OKU Fen Bilimleri Enstitüsü Dergisi
8(2): 490-504, 2025

OKU Journal of The Institute of Science and Technology, 8(2): 490-504, 2025

Osmaniye Korkut Ata Üniversitesi
Fen Bilimleri Enstitüsü
Dergisi

Osmaniye Korkut Ata University
Journal of The Institute of Science
and Technology

Melampyrum arvense L. var. *arvense* L.'nin Uçucu Yağ Bileşimi, Antioksidan Aktivitesi ve Fenolik İçeriği

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Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 06.06.2024
Kabul tarihi: 07.10.2024
Online Yayınlanma: 12.03.2025

Anahtar Kelimeler:
M. arvense var. arvense
Uçucu yağ
GS-MS
LC-MS/MS
Fenolik
Antioksidan

ÖZ

Bu çalışmada, *Melampyrum arvense* L. var. *arvense* L.'nin uçucu yağ bileşimi, antioksidan aktivitesi ve fenolik içeriği araştırılmıştır. *M. arvense* var. *arvense*'nin toprak üstü kısmı hidrodistile edilmiş ve %0,9 (v/w) açık sarımsı yağ verimi elde edilmiştir. Uçucu yağ bileşimi GC-MS ile analiz edilmiştir. Antioksidan aktiviteyi belirlemek için *M. arvense* var. *arvense*'nin tüm kısımlarından metanolik ekstraktlar hazırlanıp ve fenolik içerik LC-MS/MS ile tespit edilmiştir. GC/MS analizine göre, *M. arvense* var. *arvense* yağında 70 bileşik tanımlanmıştır. *M. arvense* var. *arvense*'deki başlıca bileşikler *n*-hekzadekanoik asit-palmitik (%17,73), fenol, 2,4-bis (1,1-dimetiletil) (%15,56) ve oktadekanoik asit-stearik asit (%9,96)'dir. Toplam fenolik ve flavonoid içeriği, DPPH• ve ABTS•+ süpürme aktiviteleri sırasıyla 33.5±2.6 mg GAE/g dw,, 76.5±1.1 mg QE/g dw, 21.5±2.7 mg Trolox/g dw ve 30.7±0.45 mg Trolox/g dw olarak tespit edilmiştir. *M. arvense* var. *arvense* ekstraktında, 23 fenolik bileşenden, kafeik asit, vanillin, 4-OH-benzoik asit, salisilik asit ve rutin tespit edilmiş olup, en yüksek oranda 4-OH-benzoik asit bulunmuştur. Bu çalışma sonucunda, *M. arvense* var. *arvense* uçucu yağında daha önce literatürde rapor edilmemiş olan yüksek seviyelerde yağ asitleri tespit edilmiştir.

Essential Oil Composition, Antioxidant Activity, and Phenolic Content of *Melampyrum Arvense* L. Var. *Arvense* L.

Research Article

Article History:
Received: 06.06.2024
Accepted: 07.10.2024
Published online: 12.03.2025

Key words:
M. arvense var. arvense
Essential oil
GC-MS

ABSTRACT

In this study, the essential oil composition, antioxidant activity, and phenolic content of *Melampyrum arvense* L.var. *arvense* L. were investigated. The aerial part of *M. arvense* was hydrodistilled, and a light yellowish oil yield of 0.9% (v/w) was obtained. Essential oil composition analyzed with GC-MS. Methanolic extracts were prepared from whole parts of *M. arvense* var. *arvense* to determine the antioxidant activity and phenolic content was detected with LC-MS/MS. According to GC/MS analysis, 70 compounds were identified in

LC-MS/MS
Phenolic
Antioxidant

the oil of *M. arvense* var. *arvense.* The major compounds in *M. arvense* var. *arvense* were *n*-hexadecanoic acid-palmitic (17.73%), phenol, 2,4-bis (1,1-dimethylethyl) (15.56%), and octadecanoic acid-stearic acid (9.96%). Total phenolic and flavonoid content, DPPH•, and ABTS•$^{+}$ scavenging activities were 33.5±2.6 mg GAE/g dw, 76.5±1.1 mg QE/g dw, 21.5±2.7 mg Trolox/g dw, and 30.7±0.45 mg Trolox/g dw, respectively. In the extract of *M. arvense* var. *arvense,* 23 phenolic compounds were identified, including caffeic acid, vanillin, 4-hydroxybenzoic acid, salicylic acid, and rutin, with the highest amount found in 4-hydroxybenzoic acid. As a result of this study, high levels of fatty acids were detected in *M. arvense* var. *arvense* essential oil, which has not been reported in the previous literature.

To Cite: Demirpolat A., Çobanoğlu DN., Düzdaban P., Çobanoğlu D., Zafar M., Ahmad M., Kılıç Ö. *Melampyrum arvense* L. var. *arvense* L.'nin Uçucu Yağ Bileşimi, Antioksidan Aktivitesi ve Fenolik İçeriği. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2025; 8(2): 490-504.

Introduction

Melampyrum genus formerly belonged to Scrophulariaceae and is now placed in the Orobanchaceae family consisting of semi-parasitic plants (Kırmızıbekmez et al., 2009). The family, formerly known as Scrophulariaceae, is not monophyletic, according to recent molecular phylogenetic analyses (Tank et al., 2006). Orobanchaceae is considered the biggest parasitic family in Angiosperms and includes organisms with a variety of parasitic lifestyles, including autotroph, hemiparasitism, and holoparasitism (Zhang et al., 2022). The *Melampyrum* genus is a family of semi-parasitic annual plants. In the Flora of Turkey, it is represented by the two species *M. arvense* and *M. pratense* (Karadağ and Tosun, 2019). There have been relatively few investigations on the chemical and pharmacological properties of the *Melampyrum* genus, in contrast to its widespread use in traditional medicine as a cardiovascular and anti-inflammatory agents, neurological diseases (Korkotian et al., 2015), anticancer (Mihoğlugil et al., 2023), rheumatic disorders, skin infection, and sedatives (Galishevskaya and Petrichenko, 2010). Due to their unique properties, these herbs are frequently used in traditional medicine as sedatives and anticonvulsants. The seeds of *Melampyrum* can be consumed by ants and small mammals, and pollinators can feedon the nectar from the blooms (Dalrymple, 2007). Natural diets rich in phenolics with antioxidant activity have sparked interest in nutrition and food science in recent decades (Lee et al., 2015). Plant secondary metabolites with an aromatic ring and at least one hydroxyl group are known as natural phenolic compounds (Tungmunnithum et al., 2018). Because their hydroxyl groups can directly contribute to antioxidant action, phenolic compounds are good electron donors (Bendary et al., 2013). Furthermore, some of them stimulate the cellular synthesis of endogenous antioxidant molecules (Côté et al., 2010). Numerous publications in the literature claim that phenolic compounds reduce the burden of oxidative disease by inhibiting free radicals, breaking down peroxides, inactivating metals, and scavenging oxygen in biological systems (Babbar et al., 2015). *Melampyrum* contains a variety of biologically active substances, including alkaloids, flavonoids, and iridoids (Korkotian et al., 2015). The flavonoids include luteolin, apigenin, quercetin, and phenol carboxylic acid derivatives (Galishevskaya and Petrichenko, 2010). *Melampyrum* extracts contain protein kinase C inhibitory, antimicrobial, antimalarial, cytotoxic, and

antiprotozoal effects, according to earlier *in vitro* investigations, and they are commonly used as animal feed (Štajner et al., 2009; Kirmizibekmez et al., 2011; Karadağ and Tosun, 2019). In contrast to the numerous articles about this genus (Háznagy-Radnai et al., 2014), little information is available on the *M. arvense* var. *arvense.*

In current study, it was aimed to evalute of essential oil compositions of *M. arvense* var. *arvense* with GC-MS. Total phenolic, flavonoid contents and antioxidant activity were detected and phenolic compounds were determined with LC-MS/MS.

Material and Methods

Plant materials

M. arvense var. *arvense* was collected from Bingol, steppe and rocky areas, in June 2019, 1450-1500 m., by A.D. with 3467 collected number. Plant samples were identified while comparing with the Flora of Turkey and East Aegean Islands (Davis, 1967). Voucher specimens were deposited in the Bingol University.

Gas chromatography/mass spectrometry (GC-MS) analysis

5 g of each plant sample were homogenized in 10 ml of hexane/isopropanol at 10.000 rpm for 30 seconds and centrifuged at 5000 rpm for 10 min. The upper part was taken and put into the test tubes by filtration. Fatty acids need to be derivatized in order to analysing by GC. Methyl esters are often preferred for derivazation. 5 ml of 2% methanolic sulfuric acid was added and vortexed. This sample was kept at 50 °C for 15 hours of methylation. After 15 hours, the tubes were removed, cooled to room temperature, and vortexed with the addition of 5 ml of 5% NaCl. The fatty acid methyl esters that had developed in the tubes were extracted with 5 ml of hexane. The hexane phase was then treated with 5 ml of 2% $KHCO_3$, and the phases were allowed to separate for one to two hours. Following the 45 °C under nitrogen evaporation of the mixture containing the methyl esters, the fatty acids below the test tubes were dissolved in 1 ml of hexane and then subjected to GC-MS analysis using amber GC vials. The essential oil compounds were identified using the Wiley and NIST mass spectral libraries, and the identified compounds of the essential oils are listed in Table 2. An Agilent brand 7890A/5970 C GC-MS instrument and a SGE Analytical BP× 90 100 m × 0.25 mm × 0.25 um column were used for fatty acid analysis. The temperature program was gradually heated from 120°C to 250°C, and the total time was set to 45 minutes. The temperature program was like this: 120°C is heated up to 250°C at 5°C/min and was expected to remain at this temperature for 19 minutes. The total time was 45 minutes. The auto samplers washed in hexane five times before shrinking and after giving the collar. Injection volume was 1 uL, split ratio was 10:1, solvent delay time was 12 minutes, carrier gas was He, and H_2 flow was 35 ml/min, flow rate was 350 ml/min, N_2 was 20.227 ml/min is automatically set by the

program. The identified fatty acid compounds ofthe studied taxa are listed in Table 2 (Demirpolat, 2022; Akman et al., 2023).

Plant material extraction for antioxidant activity and phenolic content

The whole part of *M. arvense* var. *arvense* were ground with a grinder whenever samples were dried totally. The ground plant material was then weighed at 2.5 g and dissolved in 45 ml of a 90:10 methanol water (Johnson et al., 2020). The solution was kept in an ultrasonic bath at 40 °C for 1 hour. After that, centrifugation was carried out at 3500 rpm for 15 min. It was filtered via a whatman no:1 filter paper, after centrifugation. Until the experiments were conducted, the solution was kept at -20°C.

Total phenolic content analysis

The Folin-Ciocalteu method was modified to find out the total phenolic content of the plant extract as being equivalent to gallic acid (GAE) (Slinkard and Singleton, 1977). For the gallic acid calibration curve, GA standard solutions were prepared at concentrations of 500, 250, 125, 62.5, 31.25, 15.625, and 7.812 μg/ml. 0.2 N Folin-Ciocalteau (V/V) and 7.5% Na_2CO_3 (W/V) solutions were prepared. The prepared solutions were pipetted as 50 μL sample/standard, 250 μL folin, 750 μL Na_2CO_3 and 2.5 ml distilled water and kept in the dark at room temperature for two hours. Each pipetting was done in 3 repetitions. Using a spectrophotometer with a wavelength of 760 nm, absorbances were measured. The result were expressed as mg GAE/g dry weight (dw).

Total flavonoid content analysis

The total flavonoid content of the plant extract was calculated as quercetin (QE) equivalent by modifying the study of Barros et al. (2007). For the quercetin calibration curve, QE standard solutions were prepared at concentrations of 400, 350, 300, 250, 200, 150, 100, and 50 μg/ml. 5% $NaNO_2$ was prepared by dissolving in pure water, 10% $AlCl_3$ in MeOH, and 1 M NaOH in pure water. The prepared solutions were pipetted into the reaction tube with 100 μL sample/standard, 640 μL distilled water, 30 μL $NaNO_2$, 30 μL $AlCl_3$, 200 μL NaOH, and kept in the dark at room temperature for 40 minutes. Each pipetting procedure was repeated three times. Absorbances with a wavelength of 515 nm were recorded at the end of the experimental work. The results were calculated as mg QE/g dw from the QE standard curve.

Antioxidant activity studies

DPPH• assay

The antioxidant activity of the plant extract was evaluated using the trolox standard (Brand-Williams et al., 1995). For the calibration curve, trolox solutions were prepared at concentrations of 280, 240, 200, 160, 120, 80, and 40 μM. DPPH solution was prepared with ethanol. 100 μL sample/trolox, and

900 µL DPPH free radical were added and vortexed. Each pipetting process was repeated three times. The prepared tubes were kept in the dark at room temperature for 50 minutes. At the end of the time, absorbances at the 517 nm wavelength were recorded. The DPPH free radical scavenging capacity value was calculated in mg TE/g dw.

ABTS•⁺ assay

The standard Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was prepared with methanol (Re et al., 1999). For the trolox calibration curve, trolox solutions were prepared at concentrations of 280, 240, 200, 160, 120–80 and 40 µM. 7 mM ABTS and 2.54 mM $K_2S_2O_8$ (potassium persulfate) were dissolved in distilled water and kept in the dark at room temperature for at least 12 hours. The prepared ABTS stock solution was adjusted in the absorbance range of 0.75–0.80. To prepare the reaction tube, 100 µL sample/trolox, 900 µL ABTS radical were added and vortexed, and then each pipetting process was repeated three times. The prepared tubes were kept in the dark at room temperature for 30 minutes. At the end of the time, absorbances at the 734 nm wavelength were recorded. The total antioxidant capacity value in mg TE/g dw was obtained using the ABTS calibration graph.

Phenolic compound detection

M. arvense var. *arvense* extract phenolic compounds were determined by liquid chromatography-tandem mass spectrometry (LC/MS/MS, Thermo Scientific/TSQ Quantum Access Max). A C18 column (ODS Hypersil, 4.6 ID × 250 mm 5 m) was used to separate phenolic compounds at 30 °C with a flow rate of 0.7 ml min^{-1}. The injection volume was 20 µL, and the analysis was performed over 20 minutes. As mobile phases, A (water containing 0.1% formic acid) and B (methanol) were employed. Starting with 100% A, the mobile phase mixture was run through the column for one minute. The gradient elution was then changed to be 5% A-95% B between minutes 1 and 22, and after minute 22, the 5% A-95% B phase was continued for an additional three minutes. The system was then set up to run from 0% A to 100% B for 25 to 30 minutes, at which point it was turned off. The capillary temperature was set at 300 °C, the vaporizer temperature at 350 °C, and the positive and negative spray voltages were set at 4000 V and 2500 V, respectively (Çobanoğlu et al., 2023). Table 1 contains the parameters for quantifying phenolic chemicals.

Table 1. Phenolic compound standard's parameters

Phenolic compounds	Rt (min)	MS [m/z]	MS /MS [m/z]	LOD (mg.l^{-1})	LOQ (mg.l^{-1})	Polarity
Gallic acid	8.92	169.7	80.50 126.20	0.061	0.203	-
Caffeic acid	15.27	179.7	135.20 136.20	0.047	0.157	-
Taxifolin	16.68	303.0	126.20 285.50	0.058	0.194	- -
Protocatechuic acid	12.13	153.8	110.40 92.50	0.049	0.162	-
Protocatechuic aldehyde	13.16	136.9	92.25 108.20	0.026	0.087	-
Sesamol	12.82	137.18	109.291 108.173	0.048	0.161	-
p-coumaric acid	17.00	163.9	94.30 120.20	0.116	0.387	-
Catechin	10.92	289.2	203.90 245.70	0.068	0.227	-
Epicatechin	11.26	291.5	123.30 139.30	0.045	0.151	+
Rosmarinic acid	17.82	359.18	134.30 162.20	0.029	0.095	-
Vanillin	15.87	150.91	92.30 136.10	0.023	0.076	-
Ferulic acid	17.19	193.35	134.10 178.00	0.061	0.204	-
4-OH-benzoic acid	18.12	137.90	66.60 94.60	0.031	0.104	-
Salicylic acid	18.13	137.14	65.51 93.26	0.030	0.099	-
Syringic Acid	15.45	183.07	123.2 77.3	0.192	0.643	-
Ellagic acid	19.47	300.90	284.797 174.151	0.087	0.289	-
Rosmarinic acid	17.82	359.18	134.30 162.20	0.029	0.095	-
Quercetin	20.58	301.00	152.1 179.9	0.038	0.123	-
Oleuropein	18.00	539.10	275.80 377.50	0.050	0.167	-
Rutin	18.26	609.37	300.60 301.70	0.007	0.024	-
Rezveratrol	18.45	228.98	107.20 135.10	0.030	0.099	+
Flavone	23.90	222.90	77.275 121.154	0.027	0.090	+
Kaempferol	21.68	286.97 165.00	153.00	0.055	0.184	+

Table 2. Essential oils chemical composition of *M. arvense* var. *arvense*

No	Component	RI	RT	%
1	Methyl(2E,4E,6S)-9-(butyldimethylsiloxy)-6-ethyl-,4-nonadienoate	968	5.403	1.37
5	5-Propylnonane	985	6.192	0.75
6	Dodecane	988	6.332	2.58
8	Decane, 4-methyl	990	6.436	0.21
9	Nonadecane	994	6.623	0.67
10	Methoxyacetic acid, 3-tetradecyl eter	995	6.654	0.28
12	Tetradecane	1003	7.022	2.36
14	*n*-Dodecane	1009	7.307	0.57
15	*n*-Eicosane	1016	7.608	2.56
17	Octacosane	1033	8.366	1.10
20	Octacosane, 1-iodo-	1041	8.734	2.13
21	Eicosane	1042	8.786	1.59
22	Heneicosane	1047	9.020	2.19
25	Tricosane	1056	9.424	0.21
27	*n*-Hexadecane	1061	9.674	0.11
28	*n*-Tetracosane	1062	9.710	0.74
31	*n*-Octadecane	1066	9.881	0.93
33	Docosane, 11-decyl-	1068	9.954	0.36
34	*n*-Octadecane	1069	9.990	0.46
35	Docosane	1071	10.125	1.09
38	Pentacosane	1087	10.841	0.37
39	Tetratriacontane	1089	10.934	0.33
40	Eicosane, 1-iodo-	1092	11.085	1.06
41	Octadecane, 1-iodo-	1094	11.142	0.48
43	Benzaldehyde, 4-propyl-	1100	11.448	3.69
47	Myristic acid	1129	12.724	0.52
50	3-Ethyl-3-methylheptadecane	1143	13.368	0.31
53	Heptacosane	1152	13.804	0.11
54	Cyclotetradecane	1167	14.483	0.54

Table 2 continued.

No	Component	RI	RT	%
55	3-Heptadecanol	1174	14.810	2.48
56	Docosane	1180	15.075	0.23
57	***n*-Hexadecanoic acid-Palmitic**	**1183**	**15.225**	**17.73**
59	Isopropyl palmitate	1190	15.537	1.89
62	**Phenol, 2,4-bis(1,1-dimethylethyl)**	**1208**	**16.341**	**15.56**
63	Tetracosane	1211	16.491	1.03
64	**Octadecanoic acid- Stearic acid**	**1257**	**18.572**	**9.96**
65	*i*-Propyl 16-methyl-heptadecanoate	1265	18.930	0.85
67	Linolelaidic acid(trans-9,12-omega 6)	1286	19.890	1.35
68	α- Linolelaidic acid-cis- 9,12,15	1316	21.234	1.43
69	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester	1321	21.498	2.94
70	Arachidic acid C 20:0	1350	22.790	0.39

RI: Retention indices; RI: Based on retention index; MS: Based on mass spectra matching; RT: Retention time.

Table 3. Phenolic compounds of *M. arvense* var. *arvense***.**

Phenolic compounds	µg/g dw
Gallic acid	nd*
Caffeic acid	3.45±2.3
Taxifolin	nd
Protocatechuic acid	nd
Protocatechuic aldehyde	nd
Sesamol	nd
p-coumaric acid	nd
Catechin	nd
Rosmarinic acid	nd
Vanillin	11.5±1.4
Ferulic acid	nd
4-OH-benzoic acid	25.3±3.2
Salicylic acid	14.4±1.8
Syringic Asit	nd
Ellagic acid	nd
Rosmarinic acid	nd
Quercetin	nd
Oleuropein	nd
Rutin	9.1±1.1
Rezveratrol	nd
Flavone	nd
Kaempferol	nd

nd: not detected

Results

Essential oil composition

Qualitative and quantitative differences were noted in the GC-MS analysis of *M. arvense* var. *arvense*. The aerial part of *M. arvense* var. *arvense* was hydrodistilled, obtaining yields of 0.9 % (v/w) of light yellowish oil. In the oil of *M. arvense* var. *arvense* 70 components were identified. The major compounds were *n*-hexadecanoic acid-palmitic (17.73%), phenol, 2,4-bis (1,1-dimethylethyl) (15.56 %), and octadecanoic acid- stearic acid (9.96 %) in *M. arvense* var. *arvense.* As a result of this study, high levels of fatty acids were determined in the essential oil of *M. arvense* var. *arvense* which was not previously reported in the literature. There were 25.88% alkanes in the essential oil content. The *M. arvense* var. *arvense* essential oil was weak in monoterpenes and sesquiterpenes.

TPC, TFC, DPPH• and ABTS•$^{+}$ analyses

Total phenolic compounds (TPC) concentration was determined 33.5± 2.6 mg GAE/g dw., while total flavonoid was 76.6±1.1 mg QE/g dw. To compare the antioxidant capabilities of *Melampyrum arvense* var. *arvense* methanol extract, the ABTS•$^{+}$ and DPPH•$^{+}$ tests were carried out. Plant extract had considerable levels of DPPH•$^{+}$and ABTS•$^{+}$ with 21.5±2.7 mg TE/g dw and 30.7±0.45 mg TE/g dw, respectively.

Phenolic Compounds

In current study, twenty three phenolic compounds were investigated in *Melampyrum arvense* var. *arvense* methanol extract (Table 3). Among them, caffeic acid, vanillin, 4-OH- benzoic acid, salicylic acid, and rutin, were detected 3.45±2.3, 11.5±1.4, 25.3±3.2, 14.4±1.8, 9.1±1.1 respectively as µg/100g. Other 18 phenolic compounds weren't detected in *M. arvense* var. *arvense* extract.

Discussion

Essential oil

Specific information on the essential oil composition of *Melampyrum* species is limited, and there is no comprehensive data available. It's important to note that *Melampyrum* is a diverse genus with numerous species, and the essential oil composition can differ significantly among species or variety.

Saturated fatty acids include palmitic acid, also known as *n*-hexadecanoic acid (Eastwood, 2003). In this study, *n*-hexadecanoic acid (17.73%) was recordedthe highest essential oil among the all the components. Due to the harmful effects of synthetic compounds used for chemical protection, the use of substances obtained from plant sources and showing antimicrobial activity is increasing. It is well known that several fatty acids have antibacterial and antifungal effects (Agoramoorthy et al., 2007). By directly interacting with T cells, fatty acids can influence immunological responses (Leventhal et

al., 1993). Linoleic acid has an anti-inflammatory impact by reducing the generation of inflammatory mediators (Yu et al., 2002).

Another important compound identified in our research was phenol, 2, 4-bis (1, 1-dimethylethyl) (15.56%), a naturally occurring chemical, used for numerous reasons in food, medicine, and agriculture. It contains anti-inflammatory, antifungal, antibacterial, and anti-trimethyltin (TMT)-induced cognitive impairment properties in terms of medicinal properties (Choi and Lee, 2009; Kadoma et al., 2009; Malek et al., 2009; Zhou et al., 2011; Abdullah et al., 2011; Rangel-Sánchez et al., 2014; Kim et al., 2017). It has been suggested to use it in food to stop *Aspergillus niger, Fusarium oxysporum*, and *Penicillium chrysogenum* from growing on wheat grainsand fresh apple juice from browning (Suh et al., 2011; Varsha et al., 2015). In agriculture, phenol, 2, 4-bis (1, 1-dimethylethyl) was found to have allelopathic effects on the germination and seedling growth of weedy plants under soilless conditions and it has also been reported as the defense compound of avocado root which prevents the root rot caused by *Phytophthora cinnamomi* (Zhang et al., 2011; Rangel-Sánchez et al., 2014).

In the present study, it has been found that, phenol, 2,4-bis (1,1-dimethylethyl), the second major compound obtained from *M. arvense* var. *arvense*. Phenol, 2,4-bis (1,1-dimethylethyl) was found to have antioxidant effects (María Teresa et al., 2014). Phenol-2, 4-bis (1, 1-dimethylethyl) is a chemical intermediate used to synthesize other chemical intermediates and is a precursor to many complex chemicals. It is also frequently used as an antioxidant, a light or UV stabilizer, and as an antioxidant. Phenol-2,4-bis (1,1-dimethylethyl) is a naturally occurring antimicrobial molecule that can be obtained from plant, animal, and microbial metabolites (Rangel-Sánchez et al., 2014; Pawar et al., 2016; Gao et al., 2018; Karthick and Mohanraju, 2018). Some polyphenolcarboxilic acids of the iridoide, mucilagy, saponins, carotenoids, and phytosterols were found in *Melampyrum bihariense* Kern. and *Melampyrum cristatum* L. (Munteanu, 2006).

Antioxidant Activity and Total Phenolic/Flavonoid Content

It is generally known that plants have bioactive compounds with antioxidant properties. Antioxidants are essential, because they scavenge free radicals that can damage cells and tissues (Gopinathan and Balasubramanian, 2021). Although there has been no detailed study on the antioxidant activity of *M. arvense*, previous studies on the antioxidant activities of other *Melampyrum* species showed similar results to those in the current study (Karadağ and Tosun, 2019).

Stajner et al. (2009) investigated the antioxidant and free radical scavenging capacities of the red and yellow forms of *Melampyrum barbatum* L. They discovered that, the red form of *Melampyrum barbatum* flowers had the best antioxidant capacity. According to a report, *Melampyrum arvense* contains phenolic compounds and has antibacterial, and antioxidant properties (Karadağ and Tosun,

2019). Háznagy-Radnai et al. (2014) determined that, the methanolic extract of *M. bihariense* showed excellent DPPH radical scavenging and free radical scavenging activities.

M. arvense var. *arvense* methanol extracts had considerable levels of antioxidant activity in the current study. Mihoğlugil et al. (2023) investigated the cytotoxicity of various Turkish plants against renal cancer cells. *Melampyrum arvense* was found to inhibit the growth of renal cancer cell lines as contains phenolic compounds. Although this study did not specifically focus on the antioxidant activity of *Melampyrum,* it provides additional evidence of the plant's potential antioxidant properties.

In conclusion, *M. arvense* var. *arvense* contains phenolic and flavonoid compounds, which have antioxidant activity. The total phenolic and flavonoid contents of *Melampyrum* species vary depending on the species and the region where they grow. The antioxidant activity of *Melampyrum* species can be attributed to the presence of phenolic and flavonoid compounds.

Phenolic compounds

One of the main goals of modern pharmaceutical research is to find new drugs by screening new biologically active substances of natural origin that have been widely used for centuries in traditional medicine. The use of traditional or less well-known medicinal plants to create medications with a variety of active components and therapeutic properties is particularly interesting (Korkotian et al., 2015). A wide variety of biologically active compounds, such as flavonoids, iridoids, and alkaloids, are present *M. pratense* (Vogl et al., 2013).

Galishevskaya and Petrichenko (2010) investigated the phenolic compounds of two *Melampyrum* species, *Melampyrum pretense,* and *M. nemorosum*, growing in the Ural region. The extracts contain 23 compounds including 17 flavonoids and 6 phenolcarboxylic acids. The *Melampyrum* herb contains 9 flavonoids and 5 phenolcarboxylic acids. The study also found the presence of cinaroside, luteolin, quercetin, hyperoside, and chlorogenic, caffeic, and ferulic acids in both species. In the current study, we didn'tdetect quercetin, caffeic acid. The methanol extract of *M. arvense* var. *arvense* showed the highest ratio of 4-OH benzoic acid (25.3±3.2 µg/g). Háznagy-Radnai et al. (2014) determined apigenin, and luteolin flavones from the methanolic extract of *Melampyrum bihariense*, as we didn't identify in *Melampyrum arvense* var. *arvense* methanol extract.

Conclusion

The essential oil composition, antioxidant activity, and phenolic content of *Melampyrum arvense* var. *arvense* may vary depending on the specific species or variety within the genus. However, it's worth noting that information on the essential oil composition and phytochemical profile of *Melampyrum* species is limited, and more research is needed to provide comprehensive data. As a result of this study, high levels of fatty acids were detected in *M. arvense* var. *arvense* oil, which has not been reported in the previous literature. Regarding antioxidant activity and phenolic content, *M. arvense*

var. *arvense* plant has shown potential as a source of natural antioxidants due to their phenolic compounds. Phenolic compounds are known for their antioxidant properties, which can help protect cells from oxidative damage. However, the specific antioxidant activity and phenolic content of *Melampyrum* species would require further scientific investigation to provide accurate and detailed information.

Statement of Conflict of Interest

The authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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