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Characterization of essential oil from *Matricaria sevanensis* by microwave-assisted distillation

Özge Süfer[1] · Fuat Bozok[2]

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Abstract
Matricaria sevanensis is a widespread plant species grown in Central Anatolian and helps to prevent sleep disorders, influenza and stomach upsets when consumed in tea form. In this study, the essential oil from aerial parts of *M. sevanensis* collected from Osmaniye Province of Turkey was obtained by solvent-free microwave-assisted distillation at 340 W in 30 min and its chemical compounds, some thermal characteristics, functional groups and antioxidant activity were investigated by gas chromatography/mass spectrometry (GC/MS), differential scanning calorimetry (DSC), Fourier-transform infrared (FTIR) spectroscopy and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, respectively. Also, temperature values were recorded in every 5 min of distillation process in order to visualize a profile depending on time. Oil yield was nearly 0.04% (v/w), and eighteen components were totally identified by GC/MS. Bisabolene oxide A (50.32%), (Z)-β-farnesene (24.20%), α-bisabolol oxide A (8.12%) and α-farnesene (4.06%) were found as major compounds. Three peaks were observed in DSC graph, and onset temperatures (T_o), peak temperatures (T_p) and enthalpy (ΔH) values were specified. FTIR spectra showed that several functional groups such as alcohols, alkanes, aromatics, amines and ethers were present in essential oil. Antioxidant activity (IC_{50}) of *M. sevanensis* essential oil was also computed as 156.005 μg mL^{-1} ($p < 0.05$).

Keywords *Matricaria sevanensis* · Essential oil · GC/MS · Thermal analysis · Antioxidant activity

Introduction

Genus *Matricaria* which belongs to the family Asteraceae has 22 taxa in the world, and also, 5 taxa (*M. aurea*, *M. chamomilla* var. *chamomilla*, *M. chamomilla* var. *pappulosa*, *M. recutita* and *M. sevanensis*) are grown in Turkey [1–3]. This genus is very similar to the genus *Tripleurospermum* morphologically [4]. These genera are widespread in Asia, Africa, North America and Europe [5]. Related plants consumed as chamomile tea and used in alternative medicine for people suffering from sleep disorders [6].

Essential or ethereal oils which are comprised of volatiles from plant materials are used as alternatives for synthetic compounds in various industries like cosmetics and food [7]. The characterization of essential oil from both the genera *Matricaria* and *Tripleurospermum* by gas chromatography/mass spectrometry (GC/MS) has been reported in the literature by several researchers [8–12]. Many of them were focused on the essential oil components of *Matricaria* and *Tripleurospermum* and reported major compounds at the rates of 31.20 and 61.30% as chamazulene in *M. recutita* from the south west and central of Iran [13, 14], α-bisabolol oxide B (51.42%) in *M. chamomilla* from Ankober, district of Kundi [15], α-bisabolol oxide A (21.50%) in *M. chamomilla* from Neyshabur, Iran [6], 18.80% *p*-methoxy-β-cyclopropylstyrene and 22.46% β-farnesene in *Tripleurospermum disciforme* from Fars Province and Alvand Mountain in Hamedan of Iran [8, 16] and 18.20% (Z)-β-farnesene in *T. corymbosum* from Erzurum in Turkey [17].

Microwaves supply more effective and rapid heating, and hence, equipment dimensions, thermal gradient and operation units are easily diminished [18]. Drying [19, 20],

✉ Özge Süfer
ozgesufer@osmaniye.edu.tr

[1] Department of Food Engineering, Faculty of Engineering, Osmaniye Korkut Ata University, Osmaniye, Turkey

[2] Department of Biology, Faculty of Arts and Science, Osmaniye Korkut Ata University, Osmaniye, Turkey

evaporation [21], packaging [22] and so on are the current application areas of microwaves. Unit operations like extraction and distillation could be completed within minutes instead of long times with the aid of microwaves, and greater purity, high reproducibility and preserving quality indices are some advantages of this valuable technique. Because of the harmful effects of petroleum-based solvents in the environment, solvent-free procedures have been recently combined with microwave-assisted extraction and considerably the successful results are observed [23].

Besides the identification of aromatic compounds by GC/MS, thermal specification of essential oils is also important for determining heat capacities [24], melting properties and behavior [25], boiling temperatures [26] and so on. Differential scanning calorimetry (DSC) enables to evaluate the related features confidently, and thus, it is accepted as a useful thermodynamical approach for oils and fats [26]. Moreover, chemical groups which are available in materials are easily detected by Fourier-transform infrared (FTIR) spectroscopy and this technique can be combined with thermogravimetric procedures and DSC for performing effective thermochemical conversions in several biomasses [27].

Free radicals and reactive oxygen species can be produced in human body and DNA, and protein and lipid molecules can be affected by oxidative damages [28]. Decreasing antioxidant level may cause some important diseases in individuals such as cancer, atherosclerosis, inflammation and so on [29]. Plants are the important source of bioactive substances, and they are able to be evaluated as natural antioxidative molecules [30]. The antioxidative capacity of essential oils obtained from different plants in the family Asteraceae such as *M. chamomilla* [8, 10], *M. recutita* [14], *T. insularum* [31] and *T. disciforme* [32] are found in the literature.

In previous studies, essential oil components, anticholinesterase, anti-inflammatory, antioxidant, antichemotactic antistreptococcal, antibacterial and antiseptic activities of different species belonging to the genera *Matricaria* and *Tripleurospermum* were specified using different techniques [6, 8, 10, 11, 13–15, 32]. However, there is no study available in the literature focused on essential oil compounds, calorimetric profile and antioxidant activity of *M. sevanensis* named as formerly *T. sevanense*. Therefore, present report attempts to analyze (1) volatile substances, (2) thermal properties and (3) antioxidant potential (by DPPH method) of *M. sevanensis* essential oil.

Materials and methods

Plant material

Aerial parts of *Matricaria sevanensis* (about 3 kg) were collected from Osmaniye Province (Fakıuşağı region, 37°02′26″ N, 36°13′50″ E, 150 m elev., voucher no FBozok00417) of Turkey in April, 2018. They were authenticated by the second author and deposited at the Department of Biology in Osmaniye Korkut Ata University. Fresh samples were brought to the laboratory and powdered by a blender (Loyka LKD 100, Turkey).

Reagents and chemicals

All the chemicals used were of analytical grade, and they are demonstrated in Table 1 with their brands and purity values.

Essential oil isolation by solvent-free microwave-assisted distillation

Essential oil of *M. sevanensis* was obtained by using a Clevenger apparatus containing a microwave oven (Arçelik, MD 574, Turkey) for 30 min at 340 W. The distillation experiments were repeated ten times. A schematic illustration of microwave Clevenger system is demonstrated in Fig. 1. The oil was stored in a sealed vial in freezer (at − 18 °C) until further analysis.

Gas chromatography/mass spectrometry analysis

GC/MS analysis of the oil (diluted with n-hexane) was carried out on an Agilent 7000 Series Triple Quad gas chromatograph equipped with a capillary column (HP-5MS, 30-m length × 0.25-mm diameter × 0.25-μm film thickness), an inner surface containing 5% phenyl methylpolysiloxane and a flame ionization detector (Thermo Scientific Focus). Initial temperature was set to 50 °C and increased to 240 °C at a rate of 3 °C min^{-1}. Carrier gas was helium, and the flow rate was 1 mL min^{-1}. Mass spectra were obtained at 70 eV.

The determination of essential oil compounds was conducted by their retention indices (RI) using C_8–C_{20} n-alkanes (Sigma, St. Louis, MO, USA), retention times (RT) and a comparison with current mass spectra (Wiley and NIST libraries, Kovats retention index), and they were validated by comparing calculated indices with reported ones in previous studies. In order to quantificate volatiles, percentages of individual areas were calculated relatively to total area. Furthermore, chromatographic profile of *M. sevanensis* essential oil is shown in Fig. 2.

Table 1 Reagents and chemicals used in this study

Name	Brand	Purity/%
1,1-Diphenyl-2-picrylhydrazyl (DPPH)	Sigma-Aldrich (Germany)	95.0
6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox)	Sigma-Aldrich (Germany)	97.0
Methanol	Merck (Germany)	≥ 99.9
n-Hexane	Merck (Germany)	≥ 98.5

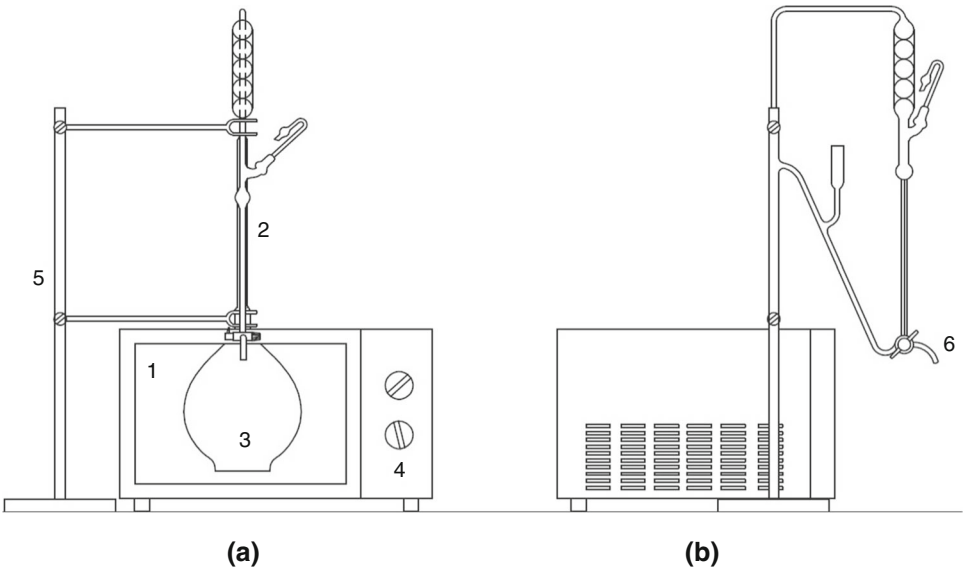

Fig. 1 Technical drawing of microwave-assisted Clevenger system. (A) Front view, (B) side view. 1) Microwave oven, 2) Clevenger apparatus, 3) sample bowl (1 L), 4) control panel, 5) monopod, 6) tap (for product collection)

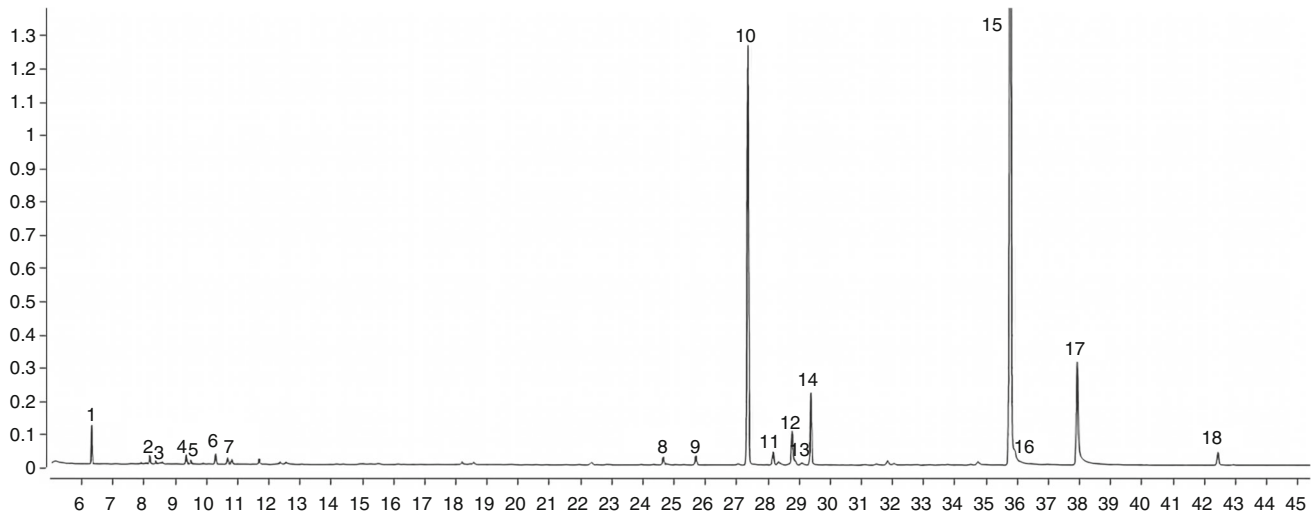

Fig. 2 Chromatographic profile of *M. sevanensis* essential oil (1:α-pinene, 2:β-myrcene, 3:octanal, 4:o-cymene, 5:L-limonene, 6:(E)-β-ocimene, 7:γ-terpinene, 8:β-elemene, 9:caryophyllene, 10:(Z)-β-farnesene, 11:α-selinene, 12:bicyclogermacren, 13:β-curcumene, 14:α-farnesene, 15:bisabolene oxide A, 16:chamazulene, 17:α-bisabolol oxide A, 18:(E)-ene-yne-dicycloether)

Temperature measurement

The temperature evolutions of both plant material and glass bowl were monitored during microwave-assisted distillation. They were measured at 5-min time intervals from a distance of nearly 10 cm by a non-contact infrared thermometer (Sinometer, BM380, China) which has the operating capability at a range of − 32 °C and 550 °C.

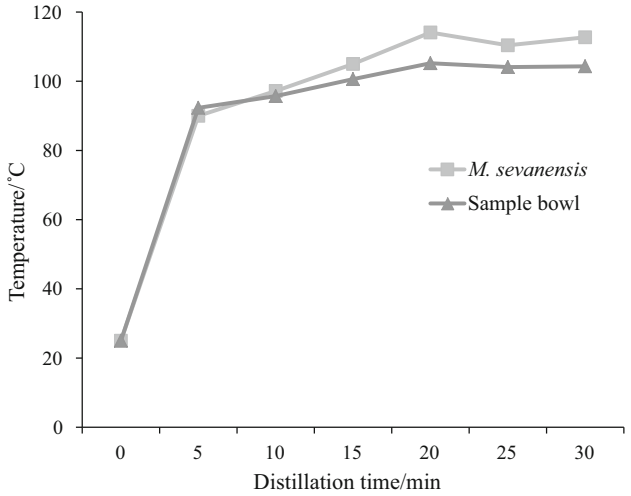

Fig. 3 Changes in temperatures of both sample and bowl versus time

Thermal analysis by DSC

DSC was performed on a Mettler Toledo DSC 3 equipment (Switzerland) under a 40 mL min^{-1} flow rate of nitrogen. A 40-μL standard aluminum sample holder was used in order to prevent the evaporation of essential oil. The initial and final temperatures were 30 °C and 350 °C, respectively, and the increment rate was 2 °C per minute (modified from Chen et al. [33]). The sample mass was 5.7 mg, and the data were recorded in every one second. STAR software (version 16.0) was used for drawing heat flow versus temperature curve.

Functional groups test by FTIR spectroscopy

The FTIR spectrum of essential oil was obtained at a wave number range of 600–4000 cm^{-1} using a FTIR spectrometer (PerkinElmer spectrum 65, USA) with an universal ATR sampling accessory. Before the experiments, essential oil was waited at a room temperature until thermal equilibrium was reached. Then, a drop of sample was applied on a cell and 16 scans were carried out for each spectrum.

Antioxidant activity test (DPPH assay)

In order to evaluate free radical scavenging activity of *M. sevanensis* essential oil, some modifications were conducted in traditional DPPH method reported by Zhang et al. [34]. First, 0.025 g 1,1-diphenyl-2-picrylhydrazyl (DPPH) was dissolved in 1000 mL of 100% (v/v) methanol. The methanolic dilutions of oil at different concentrations were prepared, and 0.1 mL sample was reacted with 2 mL of 0.25% (w/v) DPPH solution. The absorbances were read at 517 nm by using an UV–Vis spectrophotometer (Shimadzu, UV 1800, Japan) after keeping in dark for 30 min at room temperature (25 °C). Inhibition of DPPH (%) was calculated by following formula;

$$\text{Inhibition}\,\% = \frac{A_{\text{dpph}} - A_{\text{sample}}}{A_{\text{dpph}}} \times 100$$

where A_{dpph} is the absorbance of DPPH solution (as blank, nm) and A_{sample} is the absorbance of oil sample (nm). A linear curve (oil concentration vs. inhibition, %) was prepared, and IC_{50} value was calculated from the equation of that curve. The IC_{50} value is known as the required amount for an antioxidant to sweep 50% of free radical in a radical–antioxidant mixture. Also, trolox (a popular synthetic antioxidant material) was used as positive control. The analysis was carried out in duplicate, and the results were given as the mean of measurements.

Statistical analysis

Both means and standard deviations of experimental findings were calculated using Microsoft Office 2010, Excel program. The data of antioxidant activity by DPPH method were analyzed using Student's *t* test at a significance level of 0.05. SPSS version 18 (IBM, USA) was the statistical computer software.

Results and discussion

Composition of essential oil

Average yield of the oil was calculated as 0.04% (w/v). In total, eighteen components in essential oil of *M. sevanensis* were determined and the oil appeared to be rich in bisabolene oxide A (50.32%), (Z)-β-farnesene (24.20%), α-bisabolol oxide A (8.12%) and α-farnesene (4.06%) (Table 2). Comparison of essential oil compounds among *M. sevanensis* with different *Matricaria* species is shown in Table 3.

Many studies about the essential oil of different *Matricaria* species are available. According to Shams-Ardakani et al. [8], the main constituents of *M. chamomilla* were α-bisabolol oxide A (25.01%), α-bisabolol oxide B (9.43%), spathulenol (8.49%) and cis-en-yn-dicycloether (7.42%). Raal et al. [35] reported the volatile compounds of *M. perforata* collected from Estonia as (Z,Z)-matricaria ester (77.90%), (E)-β-farnesene (3.50%), matricaria ester isomer (3.50%) and matricaria lactone (3.00%). Furthermore, α-bisabolol (37.99%), β-farnesene (18.67%), α-farnesene (5.34%) and bisabololoxide (4.38%) were emphasized in *M. maritima* from Canada [36].

M. sevanensis is different from *M. recutita* and *M. chamomilla* in terms of the main volatile organic

compounds. The amounts of chamazulene (primary substance) in *M. recutita* from west and central of Iran were stated as 31.40 and 61.30%, respectively [6, 14]. α-Bisabolol oxide A (21.50%) in *M. chamomilla* from Khorasan-Razavi Province of Iran [11] and α-bisabolol oxide B (35.60%) in *M. chamomilla* from Germany [37] were found as the main components. In this study, bisabolene oxide A (50.32%) was determined as the major compound of *M. sevanensis* essential oil. These variations in chemical compositions of oil samples could be arised from collection place and time of plant, climatic constraints of location and analyze conditions. However, *M. sevanensis* is easily separated from *Matricaria*-like species with the aid of *T. disciforme* (p-methoxy-β-cyclopropylstyrene 18.80%) and *T. corymbosum* ((Z)-β-farnesene 18.20%) [8, 16].

Table 2 Volatile compounds of *Matricaria sevanensis* essential oil from Osmaniye, Turkey

No	RT	RI[a]	RI[b]	Compounds	Area/%
1	6.30	897	907	α-Pinene	1.50
2	8.17	984	984	β-Myrcene	0.39
3	8.58	1001	1002	Octanal	0.12
4	9.34	1021	1024	o-Cymene	0.40
5	9.49	1030	1034	L-Limonene	0.18
6	10.28	1050	1042	(E)-β-Ocimene	0.50
7	10.67	1062	1052	γ-Terpinene	0.29
8	24.64	1388	1397	β-Elemene	0.46
9	25.68	1417	1410	Caryophyllene	0.57
10	27.35	1439	1446	(Z)-β-Farnesene	24.20
11	28.34	1488	1475	α-Selinene	0.31
12	28.77	1492	1480	Bicyclogermacren	2.10
13	28.86	1506	1503	β-Curcumene	0.26
14	29.37	1507	1504	α-Farnesene	4.06
15	35.78	1699	1684	Bisabolene oxide A	50.32
16	35.89	1715	1715	Chamazulene	0.78
17	37.92	1738	1746	α-Bisabolol oxide A	8.12
18	42.43	1843	1843	(E)-ene-yne-dicycloether	0.86

RT: Retention time, RI[a]: Retention indices from the present study, RI[b]: Retention indices from the literature

Temperature changes during distillation process

Figure 2 depicts the temperature variations in *M. sevanensis* samples and their container depending on time. Before distillation, the specimens and glass bowl were waited at about 25 °C until thermal equilibrium was reached, and then, process was started. In first 5 min, there was a huge increment in temperature (nearly 65 °C) of both system items. The maximum temperatures (114 °C and 105 °C for sample and bowl, respectively) were observed at 20 min of distillation due to the reduced rate of water evaporation [38], and after that, fluctuations were seen as the heating progressed. Süfer and Palazoğlu [38] reported the maximum temperature of pomegranate arils during microwave vacuum drying as 88.8 °C at 150 W and 90 min. The presence of vacuum and the initial moisture content may be effective in temperature variations, although similar operations are conducted. Also, microwave power level and type of oven play a key role within this context.

There are different remarks about the effect of high temperature on chemical and bioactive compounds in previous studies. Zielinska and Zielinska [39] claimed that a dehydration temperature of 90 °C enhanced the antioxidant activity (by FRAP method) of cranberries as well as total phenolic contents. On the contrary, 40 °C was advisable for volatile compounds of *Cordyceps militaris* mushroom rather than 50, 60 and 70 °C [40]. Hence, processing conditions should be optimized for microwave-assisted distillation operations of essential oils by applying a power program [38].

Table 3 Comparison of essential oil compounds among *Matricaria sevanensis* and various *Matricaria* species in different regions

M. sevanensis	Area/%	*M. chamomilla* [6]	Area/%	*M. recutita* [13]	Area/%	*M. recutita* [14]	Area/%	*M. chamomilla* [37]	Area/%
Bicyclogermacren	02.10	Trans-β-Farnesene	05.20	α-Bisabolol	02.00	α-Bisabolol	07.41	Trans-β-Farnesene	03.97
α-Farnesene	04.06	α-Bisabolol oxide B	07.00	Z,E-Farnesol	04.80	α-Pinene	08.14	α-Bisabolene oxide A	05.04
α-Bisabolol oxide A	08.12	Spathulenol	09.40	E-β-Farnesol	05.20	β-Pinene	10.11	Chamazulene	16.68
(Z)-β-Farnesene	24.20	α-Bisabolene oxide A	10.00	Trans–trans-Farnesol	06.90	1,8-Cineole	15.20	Bisabolol oxide A	17.46
Bisabolene oxide A	50.32	α-Bisabolol oxide A	21.50	Chamazulene	61.30	Chamazulene	31.40	Bisabolol oxide B	35.63

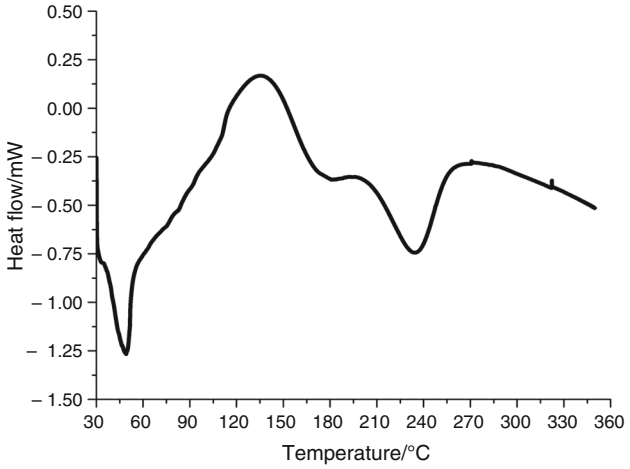

Fig. 4 Representation of DSC curve *M. sevanensis* essential oil

DSC results

According to DSC graph of *M. sevanensis* essential oil (Fig. 4), three peaks were specified and the number of peaks in oil samples may be attributed to their triacylglycerol amounts [41]. Only one peak was determined in essential oil of *Syzygium aromaticum* L. [26] and, however, five peaks in *Hylocereus undatus* seed oil [41]. Both exothermic (peak 1 and 3) and endothermic (peak 2) behaviors were monitored, and endothermic pattern is strongly related to double bonds [42] and an indicator of decomposition, although exothermic event could be characterized by oxidation reactions [43]. The peaks were generally smooth, but some roughnesses were seen locally in DSC chart because of containing probable impurities. The first, second and third onset temperatures (T_o) and peak temperatures (T_P) were recorded as 47.07 °C, 49.13 °C; 107.68 °C, 136.64 °C; 208.03 °C and 234.69 °C, respectively. The enthalpies (ΔH) of exothermic peaks were $-$ 26.98 J g^{-1} and $-$ 64.48 J g^{-1}, and peak 2 had a ΔH of 104.07 J g^{-1}.

FTIR fingerprints

Essential oils contain concentrated oils as well as fragrants, and thus, several functional groups are existing in samples [33]. Aromatics, amines, ethers, carboxyclic acid, alcohols, aldehyde carbonyl, aldehyde and alkanes were the functional ingredients found in *M.sevanensis* essential oil (Table 4), and the matchings of related groups were achieved by using previous researches [44–48]. The wave numbers of identified compounds were between 3430.99 cm^{-1} and 713.93 cm^{-1}, even though the scanning was performed in the range of 4000-600 cm^{-1}. Moreover, Fig. 5 indicates the FTIR spectra of oil and as can be seen from the graph, a large peak at 2924.22 cm^{-1} corresponded to alkanes with C–H stretch [47]. The detected constituents were intensified in the band of 1721.11-713.93 cm^{-1}.

Antioxidant activity

The IC_{50} value of *M. sevanensis* essential oil was calculated as 156.005 ± 1.533 μg mL^{-1} ($p < 0.05$). On the other hand, the IC_{50} value of synthetic antioxidant, trolox, was recorded as 0.252 ± 0.001 μg mL^{-1} ($p < 0.05$). Thus, the antioxidant activities were in the following order: trolox < essential oil. *Matricaria sevanensis* essential oil showed nearly the same antioxidant effect with *D. multicaule* (156.5 μg mL^{-1}) [49] and better antioxidative behavior than *M. recutita* (137.2 μg mL^{-1}) [14].

α-Pinene, chamazulene and limonene [50] are known as their antioxidative impacts, and the free radical scavenging ability of essential oil from *M. sevanensis* can be attributed to these monoterpene hydrocarbons as well as caryophyllene compounds like (Z)-β-farnesene which was one of the most abundant compounds (24.20%) in oil [51].

Table 4 Chemical interpretation of FTIR data

Vibrational motion	Wave number/cm^{-1}	Functional group	References
C–H bend (ortho)	713.93	Aromatics	[44, 45]
C–H bend	802.21	Aromatics	[45, 46]
C–H bend	897.74	Aromatics	[45]
C–N stretch (alkyl)	1012.69	Amines	[44]
C–O–C stretch	1090.71	Ethers	[44]
C–O–C stretch	1178.42	Ethers	[44]
C–O strech	1374.04	Carboxylic acid	[47]
C–OH bend	1448.29	Alcohols	[48]
C = O stretch	1629.79	Aldehyde carbonyl	[48]
C = O stretch	1721.11	Aldehyde	[47, 48]
C–H stretch	2924.22	Alkanes	[47]
O–H strech	3430.99	Alcohols	[44]

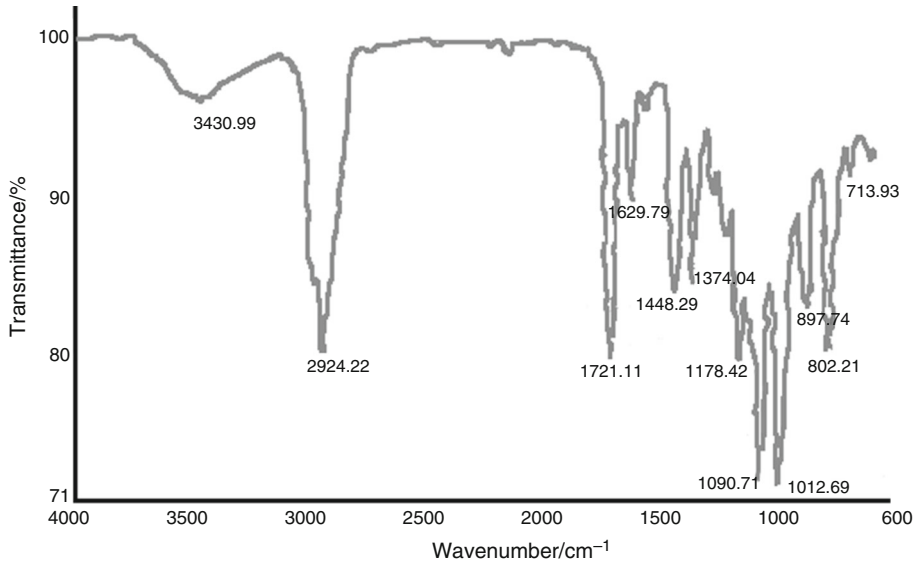

Fig. 5 FTIR spectra of *M. sevanensis* essential oil

Conclusions

Essential oils have a widespread usage potential in especially medicine and several industries. The attentions should pay into not only its volatile compounds, but also thermal characteristics and antioxidant activity because of clarifying thermal hazard risks and health beneficial effects. On the other hand, classical distillation methods require relatively longer times, more energies as well as organic solvents, and thus, microwaves could be an alternative for fast processing. The solvent-free methods may probably be helpful in environmental problems.

The essential oil of Turkish *M. sevanensis* samples was evaluated by GC/MS, and bisabolene oxide A (50.32%) and (Z)-β-farnesene (24.20%) were the most abundant volatiles. In the light of this information, (Z)-β-farnesene makes *M. sevanensis* (chamomile) tea which is consumed gladly in Turkey, a valuable functional drink due to its known anticancerogenic and antimicrobial properties. Besides that, an antioxidant potential (IC_{50}) of 156.005 μg mL^{-1} which corresponds to a significant level of bioactivity may give a chance to *M. sevanensis* essential oil for being utilized as a source of natural antioxidants instead of synthetic antioxidants. Furthermore, a total enthalpy of 12.61 J g^{-1} which means a low accumulated energy proves the safety of this product. Optimization studies should be conducted in order to recover great amounts of volatiles and bioactives at the same time. The application of a gradual microwave power program can be recommended for this purpose.

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Compliance with ethical standards

Conflict of interest The authors declare that they no conflict of interest.

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