

COMPOSITION OF *SCABIOSA OCHROLEUCA* L. EXTRACTS PREPARED BY ULTRASONIC AND MICROWAVE METHODS AND THEIR ANTIRADICAL AND ANTIOXIDANT ACTIVITY ASSESSMENT

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Abstract

Scabiosa ochroleuca L. is one of the promising Kazakh domestic medicinal plants. It has been used in traditional medicine for stomach, ostealgia, fever, tuberculosis, syphilis, eye infections, and as a wound healing agent. Despite its therapeutical applications, there is a lack of its secondary metabolites. The aim of the manuscript is to study the chemical difference between the two extracts prepared by ultrasonic and microwave methods by GC-MS analysis and their antioxidant properties. To determine the possibility of using *S. ochroleuca* L., we researched the component composition of extracts obtained by ultrasound and microwave methods of the aboveground part of *S. ochroleuca* L. using the specific GC-MS method, as well as the antiradical and antioxidant activity of the obtained extracts were researched. The GC-MS analysis of the silylized extracts revealed the presence of several major components: catechol, 2-methoxy-4-vinylphenol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and quinic acid. The antiradical activity (DPPH) showed that the ultrasonic extract had a stronger activity (83.9 %). The activity of the ultrasonic and microwave extracts at concentration of 0.75 mg/mL and 1 mg/mL, respectively, was almost the same as the standard butylhydroxyanisole (1 mg/mL), accordingly, using the FRAP method at a concentration of 1 mg/mL, the extracts showed high activity in comparison with the standard sample (ascorbic acid), which proves the prospects of studying this herb.

Rezumat

Scabiosa ochroleuca L. este una dintre plantele medicinale cu important potențial terapeutic din Kazahstan, fiind folosită în medicina tradițională pentru boli ale stomacului, osteoalgie, febră, tuberculoză, sifilis, infecții oculare și ca agent de vindecare a rănilor. În ciuda aplicațiilor sale terapeutice multiple, se cunosc prea puține informații despre metabolizii săi secundari. De aceea, scopul studiului a fost de a evalua diferența chimică dintre două extracte preparate prin metode ultrasonice și cu microunde, prin analiza GC-MS și a proprietăților antioxidante. Astfel, analiza GC-MS a extractelor a relevat prezența mai multor componente majore: catecol, 2-metoxi-4-vinilfenol, 4H-piran-4-onă, 2,3-dihidro-3,5-dihidroxi-6-metil- și acid chinic. Activitatea antiradicalică (DPPH) a arătat că extractul obținut prin metoda cu ultrasunete a prezentat o activitate mai puternică (83,9 %). Efectele extractelor cu ultrasunete și microunde la concentrație de 0,75 mg/mL și, respectiv, 1 mg/mL, au fost similare cu a butilhidroxianisolului standard (1 mg/mL); în consecință, folosind metoda FRAP la o concentrație de 1 mg/mL, extractele au prezentat activitate antioxidantă pronunțată în comparație cu proba standard (acid ascorbic).

Keywords: *Caprifoliaceae*, *Scabiosa ochroleuca* L., GC-MS, ultrasonic extraction, microwave extraction, antiradical activity, DPPH, FRAP

Introduction

The pharmaceutical industry is one of the highly profitable and rapidly growing sectors of the world economy and the driving force of the country's development, economy and social development [1]. At the present stage of development of the pharmaceutical sector [2], domestic medicinal plant raw materials

are often used. One of such promising species is *S. ochroleuca* L.

The genus *Scabiosa* (*Dipsacaceae* family) includes about 80 - 100 species. *S. ochroleuca* L. is a biennial plant which reaches 40 - 70 cm in height. Its flowers are pale yellow in colour, partially whole and toothed basal leaves, and double-pinnately dissected, 5 - 10 cm long, sessile leaves, with linear lobes [3, 4]. The

plant is distributed over South Central Europe, the Baltic states, the North of the Balkan Peninsula, Dzungaria-Kashgaria and Mongolia [5]. In the Republic of Kazakhstan, it is widespread from Altai to the Northern Tien Shan [6] and is often found in Central Kazakhstan [7].

S. ochroleuca L. is a plant of interest due to its medicinal properties. It is used in Europe and Asia in the treatment of gastric and gynaecological diseases, fever, tuberculosis and skin diseases. Its activity was certainly influenced by the presence of polyphenols [3]. The plant constituents vary by several factors, including soil composition, climatic and cultivation conditions and stage of plant life. The goal was to determine the phenolic composition of *S. ochroleuca* L. growing in Kazakhstan and its economic importance. For this purpose, GC-MS analysis had been conducted on the silylized extracts prepared by ultrasonic and microwave extraction [8].

So far, a lot of attention has been paid to study the composition and pharmacological activity of the genus *Scabiosa*. Eleven new triterpenoid saponins were isolated from *S. tschiliensis*, together with 22 known flavonoids and sterols [9]. Extracts from *S. stellata* L. contained triterpenoid saponins, sterols, fatty acids and phenolic compounds [10, 11]. Despite phenolic constituents, terpenes were described as the constituents of *Scabiosa* spp. Al-Qudah *et al.* [12] identified forty components of fresh pre-flowering oil. The aim of the manuscript is to study the chemical difference between the two extracts prepared by ultrasonic and microwave methods by GC-MS analysis and their antioxidant properties.

Materials and Methods

Plant material

The aerial parts of *S. ochroleuca* L. were collected in the Karagandy region of the Republic of Kazakhstan, between July and August 2018, in the full flowering phase. The plant was identified by botanist Ishmuratova M. Yu. (Department of Botany, Karaganda Buketov University, Karaganda, Kazakhstan). The collected material was deposited in the herbarium of the Faculty of Biology and Geography of the Academician "E.A.

Buketov" Karaganda State University, Kazakhstan. Herbarium code *S. ochroleuca* L. - 2010.06.11.01.14 (collection of Mount Karkaraly). The plant raw materials were collected according to GAAP standards [13].

Extraction of plant material

Extracts of *S. ochroleuca* L. were prepared at Karagandy Medical University using ultrasonic extraction unit (Ultrasonic bath VGT-1200, China) and a microwave bath extractor with an operating frequency of 2.45 GHz, with an adjustable power of 100 - 1000 W, equipped with a highly efficient reflux condenser (solvent-ethyl alcohol 70%). The extraction was carried out at an exposure power of 150 W in three repetitions.

GC-MS analysis

The composition of the extracts obtained by ultrasonic and microwave extraction was determined in the Laboratory "Ecology of the Biosphere" of the "Al-Farabi Kazakh" National University (Test report No. 2-580 of 10.12.2019) on a gas chromatograph with mass spectrometric detection (Agilent 6890N/5973N, Santa Clara, CA, USA) under the following analysis conditions: sample volume 0.2 µL, sample injection temperature 240°C, without flow division. Separation was carried out using a chromatographic capillary column DB-35MS with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm at a constant carrier gas (helium) velocity of 1 mL/min. The chromatographic temperature is programmed from 40°C (exposure 2 min) to 200°C with a heating rate of 10°C/min (exposure 5 min) and up to 300°C with a heating rate of 20°C/min (exposure 10 min). Detection was performed in SCAN m/z 34 - 750 mode. Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system, register and process the results and data. Data processing included the determination of retention times, peak areas, as well as processing, spectral information obtained using a mass spectrometric detector. To decode the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550 thousand) [14]. The research results are shown in Table I.

Table I

The quantitative composition of the extracts of *S. ochroleuca* L.

No.	Compound	Ultrasonic extraction		Microwave extraction	
		Retention time, min	%	Retention time, min	%
1.	L-Lactic acid	7.6	28.0	7.4	25.2
2.	1,2-Cyclopentanedione	7.8	1.8	8	3.1
3.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	8.0	0.1	-	-
4.	Butanoic acid, 4-hydroxy	-	-	8.5	1.2
5.	Benzeneacetaldehyde	9.7	0.5	-	-
6.	Glycerin	-	-	9.9	2.0
7.	2-Hydroxy-gamma-butyrolactone	-	-	10	1.0
8.	Succindialdehyde	10.1	5.4	-	-
9.	2-Pyrrolidinone, 1-methyl	-	-	10.6	0.2
10.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	11.5	3.7	11.4	1.3

No.	Compound	Ultrasonic extraction		Microwave extraction	
		Retention time, min	%	Retention time, min	%
11.	Cyclopropylcarbinol	11.6	2.5	11.5	1.8
12.	Catechol	12.1	3.0	12.1	1.6
13.	Benzofuran, 2,3-dihydro	12.2	1.26	12.2	1.2
14.	1,4:3,6-Dianhydro- α -d-glucopyranose	13.0	0.2	13	0.3
15.	Hexane, 3-bromo	-	-	13.2	0.4
16.	2,5-Dimethylcyclohexanol	-	-	13.6	0.1
17.	1,6-Octadiene, 3-ethoxy-3,7-dimethyl	13.6	0.1	-	-
18.	2-Methoxy-4-vinylphenol	13.7	0.4	13.7	0.8
19.	Geranyl- α -terpinene	-	-	13.9	0.1
20.	Eugenol	14.1	0.5	14.1	0.6
21.	Phenol,2,6-dimethoxy-	14.6	0.2	14.6	0.3
22.	Heptan-2-ol, 5-(2-tetrahydrofurfuryl)	-	-	14.8	0.4
23.	Benzoicacid, 2-formyl-, methylester	14.9	0.2	14.9	0.3
24.	Z-10-Pentadecen-1-ol	-	-	15.3	0.3
25.	4-(2,6,6-Trimethyl cyclohexa-1,3-dienyl)but-3-en-2-one	15.5	0.1	15.5	0.1
26.	L-Proline, 5-oxo-, methyl ester	15.7	0.1	-	-
27.	Isopulegol	15.8	1.7	15.8	1.7
28.	6-Hydroxy hexahydrocyclopenta[b]furan-2-one	16.0	1.9	16	1.8
29.	1-Methylcyclohexylcarboxylic acid	-	-	16.1	0.8
30.	Trans,trans-2,6-dimethyl-2,6-octadiene-1,8-diol	16.3	2.0	16.3	2.1
31.	9-Oxabicyclo nonan-2-ol, acetate	-	-	16.5	0.4
32.	2-Undecenal	-	-	16.7	0.7
33.	2-Methylindoline	16.9	0.1	-	-
34.	Fumaric acid, 2,4-dimethylpent-3-yl ethyl ester	17.1	0.6	17.1	0.9
35.	2 <i>H</i> -1-Benzopyran-3,4-biol, 2-(3,4-dimethoxyphenyl) -3,4-dihydro-6-methyl-, (2 α ,3 α ,4 α)-	17.3	1.0	-	-
36.	Tributylphosphate	-	-	17.4	6.5
37.	Ethyl ester of 7-chloro-7-oxoheptanoic acid	17.7	16.3	17.7	22.1
38.	9-Oxabicyclo [3.3.1]nonane-2,6-diol	18.4	4.9	18.3	3.1
39.	Quinic acid	19.5	16.2	19.4	10.8
40.	4-((1 <i>E</i>)-3-Hydroxy-1-propenyl)-2-methoxyphenol	20.1	1.5	20.1	2.0
41.	<i>N</i> -Hexadecanoicacid	20.8	1.6	20.8	1.5
42.	Ethyl (+)-camphorcarboxylate	-	-	21.5	0.1
43.	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo [4.1.0]hept-1-yl)-propenyl ester	21.5	0.3	-	-
44.	3-Phenpropionic acid, 4'-hydroxy-2'-nitro-, methyl (ester)	-	-	22.4	0.3
45.	Dibutylphthalate	-	-	23.1	0.1
46.	Phytol	23.4	0.1	-	-
47.	Ethylolate	24.5	0.1	-	-
48.	9,12-Octadecadienoic acid, ethyl ester	24.6	0.8	-	-
49.	Linoleicacidethylester	-	-	24.6	0.4
50.	Ethyl 9,12,15-octadecatrienoate	24.9	0.6	-	-
51.	9,12,15-Octadecatrienoic acid	-	-	24.9	0.3
52.	9 <i>H</i> -Pyrido[3,4- <i>b</i>]indole	-	-	25.8	0.7
53.	Eicosanoicacid, ethylester	26.4	0.1	-	-
54.	9-Octadecenamide	-	-	27.1	0.2
55.	Diisooctylphthalate	27.9	0.1	27.9	0.2
56.	9-Octadecene,1,1'-[1,2-ethanediylbis(oxy)]bis	-	-	28.1	0.3
57.	Hexacosane	28.7	1.1	28.7	0.3
58.	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	28.7	0.1	-	-
59.	Eicosane	29.8	0.1	-	-
60.	Vitamin E	32.0	0.1	32	0.1
61.	Campesterol	34.0	0.2	65	0.1
62.	Oleic acid	-	-	-	-
63.	Stigmasterol	34.4	0.2	-	-
64.	γ -Sitosterol	35.3	0.6	87	0.4

Antiradical assay

The antiradical activity of *S. ochroleuca* L. extracts obtained by ultrasonic and microwave extraction was compared with the antiradical activity of a standard, butylhydroxyanisole (BHA). The research was carried out in the laboratory of the Institute of Research Institute of New Chemical Technologies at the "L.N. Gumilyov" Eurasian National University by the method of colorimetry of free radicals based on the reaction of DPPH with an antioxidant sample [15]. To determine DPPH inhibition, alcoholic solutions of carbon dioxide extracts of pale yellow scabiose were prepared in the concentration range of 0.1; 0.25; 0.5; 0.75 and 1.0 mg/mL. For the study, 3 mL of 6×10^{-5} M radical solution were added to 0.1 mL of an extract. The tubes were incubated in the room temperature in a rack wrapped in black polyethylene. After vigorous stirring, the solutions remained in the dark for 30 minutes, then the optical densities were measured at 520 nm. The values of the antiradical activity (ARA) of the studied objects were determined by the formula (1):

$$\text{ARA (\%)} = A_0 - \frac{A_t}{A_0} * 100, \quad (1)$$

where, A_0 - is the optical density of the control sample; A_t - is the optical density of the working sample.

Method for determining antioxidant activity using FRAP

0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium (III) hexacyanoferrate solution

were added to 0.1 mL of the studied substances in the concentration range of 0.25, 0.5, 0.75 and 1.0 mg/mL. The reaction mixture was incubated for 20 minutes at 50°C; the reaction was stopped by adding 0.25 mL of 10% trichloroacetic acid solution. The mixture was centrifuged for 10 minutes (3000 rpm). The top layer of 0.5 mL was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1 % FeCl_3 . The optical density was measured at 700 nm. The antioxidant activity (AOA) of the samples was compared with the AOA of ascorbic acid. [16].

Results and Discussion

The composition of the two extracts was determined by the first silylation followed by GC-MS analysis. Forty-seven components were identified in the microwave extraction, and forty-two in the ultrasonic extraction. All together 63 components were identified (Table II). In Ultrasonic extract the major components were: lactic acid (28.0%), quinic acid (16.2%), catechol (3.0%), succindialdehyde (5.4%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (1.5%), and 4H-pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl (3.7%); while in the microwave extract the major components were: catechol (1.6%), quinic acid (10.8 %), ethyl ester of 7-chloro-7-oxoheptanoic acid (22.1%), 4- ((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.0%) and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (1.3%).

Table II

Changes in the optical density of the solutions under study with changes in concentration

No.	Test substances	Optical density values by concentration (mg/mL)				
		0.1	0.25	0.5	0.75	1.0
1.	Butylhydroxyanisole (BHA)	0.362 ± 0.0000	0.1333 ± 0.0010	0.1257 ± 0.0001	0.1202 ± 0.0035	0.1145 ± 0.0004
2.	Ultrasonic alcohol extract (USE)	0.4617 ± 0.0163	0.3807 ± 0.0000	0.2649 ± 0.0000	0.1686 ± 0.0168	0.1277 ± 0.0059
3.	Microwave alcohol extract (MVE)	0.4477 ± 0.0189	0.3569 ± 0.0034	0.2193 ± 0.0000	0.1302 ± 0.0500	0.1085 ± 0.0000

Figure 1 shows the comparison of the percentage share of *S. ochroleuca* L. metabolites depending on the chemical groups and type of extract. Among the identified components: acids predominate in the ultrasonic extract (USE) with a 14.6% share (and in the microwave extract [MVE] with 38.0%). The following groups include ketones 56% (MVE 11.5%), phenols 5.0% (MVE 4.8%) and aldehydes 5.9% (absent in MVE). At the same time, tocopherol was determined in the MVE at 0.1% (no representation in USE). Moreover, the MVE contained alcohols 4.4% (USE 2.7%), terpenoids 5.0% (USE 3.6%), ethers 30.5% (USE 18.3%), and fatty acids 2.5% (USE 2.2%).

The identified components of *S. ochroleuca* L. are found in other medicinal plants with various pharmacological activities. For example, catechol was identified in the herb *Elsholtzia communis* and showed high antioxidant properties [17]. Also, 1,2-cyclopentanedione (1.8%) and 2,3-dihydro-3,5-dihydroxy-6- methyl-4H-pyran-4-one (3.7%) whose presence was determined in this study show a noticeable antioxidant capacity [18, 19]. In the work of El-Askary *et al.* [20]: quinic acid derivatives were proved for hepatoprotective, anti-hyperglycemic and antioxidant activity in *in vivo* tests on mice.

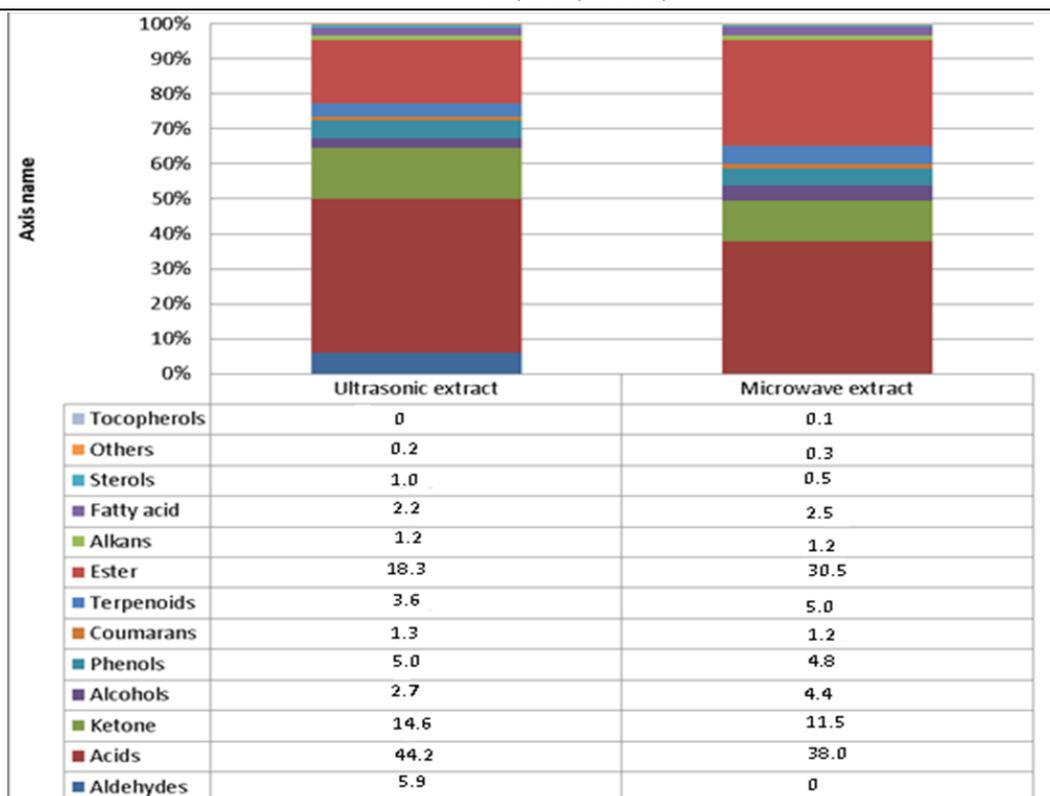


Figure 1.

The difference in the chemical composition of extracts of *S. ochroleuca* L.

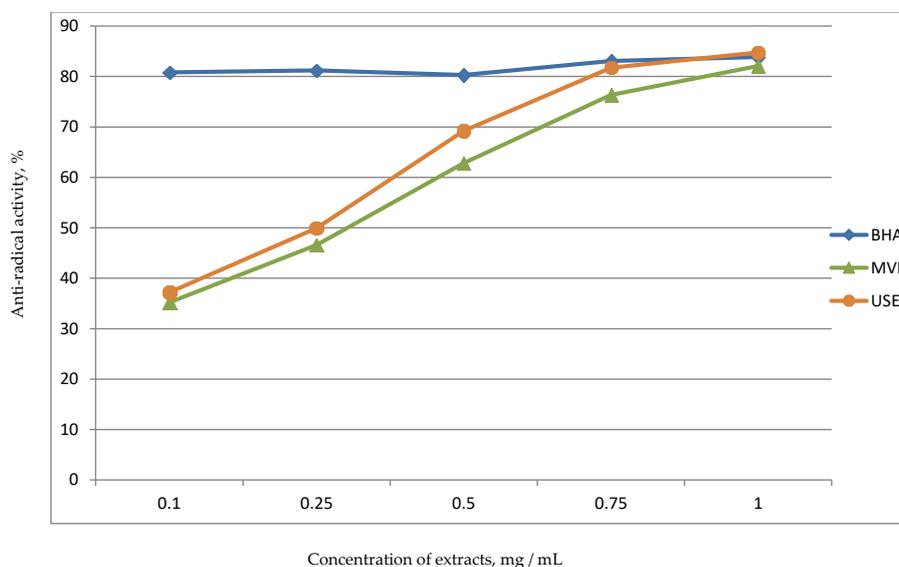


Figure 2.

Dynamics of radical activity associated with changes in the concentration of substances

The optical density of the test solutions, depending on the concentration, was measured on a JacsoV660 UV-Vis spectrophotometer at a wavelength of 520 nm (Table II). The antiradical activity of the solutions of the test sample was compared with the antiradical activity of butylhydroxyanisole (BHA - concentration 1 mg/mL).

The study of antiradical activity by the DPPH method. In the course of the study, at concentrations of 0.75 and 1 mg/mL, the activity was equal to the used reference (BHA) at the same concentration. MVE extracts of *S. ochroleuca* L. also showed the same antiradical activity, but at a higher concentration (1 mg/mL) (Table III, Figure 2).

Table III

Anti-radical activity of experiments (%)

No.	Test substances	Sample concentration (mg/mL)				
		0.1	0.25	0.5	0.75	1.0
1.	Butylhydroxyanisole (BHA)	80.8	81.2	80.3	83.1	83.9
2.	Ultrasonic alcohol extract (USE)	37.2	50.0	69.2	81.7	84.8
3.	Microwave alcohol extract (MVE)	35.2	46.6	62.8	76.3	82.1

The FRAP (Ferric Reducing Antioxidant Power Assay) method

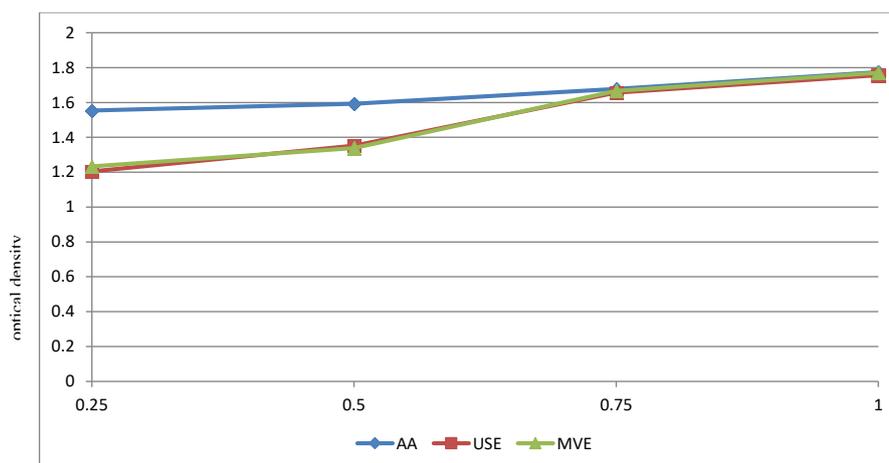
The FRAP method is based on the reduction of Fe^{3+} ions to Fe^{2+} with the help of antioxidants. Reduction reaction $\text{K}_3[\text{Fe}(\text{CN})_6]$ with antioxidants, which is accompanied by the formation of a yellow compound, namely $\text{K}_4[\text{Fe}(\text{CN})_6]$. The measurements are based on the ability of antioxidants to suppress the oxidative effect of the formed reaction particles in the reaction

mixture. Ascorbic acid was used as a comparison drug. The samples were tested at concentrations of 0.25, 0.5, 0.75 and 1 mg/mL (Table III). Based on the data analysis, it can be seen that ultrasonic and microwave extracts of *S. ochroleuca* L. at a concentration of 1 mg/ml presented the highest antioxidant activity compared to the standard ascorbic acid solution (Table IV).

Table IV

Change in the optical density of solutions depending on the working solutions concentration

No.	Test substances	Optical density value at concentration (mg/mL)			
		0.25	0.5	0.75	1.0
1	Ascorbic acid (AA)	1.5539 ± 0.0250	1.5928 ± 0.0022	1.6775 ± 0.0153	1.7738 ± 0.0169
2	Ultrasonic alcohol extract (USE)	1.2046 ± 0.0200	1.3517 ± 0.0090	1.6561 ± 0.0061	1.7571 ± 0.0007
3	Microwave alcohol extract (MVE)	1.2328 ± 0.0330	1.3382 ± 0.0028	1.6666 ± 0.0120	1.7701 ± 0.0470

**Figure 3.**

Effect of substance concentration on changes in antioxidant activity

The results of the conducted research of ultrasonic and microwave extracts of the herb *S. ochroleuca* L. showed high antiradical and antioxidant activities.

So far, a lot of attention has been paid to the studies on the composition and pharmacological activity of the genus *Scabiosa*.

The highest antiradical activity in comparison with the standard butylhydroxyanisole (83.9%), the ultrasonic extract of *S. ochroleuca* L. at a concentration of 1.0 mg/mL showed 84.8%, while the microwave extract at the same concentration showed 82.1% (Table III).

The highest antioxidant activity of *S. ochroleuca* L. extracts by the FRAP method was obtained through a microwave extract at a concentration of 1.0 mg/mL of 1.7701 compared to the ascorbic acid standard

(1.7738). Ultrasound extract of Pale yellow Scabiosa also has a high antioxidant activity compared to ascorbic acid (1.7571) (Table IV).

Extracts from *S. stellata* L. contained triterpenoid saponins, scabiostellatosides, sterols, fatty acids (with linoleic, palmitic and linolenic acids as the major representatives) and more than 25 phenolic compounds [21, 22]. Among the latter, interesting new derivatives of kaempferol, e.g. kaempferol-3-O-(4'',6''-di-Ep-coumaroyl)- β -D-galactopyranoside was described. Despite phenolic constituents, terpenes were described as the constituents of *Scabiosa* spp. Al-Qudah and coinvestigators [23] pointed out more than forty constituents with E-salvene (54.90%), apicolin (20.98%), ethylisovalerate (18.32%), and myrtenal (7.6%) as the main components of fresh pre-flowering oil.

Additionally, the macro- and microelement profile of the plant was determined and indicated that *S. ochroleuca* as a source of chromium, molybdenum, nickel etc. The amino acid composition of its extracts was performed [3] which revealed the presence of 15 amino acids in the extracts (aboveground part).

As the therapeutic potential of *S. ochroleuca* L. herb is significant, there is still a need for compositional studies on this particular plant species [9].

The presented data confirm the direct influence of phenolic constituents on antioxidant activity. According to the literature, other *Scabiosa* species were proved to exhibit antiradical properties. For example, the antiradical activity of *S. stellata* L. is one of the most studied [17]. In our previous study [5] the antiradical activity of the carbon dioxide extract of *S. ochroleuca* L. did not indicate any strong antiradical activity. As other investigated species, e.g. *S. tschiliensis* L. showed the highest antioxidant potential with an IC₅₀ value of 8.47 + at a concentration of 0.23 mg/mL, (which was almost equal to the IC₅₀ value of vitamin C (7.60 at a concentration of 0.61 mg/mL) [23]. Therefore, the authors decided to perform once more time the antioxidant activity determination of *S. ochroleuca* L. but using different polarity extracts. Antioxidant activity of *S. prolifera* L. was tested by DPPH, ABTS and FIC methods. Preliminary results have shown that the butanol fraction is the most active. BHA, α -tocopherol, and EDTA were used as positive controls. Also, the antioxidant activity of *S. tschiliensis* (DPPH) was evaluated [24, 25].

Conclusions

The compositional and antiradical studies were performed on the extracts of *S. ochroleuca* L. prepared by ultrasonic and microwave techniques. The GC-MS analyses of the silylated extracts revealed their composition. The ultrasonic extract contained mostly ketones (14.6%), phenols (5.0%), and aldehydes (5.9%). It is interesting to indicate the absence of aldehydes in the microwave extract. Also, the antiradical activity of ultrasonic extracts was slightly higher than of the microwave. And according to the FRAP method, the extracts proved their high antioxidant activity compared to the standard sample.

Conflict of interest

The authors declare no conflict of interest.

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