Plants Studies. 2016; 4:288-300.