Cumhuriyet Sci. J., 43(2) (2022) 171-175
DOI: https://doi.org/10.17776/csj.962969

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Total Phenolic Content, Free Radical Scavenging Activity and Antibacterial Activity of Some *Buplerum* Species

Hatice Taner Saraçoğlu [1,a,*], Mehtap Akın [1,b], Ahmet Ünver [2,c]

[1] *Department of Biology, Faculty of Science, Selçuk University, 42075, Konya, Türkiye*
[2] *Department of Food Engineering, Faculty of Engineering and Architecture, Necmettin Erbakan University, Konya, Türkiye*
**Corresponding author*

Research Article

History
Received: 05.07.2021
Accepted: 21.05.2022

Copyright
©2022 Faculty of Science, Sivas Cumhuriyet University

ABSTRACT

The aim of this research was to determine the total phenolic content, free radical scavenging activity and antibacterial activity of some *Bupleurum* species. In the study, twelve *Bupleurum* L. (Apiaceae) species were extracted using a mixture of methanol-water and tested for total phenolic content, free radical scavenging activity and antibacterial activity. Total phenolic content of *Bupleurum* species extracts ranged between 56.64 and 317.54 mg gallic acid equivalent (GAE)/g dry extract. The free radical scavenging activity of extracts was determined using the IC_{50} method, and the results for the samples ranged from 0.39 to 3.41 mg/ml. The antibacterial properties of *Bupleurum* species extracts were tested using the microdilution method against a total of eleven bacterial strains in this study; no inhibition was observed in the range of the investigated extract concentrations. *Bupleurum* species were contained phenolic compounds and had some free radical scavenging potential, but they did not show inhibitory impact on bacteria in the investigated extract concentration range. All the analyses were made in three replications. Results were provided as a mean of the replicates. The methods for results were looked at by utilizing the single direction and multivariate investigation of difference (MANOVA) followed by Duncan's different reach tests. The contrasts between singular methods were considered to be critical at $P < 0.05$.

Keywords: Antibacterial activity, Antioxidant activity, Bupleurum species, Extract, Microdilution.

[a] htaner@selcuk.edu.tr https://orcid.org/0000-0001-9502-3739
[b] makin@selcuk.edu.tr https://orcid.org/0000-0003-2554-236X
[c] unveraet@erbakan.edu.tr https://orcid.org/0000-0002-6310-809X

Introduction

The Apiaceae (Umbelliferae) family includes high economic and medicinal plants [1]. Extracts and essential oils of *Bupleurum* use in traditional medicine for their antiseptic and anti-inflammatory activity [2].

In Turkey, the genus *Bupleurum* of the Apiaceae (Umbelliferae) family has 49 taxa, 21 of which are endemic [3, 4]. Recent studies are generally focused on the hepatoprotective activity of the plant commonly used in China. *Bupleurum kaoi* Liu has been utilized as a main component in traditional Chinese herbal therapy to treat hepatitis. *B. kaoi* Liu (Chao et Chuang), one of numerous variants of this species, is native to Taiwan. Only the root is utilized in Chinese medicine. As aerial portions such as leaves and stems also contain bioactive components, using these tissues in the creation of health-promoting goods is both practicable and cost-effective. Leaf infusion is one of the most economically feasible leaf products [5]. *B. kaoi* Liu (Chao et Chuang) is a traditional Chinese plant that has been used as a herbal drink in the Far East for many decades [6]. *B. kaoi*, according to Lin et al. [6] and Ohtsu et al. [7] has a wide range of actions, including hepatoprotective and antioxidant properties.

Free radicals are hazardous wastes of cellular metabolism and transition-metal ions, and they appear to play a role in biomacromolecule destruction *in vivo* [8]. Antioxidants may protect our system from oxidative damage linked to diabetes, cancer, cardiovascular disease, and neurological disorders such as Alzheimer's and Parkinson's disease [9]. Several naturally occurring antioxidative substances derived from plants have been found as active oxygen scavengers, free radical inhibitors, or reducing agents *in vitro* [10, 11].

B. heldreichii, *B. sulphureum*, *B. lycaonicum* and *B. pauciradiatum*, which are among our study materials, are known as "tavşan kulağı" in our country [12, 13, 14].

B. rotundifolium species is known by the names "değirmi yapraklı tavşan kulağı", "yuvarlak yapraklı tavşan kulağı", "sarıgayşeik" and "gıcır" [15, 16, 17].

B. croceum species is known as "altuni tavşan kulağı" and "tavşan kulağı", while *B. lancifolium* is known as "sivri tavşan kulağı" and "tavşan kulağı" [18, 12, 17, 14].

Bupleurum species are not widely used in our country. There are limited studies on antimicrobial activity and antioxidant activity of Bupleurum species growing in Turkey.

The purpose of this research was to evaluate the antibacterial activity, total phenolic content and free radical scavenging activity of the methanol-water extract of some *Bupleurum* species.

Materials and Methods

Plant Materials

Twelve samples of *Bupleurum* taxa collected from Turkey (Table 1) between May and August 2009 during their blossoming period and identified by Tuna Uysal from Selcuk University in Turkey used Flora of Turkey and East Aegean Islands Book [3].

A voucher specimen was placed in the Herbarium of the Faculty of Science at Selcuk University in Turkey (HT1001 KNYA, 1011, 1008, 1006, 1005, 1010, 1007, 1003, 1004, 1002, 1014 and 1009). The aerial parts were air-dried and grinded.

Table 1. Sampled taxa of *Buplerum*

Species	Location of the samples
Buplerum rotundifolium L.	Konya: Bozkir, Cemetery of Bozkir, 1100 m, HT1001 KNYA
Buplerum falcatum L. subsp. cernuum (Ten.) Arcang.	Konya: Between Altinapa and Basarakavak, Han position, 1280 m, HT1011 KNYA
Buplerum cappadocicum Boiss.	Karaman: Nearby Sertavul Gateway, 1400 m, HT1008 KNYA
Buplerum heldreichii Boiss.&Bal.*	Konya: Karapinar, Erosion area, 960 m, HT1006 KNYA
Buplerum turcicum Snogerup*	Konya: Salt lake, Nearby Yavsan Memlehasi, 910 m, HT1005 KNYA
(Syn.) Buplerum intermedium Poiret Bupleurum subovatum Link ex Spreng.	Karaman: Ermenek, Ermenek-Kazanci road intersection, 1450 m, HT1010 KNYA
Buplerum croceum Fenzl	Konya: Altinapa, Nearby Degirmenkoy, 1200 m, HT1007 KNYA
Buplerum sulphureum Boiss.&Bal.*	Konya: Konya-Beysehir road, Quarry position, 1250 m, HT1003 KNYA
Buplerum lycaonicum Snogerup*	Konya: Konya-Beysehir road, Quarry position, 1250 m, HT1004 KNYA
Buplerum pauciradiatum Fenzl*	Karaman: Baskişla, Yaylapinar position, 1450 m, HT1002, KNYA
Buplerum zoharii Snogerup*	Icel (Mersin): Mut-Ermenek roadside, 250 m, HT1014 KNYA
Buplerum lancifolium Hornem.	Karaman: Ermenek, Ermenek-Kazanci road intersection, 1125 m, HT1009 KNYA

The Preparation of Extracts

For the separation of non-polar compounds, mainly oils, the materials (20 g) were extracted with petroleum ether using a Soxhlet device at 40-60 °C. The petroleum ether was evaporated at 40 °C. The residue was mixed with 100 ml methanol-water mixture (70% methanol + 30% water), stirred for 30 min and filtrated. The filtrate was collected and this process was repeated for three times and the collected extracts were concentrated on a rotary evaporator in vacuum at 40 °C. Then the extracts were lyophilized and stored until the analysis [19].

Antimicrobial Assay

The minimum inhibitory concentrations (MICs) of extracts, obtained by methods of microdilution identified by the National Committee for Clinical Laboratory Standards [20]. In microbiological tests, standard 11 bacterial strains that could be in humans, animals and foods as hazardous contaminants were used. These bacteria were *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 29988, *Escherichia coli* ATCC 25923, *Escherichia coli* ATCC 3166 09:K35:K99, *Streptococcus salivarius* RSHE 606, *Proteus mirabilis* ATCC 43071, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* ATCC 29853. Bacterial strains were taken from the Biotechnology Laboratory in Selcuk University. Bacterial cultures were activated for 24 hours at 37 °C in Mueller Hinton Broth (MHB, Merck). The cultures grown in the liquid medium were standardized to 10^8cfu/ml at the conclusion of the incubation period (McFarland No: 0.5). The extracts were dissolved in 25% dimethylsulfoxide (DMSO) and diluted to the maximum concentration of 4 mg/ml to be evaluated, followed by a two-fold serial of dilutions in concentrations ranging from 2 mg/ml to 1.95 g/ml. Antibacterial activity was determined using the microdilution technique. A pre-sterilized micro titration dish (Brand) with 96 "U" type wells was used for antibacterial tests. At a microtitration petri plates, serial solutions of the extracts were made. The wells were filled with 100 µL of each microbial solution. The negative control was the last well, which contained serial dilutions of antibacterial agents without microorganisms. The solvent DMSO was used as a negative control, and Chloramphenicol (Sigma) was used as a positive control.

Total Phenolic Content

The results were expressed as mg *GAE/g dry extract* using the Folin-Ciocalteu colorimetric method. As a standard

phenolic compound, gallic acid was used. In a 25 ml volumetric flask containing 9 ml distilled water, 1 ml of standard solution of gallic acid or 1 ml of diluted samples were added. Also, blank was made with addition of 1 ml of distilled water. The mixture was stirred after adding 1 ml of Folin-Ciocalteu reagent. After 5 minutes, 10 ml solution of 7% Na_2CO_3 was added, mixed thoroughly for 2 hours. At 760 nm, the absorbance was measured. Using an equation derived from a standard gallic acid graph, the content of total phenolic compounds in samples were determined as milligrams of gallic acid equivalent in gram dry extracts [21].

Free Radical Scavenging Activity

Extracts were diluted to nine concentrations (0.1, 0.3, 0.6, 0.9, 1.0, 1.5, 2.0, 3.0 and 4.0 mg/ml) with 70% methanol mixture. To make, DPPH was weighted and dissolved in ethanol 0.004% (w/v) solution. A magnetic stirrer was used to make that the mixture dissolved equally. A solution of 0.004% DPPH (1 ml) was applied on each test tube containing 50 µL dilutes. The test tubes were then placed in the dark for 30 minutes. At the same time, the blank test tubes were treated with DPPH, which contained only 70% methanol. After 30 min, the absorbances of each test tube were measured by a UV spectrophotometer at 517 nm. IC_{50} values were calculated from percent inhibition versus concentration graphs. IC_{50} (mg/ml) value is the minimum extract required to inhibit the 50% of 1,1-diphenyl-2-picrylhydrazyl [22].

Statistical Analyses

All experiments were performed in three replications. The mean of the data was recorded. The results of multivariate analysis of variance (MANOVA) and Duncan multiple range test were given in this part. Since p-values of these tests are less than 0.05, it can be concluded that the individual mean differences are statistically significant [23].

Results and Discussion

The total phenolic content of the extracts of *Bupleurum* species ranged from 56.64 to 317.54 mg GAE/g dry extract. The detected total phenolic content of *Bupleurum cappadocicum* Boiss. was the highest while it was lowest for the *Bupleurum pauciradiatum* Fenzl. The IC_{50} values of the samples have ranged between 0.39-3.41 mg/ml. The lowest free radical scavenging activity was detected for the *Bupleurum pauciradiatum* which was in correlation with total phenolic content. The total phenolic content of *B. cappadocicum*, *B. zoharii* Snogerup, and *B. lycaonicum* Snogerup was higher in comparison to other samples and their free radical scavenging activities was also higher. While the total phenolic content of *Bupleurum turcicum* Snogerup was three times of *Buplerum intermedium* Poiret, their free radical scavenging activities were similar (Table 2).

It was seen that the mixture of methanol-water extracts of twelve *Bupleurum* species, studied surprisingly did not present any antibacterial activity against the test microorganisms used in the study.

Sökmen et. al. [24, 25] studied on antimicrobial activity of *Bupleurum sulphureum* Boiss&Bal. They tested the activity of the methanol extracts of culture and methanol extract of aerial parts. They were examined through the method of Minimal Inhibitory Concentration (MIC) Test. They reported no antimicrobial activity against *B. cereus*, *E. coli*, *S. aureus* for both mentioned extracts.

Bazzaz and Haririzadeh [26] studied for the determination of antimicrobial activity of 306 plants representing 52 families. They examined the method of cylinder plate assay. According to their results the methanol extract of the total parts of *Bupleurum rotundifolium* L. had no antimicrobial activity towards to *E. coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 4027. Their findings are in accordance with our findings for the aerial parts of the *Bupleurum rotundifolium*.

Shafaghat [27] prepared the methanol extract of *B. lancifolium* plant collected from Iran. The antimicrobial activities of the extracts were determined by the disc diffusion method. It has been reported that methanol extract has antimicrobial activity against *E. coli* ATCC 25922, which we also used in our study.

Jaradat et al. [28] obtained different extract of *B. lancifolium* plant collected from Palestine. In their study, they reported that methanol extract had antibacterial activity against *E. coli* ATCC 25922 strain, which we also used in our study.

Ertan [29] studied on antimicrobial activity and antioxidant activity of *B. lycaonicum*. According to researcher's results, the methanol extract of *B. lycaonicum* had antibacterial activity against *E. coli* ATCC 25922 and antioxidant activity. However, we did not detect antibacterial activity on the same bacteria.

Tseng et. al. [5] studied the infusion prepared from the leaves of *B. kaoi*. They reported the IC_{50} of DPPH free radical scavenging activities of leaf infusion as 0.46 mg/ml. Also, they expressed as a conclusion that the leaf infusions possessed antioxidant activities and hepatoprotective capacity. Their results on free radical scavenging activity of the leaf extracts are higher than the *Bupleurum* species we studied except for *Bupleurum lycaonicum* and *Bupleurum zoharii*. The higher free radical scavenging activity may be due to the higher amounts of phenolic substances.

Wang et. al. [30] applied supercritical carbon dioxide extraction at four different pressures on ethanol extraction of roots of *B. kaoi*. They stated that the scavenging activity of 10 g/l fractions of supercritical carbon dioxide extracts on DPPH were ranged between 53%-76%. They also noticed that the phenolic content of the root extracts from 4.7 to 18.3 mg GAE/g dry extract. Their findings on scavenging activity of root extracts are lower than the results we obtained for extracts of aerial part. Besides, the total phenolic content they expressed for root extracts are lower than our findings on aerial parts of *Bupleurum* species.

There are limited studies on free radical scavenging activity and total phenolic content of Bupleurum species. Limited studies are generally focused on Bupleurum kaoi. Our results may be valuable data for the further studies.

Table 2. Total phenolic content and antioxidant activity of *Bupleurum* species

Sample	Total phenolic content (mg/g dw of extract)	DPPH (IC50)(mg/ml)
Bupleurum rotundifolium	202.39±2.57 e	1.31±0.02 e
Bupleurum falcatum subsp. cernuum	210.57±1.43 e	1.08±0.01 d
Bupleurum cappadocicum	317.54±2.52 h	0.71±0.03 b
Bupleurum heldreichii	157.54±2.06 d	1.27±0.02 e
Bupleurum turcicum	208.45±2.67 e	1.61±0.02 f
Bupleurum intermedium	73.00±0.82 b	1.61±0.03 f
Bupleurum croceum	74.82±0.57 b	2.97±0.02 h
Bupleurum sulphureum	109.66±1.47 c	2.66±0.06 g
Bupleurum lycaonicum	288.75±0.88 f	0.41±0.01 a
Bupleurum pauciradiatum	56.64±0.57 a	3.41±0.04 ı
Bupleurum zoharii	301.18±1.40 g	0.39±0.02 a
Bupleurum lancifolium	151.18±2.39 d	0.94±0.01 c

Acknowledgment

An earlier version of this study entitled by "Determination of total phenolic content, antibacterial activity and free radical scavenging activity of some *Bupleurum* species" was presented by the same authors at European Biotechnology Congress, 05-07 May, 2016, Riga, Latvia.

Conflicts of interest

All authors declare that they have no conflict of interest.

References

[1] Kubeczka K.H., Aromatic plants, basic and applied aspects. The Hague, Boston, London: Martinus Nijhoff Publishers, (1982) 165.

[2] Nose M., Amagaya S., Ogihara Y., Corticosterone secretion-inducing activity of saikosaponin metabolites formed in the alimentary trac., *Chemistry Pharmavcy Bulletin,* 37 (1989) 2736-40.

[3] Davis P.H., Turkey and the East Aegean Islands. University Press: Edinburgh, 4 (1982) 393-418.

[4] Güner A., Özhatay N., Ekim T., Başer K.H.C., Flora of Turkey and the East Aegean Islands. University Pres: Edinburgh, 11 (2000) 143-144.

[5] Tseng C.Y., Liu C.T., Chen W.L., Weng Y.M., Antioxidative properties and in vitro hepato-protective activity of *Bupleurum kaoi* leaf infusion, IFT Annual Meeting, Las Vegas, NV, July 12-16, (2004).

[6] Lin C.C., Chiu H.F., Yen M.H., Wu C.C., Chen M.F., The pharmacological and pathological studies on Taiwan folk medicine (III): The effects of *Bupleurum kaoi* and cultivated *Bupleurum falcatum* var. *komarowi*, *Amer. J. Chinese Med*., 18 (1990) 105-112.

[7] Ohtsu S., Izumi S., Iwanaga S., Ohno N., Yadomae T., Analysis of mitogenic substances in *Bupleurum chinenese* by ESR spectroscopy, *Biol. Pharm. Bull.*, 20 **(**1997) 97-100.

[8] Muller F.L., Lustgarten M.S., Jang Y., Richardson A., Van Remmen H., Trends in oxidative aging theories, *Free Radic. Biol. Med.*, 43 (2007) 477-503.

[9] Lin M.T., Beal M.F., The oxidative damage theory of aging, *Clin. Neurosci. Res.*, 2 (2003) 305-315.

[10] Yen G.C., Duh P.D., Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species, *J. Agric. Food Chem.*, 42 (1994) 629-632.

[11] Duh P.D., Antioxidant activity of burdock (*Arctium lapa* Linne): its scavenging effect on free radical and active oxygen, *J. Amer. Oil Chem. Soc.*, 75 (1998) 445-461.

[12] Anonymous, Available at: http://www2.cedgm.gov.tr/icd_raporlari/ispartaicd2008.pdf, Retrieved May 20, 2011.

[13] Bayram A., Isparta'nın korunan alanları, *Gülçevrem*, 1(1) (2007) 1-34.

[14] Yıldızbakan A., Gündoğdu E., Fakir H., Akgün C., Ulusoy H., Cehennemdere yaban hayatı geliştirme sahasında yaban keçisi *Capra aegagrus* Erxleben 1777'nin yayılışı ve habitat kullanımı, Doğu Akdeniz Ormancılık Araştırma Enstitüsü, Çevre ve Orman Bakanlığı Yayın No: 433, DOA Yayın No:59, SBN 978-605-393-100-3, Tarsus,. (2011) XI+43 s.

[15] Cansaran A., Kaya Ö.F., Contributions of the ethnobotanical investigation carried out in Amasya district of Turkey (Amasya-Center, Bağlarüstü, Boğaköy and Vermiş villages; Yassıçal and Ziyaret towns), *Biological Diversity and Conservation*, 3(2) (2010) 97-116.

[16] Kordali Ş., Zengin H., Bayburt ili buğday ekim alanlarında bulunan yabancı otların rastlama sıklığı, yoğunlukları ve topluluk oluşturma durumlarının saptanması, *Atatürk Üniv. Ziraat Fak. Derg.,* 38(1) (2007) 9-23.

[17] Yıldırım A., Ekim T., Orta Anadolu Bölgesi yabancı ot florası, *Bitki Koruma Bülteni*, 43(1-4) (2003) 92-93.

[18] Anonymous, Available at: http://www2.cedgm.gov.tr/icd_raporlari/mersinicd2006.pdf, Retrieved May 20, 2011.

[19] Tunalıer Z., Öztürk N., Koşar M., Başer K.H.C., Duman H., Kırımer N., Bazı *Sideritis* türlerinin antioksidan etki ve fenolik bileşikler yönünden incelenmesi, 14. Bitkisel İlaç Hammaddeleri Toplantısı. Bildiriler, 29-31 Mayıs 2002, Eskişehir, (2002).

[20] Wayne P.A, Performance standards for antimicrobial susceptibility testing; Ninth informational supplement, NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, (2008) 120-126.

[21] Slinkard K., Singelton V.L., Total phenolic analysis, automation and comparison with manual methods, *Amer. J. Enol. Vitic.,* 28 (1977) 49-55.

[22] Gyamfi M.A., Yonamine M., Aniya Y., Free radical scavenging action of medical herbs from Ghane: *Thonningia sanguinea* on experimentally-induced liver injuries, *General Pharm.*, 32(6) (1999) 661-667.

[23] Püskülcü H., İkiz F., Introduction statistic. Bornova-İzmir, Turkey: Bilgehan Press , (in Turkish) (1989).

[24] Sökmen A., Jones B.M., Erturk M., Antimicrobial activity of extract from the cell cultures of some Turkish medicinal plants, *Phytother. Res.*, 13 (1999) 355-357.

[25] Sökmen A., Jones B.M., Erturk M., The in vitro antibacterial activity of Turkish medicinal plants, *J. Ethnopharmcol.*, 67 (1999) 79-86.

[26] Bazzaz B.S.F., Haririzadeh G., Screening of Iranian plants for antimicrobial activity, *Pharm. Biol.*, 41(8) (2003) 573-583.

[27] Shafaghat A., Antioxidant, antimicrobial activities and fatty acid components of leaf and seed of *Bupleurum lancifolium* Hornem., *Journal of Medicinal Plants Research*, 5(16) (2011) 3758-3762.

[28] Jaradat N., Al-Masri M., Al-Rimawi F., Zaid A.N., Saboba M.M., Hussein F., Aker A., Qasem D., Hejazi S.. Investigating the impacts of various solvents fractions of *Bupleurum lancifolium* on the antimicrobial and antioxidant potentials, *J. Intercult Ethnopharmacol.*, 6(4) (2017) 401-406.

[29] Ertan S., *Bupleurum lycaonicum* Snogerup bitki türünün biyolojik aktivitesi, Yüksek lisans tezi, Gazi Üniversitesi, Fen Bilimleri Enstitüsü, (2017).

[30] Wang B.J., Liu C.T., Tseng C.Y., Yu Z.R., Antioxidant activity of *Bupleurum kaoi* Liu (Chao et Chuang) fractions fractionated by supercritical CO_2, *LWT-Food Sci Tech.*, 38 (2005) 281-287.