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STUDY OF ANTIOXIDANT, CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS OBTEINED FROM *ACHILLEA MARITIMA* (L.) EHREND. & Y.P.GUO (ASTERACEAE)

© ***S. Bilgi, A. Beyatli****

University of Health Sciences, Selimiye Mah. Tıbbiye Cad., 38, Üsküdar/Istanbul, 34668 (Turkey), e-mail: ahmet.beyatli@sbu.edu.tr

Various extracts obtained from *Achillea maritima* analyzed with HPLC-DAD method to detect phenolic compounds. Total phenolic content (954.94±38.36 mg/g sample) and antioxidant activity results of *A. maritima* (604.15±11.57 mg trolox/g sample for DPPH method, 347.65±8.98 mg trolox/g sample for ABTS method, 486.31±9.99 mg trolox /g sample for FRAP method) were found in ethyl acetate extract the most. In addition, phenolic acid and ferulic acid was determined as the major phenolic substance (47.97±0.07 mg/g sample). The ethanol and chloroform phases showed MIC values of 2500 µg/mL against bacteria and fungus, and the hexane phase showed MIC values of 19, 39, 156 µg/mL against *S. aureus*, *E. fecalis* and *P. aeruginosa*, respectively. Ethyl acetate phase has a various activity against each bacteria. Butanol phase was found to be more effective against Gram-negative (MIC 625 µg/mL and 39 µg/mL) than Gram-positive ones (MIC 1250 µg/mL). The water phase had a stable MIC (625 µg/mL) against all strains. In MTT test, extracts showed different levels of cytotoxicity (240–820 µg/mL) against K562 and Saos cell lines, while extracts were non-toxic against NIH-3T3. In Brine shrimp assay ethanol, hexane and chloroform extracts had a moderate cytotoxic effect.

Keywords: *Achillea maritima*, Antioxidant, Antimicrobial, Cytotoxicity, HPLC.

Introduction

Since ancient times, herbs have been the basis of nearly all medical treatments, until synthetic drugs were developed in the nineteenth century [1]. In addition to their traditional use, plants also used effectively in modern methods. Research has revealed potential of natural products to be used as medicine. Many people still use plant-based folk medicine in different regions of the world [2].

For decades, researchers have been trying to develop new broad-spectrum antibiotics against infectious diseases caused by microorganisms. Long-term use of these broad-spectrum antibiotics leads to the emergence of drug resistance. Antibiotic discovery methods based on synthetic substances are not keeping pace with the evolution of resistance. Therefore, new strategies to control microbial infections are very important. Plant secondary metabolites have already demonstrated their potential as antimicrobials when used alone and as synergists or potentiators of other antimicrobial agents. Screening of plant extracts with approved methods is a primary source of discovery for the identification of molecules potentially useful against infectious diseases [3].

Türkiye's flora is extremely diverse, with over 3000 medicinal and aromatical plant species. More than 100 species of *Achillea* L. (Asteraceae) exist in the world, with origins in Southwest Asia and Southeast Europe and extensions through Eurasia to North America [4]. The main habitats of the *Achillea* species are concentrated in different parts of Iran, Türkiye, Serbia and the eastern parts of Europe. The genus is represented by 48 species belonging to 54 taxa, 24 of which are endemic in Türkiye [5]. *Achillea* is a medicinal plant that has been widely used throughout the world since ancient times. Phytochemical studies of *Achillea* species have shown that many compounds of this genus are largely bioactive. Most of *Achillea* species have therapeutic effects [6].

Bilgi Selçuk – Biologist, Graduate Student,
e-mail: selcuk1357@hotmail.com
Beyatli Ahmet – Assistant Professor of the Department of medicinal and aromatic plants,
e-mail: ahmet.beyatli@sbu.edu.tr

* Corresponding author.

A. maritima (L.) Ehrend. & Y.P. Guo, a small shrub with almost-white-colored stems and leaves covered in a pilosity. The plant can reach a height of 40-50 cm. Leaves oblong-lanceolate 10–20 × 3–7–5 mm, subacute apex, sessile, semi-wide at base, margins slightly serrated. The flowers have small, hemispherical flower heads with several stalks, oval-broad white cotton scales [7]. The aim of this study is to examine the biological activities (antioxidant, antimicrobial, cytotoxic) of different extracts (ethanol, hexane, chloroform, ethyl acetate, butanol, remaining water phase) obtained from *A. maritima* and to analyze the compounds contained in it with HPLC-DAD.

Materials and methods

Materials. Rosmarinic acid, rutin, p-coumaric acid, resveratrol, luteolin, apigenin, campherol and chlorogenic acid were purchased from Sigma-Aldrich, 99% (St. Louis, Missouri, USA). Caffeic acid, ferulic acid, protocatechuic acid, epigallocatechin gallate, epicatechin gallate, naringenin, campherol-3-glycoside, and vanillic acid (99%) analytical grade of hydrochloric acid, HPLC grade of methanol, butanol, ethyl acetate, acetonitrile, hexane and formic acid were purchased from Merck (Darmstadt, Germany). All standard solutions were prepared in methanol.

Plant material and extraction. Aerial parts of *A. maritima* were collected near Şile District, Istanbul, (41°11'03.6′ N and 29°30'27.7′ E) in June 2020. The plant was authenticated by Dr. İlker Genç at Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University (voucher number: ISTE-117218). The shade dried plant was ground, then 250 g of plant material was kept in 80% (v/v) ethanol medium for 72 hours. It was then filtered through filter paper and the solvent was removed in a rotary evaporator at 40 °C. To the remainder, 60 mL of deionized water was added and the mixture was successively extracted with organic solvents of hexane, chloroform, ethyl acetate and butanol [8].

Determination of Total Phenolic Content. Gallic acid was used as a standard substance in total phenolic substance determination studies. Gallic acid solutions of different concentrations were prepared to create a calibration chart. Lowry A solution as 2% Na_2CO_3 (%, w/v) in 0.1 M NaOH and 0.5% $CuSO_4$ (%, w/v) in 1% $NaKC_4H_4O_6$ (%, w/v) The Lowry B solution was prepared in this way. Lowry C solution was prepared by mixing Lowry A and Lowry B at a ratio of 50 : 1 (v/v). 0.1 mL sample/standard and 1.9 mL distilled water, 2.5 mL Lowry C solution and 0.25 mL Folin-Ciocalteu reagent were added to the analysis tubes. After 30 min in the dark, absorbances of the samples and standards were measured at 750 nm. Absorbance versus gallic acid concentration was plotted and a calibration graph was drawn. Then, the absorbance of the samples was substituted in the graphic equation and the determination of the total phenolic substance was determined in mg gallic acid/g sample [9, 10].

In vitro antioxidant activity. Trolox was used as a standard substance in DPPH antioxidant capacity determination studies. Calibration chart was created using different concentrations of trolox solutions. 0.1 mL of sample/standard, 0.18 mL of 1 mM DPPH (prepared with methanol) solution was added to the analysis tubes and methanol was added to give a total volume of 3 mL. Analysis tubes were kept in the dark for 30 minutes and their absorbance was measured at 515 nm (sample). As a result of the measurements, % inhibition values were calculated for the samples.

$$\% \text{ Inhibition} = [A_{blank} - A_{sample} / A_{blank}] \times 100$$

Using the calibration chart of the standard, the antioxidant activity values of the extracts were calculated as "mg trolox/g sample" [11].

In ABTS antioxidant activity determination studies [10, 11], trolox was used as a standard substance. It was mixed with 0.746 mM ABTS and 0.245 mM $K_2S_2O_8$ in water. After 24–48 hours in the dark, ABTS solution was diluted 1 : 10. 0.1 mL of sample/standard, 3.9 mL of ethanol and 1 mL of diluted ABTS solution were added to the analysis tubes and their absorbance was measured at 734 nm after 6 minutes (Sample). As a result of the measurements, % inhibition values were calculated for the samples.

$$\% \text{ Inhibition} = [A_{blank} - A_{sample} / A_{blank}] \times 100$$

Using the calibration chart, the antioxidant activity values of the extracts were calculated as "mg trolox/g sample".

Trolox was used as a standard for the determination of antioxidant activity by the FRAP method, which is based on the reduction of Fe3+-TPTZ (ferrictripyridyltriazine) complex to Fe^{2+} with the presence of antioxidants in

acidic medium [12]. After adding 0.1 mL of sample/standard and 2.9 mL of FRAP reagent to the analysis tubes, they were kept in the dark for 30 minutes, and the absorbances of the samples/standards were read at 593 nm. Using the obtained line equation, antioxidant capacity values of the extracts were determined in mg trolox/g sample.

Quantitative Determination of Phenolic Compounds by HPLC. It used a vacuum degasser, binary pump, autosampler, and diode-array detector from an Agilent 1200 HPLC system (Waldbronn, Germany). A waters-derived XBridge C18 (4.6 mm 250 mm, i.d. 3.5 mm) column was used for the chromatographic separations. Acetonitrile 99% and 1% formic acid in water (solvent A) make up the mobile phase (solvent B). Gradient conditions are as follows: 13% B for the first 10 minutes, 41.5 B for the next 20, 70 B for the next 25, and 10 B for the final 35 min of the race. Each analysis began with a 10-minute equilibration of the column at 25 °C. The injection volume was 10 L, and the flow rate was 0.5 ml/min. Using Chemstation for LC, data gathering and preprocessing were carried out (Agilent). For protocatechuic acid, epigallocatechin gallate, epicatechin gallate, campherol-3-glycoside, vanillic acid and naringenin the monitoring wavelength was 280 nm, for resveratrol it was 306 nm, for luteolin, apigenin, 340 nm, while for rosmarinic acid, rutin, chlorogenic acid, caffeic acid, chlorogenic acid, p-coumaric acid, campherol and ferulic acid, it was 360 nm. Using standards for phenolic compounds as a guide, peaks were discovered. Retention durations and UV spectra were compared to identify the peaks.

Antimicrobial activity. Five reference strains were used in this study; two Gram-positive strains of cocci (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299), two Gram-negative bacillus strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATT 27853) and one fungus; (*Candida albicans* ATCC 90028). All strains were obtained from Istanbul University Faculty of Medicine, Department of Medical Microbiology.

MIC (minimum inhibitory concentrations) was determined by the broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) definition [13]. All bacterial strains were cultured on tryptic soy agar (OXOID, Turkey) medium, while fungus cells were cultured on Sabouraud dextrose agar (OXOID, Turkey) and incubated aerobically at 35 °C for 24–48 hours. Bacterial cultures were then suspended in sterile saline (0.85% NaCl) and adjusted to 0.5 McFarland turbidity (10^8 cfu/mL). Negative controls (medium containing only plant extract), positive controls (medium containing bacteria only), and plant extracts (5000–90 µg/mL) were placed in U-bottom, 96-well microplates at a final inoculum concentration of $1x10^5$ CFU/mL. Mueller Hinton Broth (OXOID, Turkey) and RPMI 1640 (Thermo Fischer Scientific, Turkey) were used to prepare serial dilutions of the plant extract for bacterial and fungus cells, respectively. All inoculated plates were incubated at 35 °C for 24–48 hours and MIC values were determined.

Cytotoxic Activity. In this study, NIH-3T3 (mouse embryonic fibroblast), K562 (chronic myeloid leukemia) and Saos (Human Osteosarcoma) cell lines from ATCC (American Type Culture Collection) were used. The cytotoxic effect on cells after the application of plant extracts at different concentrations for 3 days was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method [14]. After the stock solutions of the prepared extracts were prepared in DMSO (10 mg/mL), they were diluted with medium at desired concentrations and cultured with the used cells in a 5% CO_2 incubator for 3 days. At the end of the time, 10 µl of MTT (5 mg/mL) was added and 4 hours later, formazan was dissolved with isopropyl alcohol and the absorbance of the wells in the plate was measured at 570 nm in the ELISA reader. Each experiment was repeated three times.

Brine shrimp lethality assay (BSLA) is a method developed by Mclaughlin et al [15] to determine whether plant extracts of medicinal species are cytotoxic. Eggs of brine shrimp (*Artemia* sp., Artemiidae) were incubated in a culture dish containing brine (1% NaCl) for 36 hours. After 48 hours, Artemia larvae were collected from the culture dish. Plant extracts from stock solution 0; 31.25; 62.5; 125; 250; it was prepared as 500 and 1000 (µg/mL). Ten brine shrimp larvae are placed in each bottle using a plastic pipette with a 2 mm diameter tip. 24 hours later the survived were counted under a stereomicroscope. Results were analyzed with the help of Polo-PC probit package program, and LC50 values were determined. All experiments were performed three times [16].

Results and Discussion

Among the extracts obtained in our study, the highest total phenolic content was found in the ethyl acetate extract. This was followed by hexane > water phase > chloroform > ethanol > butanol, respectively. It was observed that there were differences in the total amount of phenolic substances depending on the solubility of the phenolic compounds in the structure of the plant. Accordingly, it can be interpreted that the most phenolic substance is in the ethyl acetate phase (Table 1).

The antioxidant activity results of the extracts of the *A. maritima* were determined by three different methods such as DPPH, ABTS and FRAP. The antioxidant activity results of the extracts according to the DPPH method were found as ethyl acetate > ethanol > butanol > hexane > water > chloroform, respectively, and according to ABTS and FRAP methods, ethyl acetate > water > chloroform > ethanol > butanol > hexane. When compared in general, the most effective extract in terms of total phenolic substance and antioxidant activity results is the extract obtained with ethyl acetate solvent (Table 2).

Phenolic substances found in *A. maritima* as a result of HPLC-DAD analysis; caffeic acid, rutin, p-coumaric acid, ferulic acid, rosmarinic acid, resveratrol, luteolin, apigenin, campherol, protocatechuic acid, epigallocatechin gallate, epicatechin gallate, naringenin, chlorogenic acid, campherol-3-glycoside, and vanillic acid (Fig. 1, 2). Considering the solvent extracts of different polarities prepared from the *A. maritima*, ferulic acid is present in all extracts. For the determined phenolic substances (Table 3), the highest amount of ferulic acid is 47.97±0.07 mg/g, as an example, in the ethyl acetate phase. The highest ethyl acetate extract was found in total phenolic substance and antioxidant activity results, and the highest phenolic substance in ethyl acetate extract was determined by HPLC-DAD device in terms of phenolic substance content. Therefore, spectroscopic (total phenolic substance and antioxidant activity results) and chromatographic results seem to complement each other.

Table 1. Total phenolic content of different extracts of *A. maritima*

Extract	mg gallic acid/g sample
Ethanol	309.35±16.14
Hexane	688.24±10.25
Chloroform	334.86±4.04
Ethyl acetate	954.94±38.36
Butanol	103.51±8.71
Water Phase	507.46±4.19

Table 2. Antioxidant activity of different extracts of *A. maritima* using different method

Extract	DPPH (mg Trolox/g)	ABTS (mg Trolox/g)	FRAP (mg Trolox/g)
Ethanol	601.41±8.34	98.36±1.35	74.70±1.92
Hexane	465.82±17.18	14.73±0.62	10.24±5.93
Chloroform	349.11±1.63	125.23±6.23	128.31±7.68
Ethyl acetate	604.15±11.57	347.65±8.98	486.31±9.99
Butanol	541.49±18.49	24.72±2.98	19.50±0.48
Water Phase	461.57±0.68	131.37±3.24	293.02±2.00

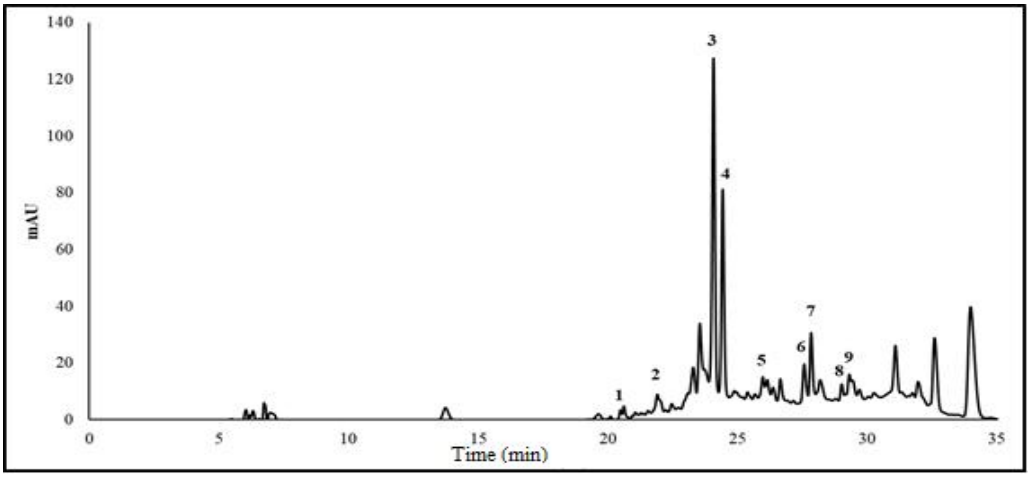

Fig. 1. HPLC-DAD chromatogram of ethanol extract (1 – caffeic acid, 2 – routine, 3 – p-coumaric acid, 4 – ferulic acid, 5 – rosmarinic acid, 6 – resveratrol, 7 – luteolin, 8 – apigenin, 9 – campherol)

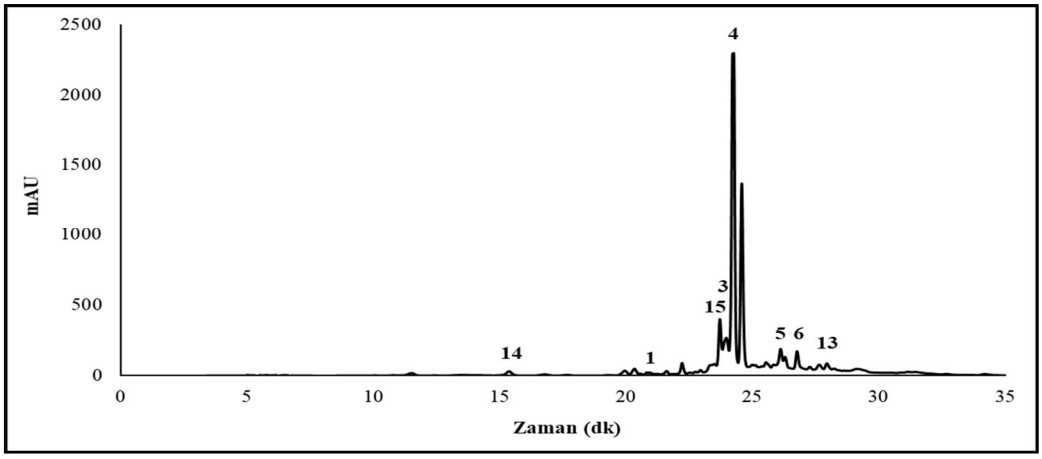

Fig. 2. HPLC-DAD chromatogram of ethyl acetate extract (1 – caffeic acid, 3 – p-coumaric acid, 4 – ferulic acid, 5 – rosmarinic acid, 6 – resveratrol, 13 – naringenin, 14 – chlorogenic acid, 15 – campherol glycoside)

Table 3. Amounts of phenolic substances in *A. maritima* extracts (mg/g sample)

Phenolic compound	Ethanol	Hexane	Chloroform	Ethyl acetate	Butanol	Water Phase
Protocatechuic acid	nd	nd	0.14±0.02	2.59±0.01	nd	0.58±0.02
Vanilic acid	nd	nd	nd	nd	nd	0.38±0.01
Epigallocatechin gallate	nd	nd	13.45±0.01	18.91±0.19	nd	nd
Epicatechin gallate	nd	nd	13.38±0.04	nd	nd	nd
Naringenin	nd	nd	12.03±0.29	9.24±0.02	nd	nd
Chlorogenic acid	nd	nd	0.15±0.01	5.45±0.33	2.19±0.08	8.19±0.04
Caffeic acid	0.79±0.01	nd	nd	1.61±0.06	nd	0.48±0.01
p-coumaric acid	0.38±0.01	nd	0.43±0.01	10.12±0.06	0.01±0.01	5.51±0.04
Ferulic acid	5.05±0.01	0.14±0.01	1.39±0.01	47.97±0.07	0.17±0.01	11.05±0.01
Rosmarinic acid	3.12±0.01	nd	3.56±0.01	16.84±0.08	nd	nd
Resveratrol	0.34±0.01	nd	nd	1.81±0.02	nd	0.20±0.01
Routine	1.04±0.01	nd	nd	nd	nd	6.52±0.05
Campherol-3-glycoside	nd	0.01±0.01	nd	40.38±0.01	nd	nd
Luteolin	1.29±0.02	nd	2.76±0.01	nd	nd	nd
Campherol	0.42±0.01	nd	1.04±0.01	nd	nd	nd
Apigenin	0.42±0.01	3.13±0.01	nd	nd	nd	nd

Mean ± standard deviation, nd: not detected

Ethanol and chloroform phases showed similar effects against bacteria and fungus (MIC 2500 µg/mL). The hexane phase was found to be more effective than the others against Gram-positive bacteria such as *S. aureus* (MIC 19 µg/mL) and *E. fecalis* (MIC 39 µg/mL) and *P. aeruginosa* bacteria (MIC 156 µg/mL). Ethyl acetate phase, on the other hand, had a specific effect for each bacteria. Contrary to what is known, the butanol phase was found to be more effective against Gram-negative rods (MIC 625 µg/mL and 39 µg/mL) than against Gram-positive bacteria (MIC 1250 µg/mL). The water phase had a stable MIC (625 µg/mL) against all strains (Table 4).

In the MTT test, ethanol and chloroform phases showed strong cytotoxic effects against K562 and Saos cells (240, 280 and 210, 240 µg/mL, respectively). Although the ethyl acetate phase was less than the other two extracts, it still showed strong activity (470, 360 µg/mL). To a lesser extent, hexane, water and butanol phases were effective. Against the NIH-3T3 cell, the activity of the ethanol> ethyl acetate> chloroform phases, respectively, is very low. The hexane, butanol and water phases showed no cytotoxicity (IC50 > 2.5 mg/mL) (Table 5). It was determined that the extracts showed different levels of cytotoxicity in K562 and Saos cell lines, and the extracts were not toxic against healthy cells.

In Brine shrimp toxicity assay it is seen that the number of viabilities did not change much in ethyl acetate, butanol and water phase compared to the negative group. Accordingly, cytotoxic effects of ethyl acetate, butanol and water phase extracts prepared from *A. maritima* were not observed on brine shrimp. However, ethanol, hexane and chloroform extracts had a moderate cytotoxic effect on brine shrimp (Table 6).

Table 4. MICs of *A. maritima* extracts against different microbial strains

Microorganisms	Tested plant extracts (MIC µg/mL)					
Bacteria	Ethanol	Hexane	Chloroform	Ethyl acetate	Butanol	Water Phase
S. aureus	2500	19	2500	1250	1250	625
E. faecalis	2500	312	2500	78	39	625
E. coli	2500	156	2500	625	625	625
P. aeruginosa	2500	39	2500	156	1250	625
Fungi						
C. albicans	2500	<9	2500	9	<9	625

Table 5. Cytotoxic activity (IC50) of *A. maritima* extracts against different cell lines

Extract	IC50 (µg/mL)		
	NIH-3T3	K562	Saos
Ethanol	1200	240	280
Hexane	>2500	750	560
Chloroform	1900	210	240
Ethyl acetate	1800	470	360
Butanol	>2500	820	780
Water Phase	>2500	700	790

Table 6. Results of Brine shrimp Lethality Assay of *A. maritima* extracts

Extract	LC50 (µg/mL)	Category of toxicity
Ethanol	281	Moderate toxicity
Hexane	342	Moderate toxicity
Chloroform	328	Moderate toxicity
Ethyl acetate	2426	Non-toxic
Butanol	1622	Non-toxic
Water Phase	5703	Non-toxic

Conclusion

Extracts of *A. maritima* species obtained using different solvents were found to be high in terms of total phenolic substance and antioxidant activity. It was also found to be rich in phenolic content. In our study, different phases of the extracts were effective against the selected microorganism and cell lines. This shows that it has potential as a new drug source for antimicrobial and anti-cancer drugs. High activity against microbial strains is particularly important because the development of drug resistance considered one of the major public health concerns nowadays at world. In terms of brine shrimp toxicity, more suitability or in another words safe phases of the plant extract were determined.

References

1. Exarchou V., Nenadis N., Tsimidou M., Gerothanassis I.P., Troganis A., Boskou D. *J. Agric. Food Chem.*, 2002, vol. 50(19), pp. 5294–5299. DOI: 10.1021/jf020408a.
2. Zengin G., Bulut G., Mollica A., Haznedaroglu M.Z., Dogan A., Aktumsek A. *Food Chem. Toxicol.*, 2017, vol. 107, pp. 597–608. DOI: 10.1016/j.fct.2017.03.037.
3. Karaalp C., Yurtman A.N., Karabay Yavasoglu N.U. *Pharm. Biol.*, 2009, vol. 47(1), pp. 86–91. DOI: 10.1080/13880200802448682.
4. Abdel-Rahman R.F., Alqasoumi S.I., El-Desoky A.H., Soliman G.A., Paré P.W., Hegazy M.E. *S. Afr. J. Bot.*, 2015, vol. 98, pp. 122–127. DOI: 10.1016/j.sajb.2015.02.009.
5. Başer K.H.C. *Nat. Volatiles Essent. Oils.*, 2016, vol. 1, pp. 1–14.
6. Saeidnia S., Gohari A., Mokhber-Dezfuli N., Kiuchi F. *Daru*, 2011, vol. 19(3), pp. 173–186.
7. Davis P.H., Tan K. *The Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, 1988.
8. Hossain M.A., Al-Hdhrami S.S., Weli A.M., Al-Riyami Q., Al-Sabahi J.N. *Asian Pac. J. Trop. Biomed.*, 2014, vol. 4, pp. S368–S372. DOI: 10.12980/APJTB.4.2014C1051.
9. Şahin S., Oran S., Şahintürk P., Demir C. Öztürk Ş., *J. Food Biochem.*, 2015, vol. 39(4), pp. 471–477. DOI: 10.1111/jfbc.12142.
10. Singleton V.L., Orthofer R., Lamuela-Raventós R.M. *Methods in Enzymology*, 1999, vol 299. pp. 152–178.
11. Şahin S., Işık E., Aybastıer Ö., Demir C. *J. Chemom.*, 2012, vol. 26(7), pp. 390–399. DOI: 10.1002/cem.2450.
12. Benzie I.F., Strain J.J. *Anal. Biochem.*, 1996, vol. 239(1), pp. 70–76. DOI: 10.1006/abio.1996.0292.

13. CLSI. *Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute 28th ed.* CLSI supplement M100. Wayne, PA (USA), 2018.
14. Mosmann T. *J. Immunol. Methods*, 1983, vol. 65(1-2), pp. 55–63.
15. McLaughlin, J.L., Chang, C. J., Smith D.L. *Studies in Natural Products Chemistry.* Elsevier, 1991, pp. 383–409.
16. Bussmann R.W., Malca G., Glenn A., Sharon D., Nilsen B., Parris B., Dubose D., Ruiz D., Saleda J., Martinez M., Carillo L., Walker K., Kuhlman A., Townesmith A. *J. Ethnopharmacol*. 2011, vol. 137(1), pp. 121–140. DOI: 10.1016/j.jep.2011.04.071.

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