Original Article

The Total Phenolic Contents and Antioxidant Activities of Endemic Species *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis (Lamiaceae) from Turkey

Emel Sönmez*, Yavuz Bülent Köse

Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 26470 Eskişehir, TURKEY.

ABSTRACT

Background: *Ajuga* species are anthelmintic, diuretic, antifungal, and antimycobacterial agents.Lamiaceae members *Ajuga postii* Briq. and *Ajuga relicta* P.H.Davis are local endemic species of Turkey. **Aim:** To give information about the total phenolic contents and antioxidant activities of the species. **Methods:** The total phenolic contents of the extracts have been quantified with Folin Ciocalteu colorimetric method, and the antioxidant activities of the extracts have been tested with DPPH, ABTS, and β-carotene / Linoleic acid assays. **Results:** Deodorized water extracts of the two species have been observed with the highest phenolic contents. 70% methanol extracts of *A. relicta* have the highest rates for the three antioxidant activity tests. However, different extracts of *A. postii* have been observed with different activity rates in all three antioxidant assays. **Conclusion:** The species are in restricted areas and can become extinct over time, therefore important of the study increases.

Key words: ABTS, Ajuga, β-Carotene / Linoleic Acid Assay, DPPH, Total Phenolic Content.

INTRODUCTION

Reactive oxygen species (ROS) are natural byproducts of oxygen for instance peroxides, superoxide, hydroxyl radical and singlet oxygen, and cause to cancer, age-related and pathogenesis diseases.[1] These destructive effects caused by oxygen are eliminated with antioxidants. Plant species have phenolic compounds and act as antioxidant agents.[2] Phenols, alkaloids, triterpenoids result from the secondary metabolites of the plants and protect the plants against pathogens, UV radiation and herbivorous.[3] As antioxidants, not only phenolic compounds got from plants are used but also produced products. BHT (Butylatedhydroxytoluene) and BHA (Butylatedhydroxyanisole) are the produced antioxidants, but they cause damage to the liver.[4]

Lamiaceae is known as the antioxidant and antimicrobial agent. Therefore, the species of the family are used in pharmacy and cosmetic industry,[5] and as antibacterial agents to extend the shelf life of the meat and fish products.[3] *Ajuga* L. is one of the members of Lamiaceae. *Ajuga* is used as anthelmintic, diuretic, antifungal, anti-inflammatory and antimycobacterial agents; used for fever, toothache, dysentery, malaria, hypertension, diabetes and gastrointestinal disorders in traditional medicine.[6-10] Thus, discussed effects result from chemical contents of the species. The found chemicals are phytoecdysteroids, diterpenoids, triterpenes, anthocyanidin-glucosides, iridoid glycosides, flavonoids and essential oils.[11] *Ajuga* has represented to 13 species and 10 subspecies in Turkey.[12]

Ajuga postii Briq. and *Ajuga relicta* P.H.Davis are local endemic species of Turkey. The species were studied for iridoid glucoside reptoside,[13] four steroids, two triterpenoids, two diterpenoids, monoterpene, and iridoid.[14] In the present study, the total

Submission Date: 15-03-2017;
Revision Date: 23-03-2017;
Accepted Date: 10-04-2017

DOI: 10.5530/ijper.51.4.103

Correspondence:
Emel Sönmez,
Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 26470, Eskişehir, TURKEY.
Tel: +90 (222) 335 0580 / 3703;
Fax: +90(222) 335 0750
E-mail: emels@anadolu.edu.tr

www.ijper.org

phenolic contents and antioxidant activities of *A. postii* and *A. relicta* extracts have been studied for the first time. The important of the study is taxa are found in limited areas and can become extinct in the years. The aim of the paper is to give information about the total phenolic contents and antioxidant activities of the species for the pharmacological studies.

MATERIALS AND METHODS

Plant materials

The aerial parts of *A. postii* were collected from İçel: Çamlıyayla, Namrun Castle, 1350 m, K 37° 16.8' 54.5" D 34° 60.1' 16.5"(10.07.2015) and aerial parts of *A. relicta* were collected from Kahramanmaraş: Çimen Mountain, Yavşan Hill, Pekmezpınarı, 1500 m, K 37° 28' 42" D 36° 42' 21"(03.06.2015). The plant materials were dried at room temperature in the shadows.

Preparation of the extracts

Powdered dried aerial parts of the plants were extracted in Soxhlet apparatus for hexane, ethyl acetate, and methanol about 8 hours for each solvent. The extracts were concentrated in rotavapor (<40°C). 70% methanol extracts and deodorized waters of plant materials were lyophilized. All the extracts were stored at -20°C until the analyses (1A (hexane extract of *A. relicta*), 1B (ethyl acetate extract of *A. relicta*), 1C (methanol extract of *A. relicta*), 2A (70% methanol extract of *A. relicta*), 2B (deodorized water of *A. relicta*), 3A (hexane extract of *A. postii*), 3B (ethyl acetate extract of *A. postii*), 3C (methanol extract of *A. postii*), 3D (70% methanol extract of *A. postii*), 3E (deodorized water of *A. postii*).

Determination of the total phenolic contents

The total phenolic contents of the extracts were quantified with using Folin-Ciocalteu colorimetric method. All samples and gallic acid as standard were dissolved in the methanol. 50 µL of the sample, 4 ml of distilled water, 250 µL Folin-Ciocalteu reagents and 750 µL sodium carbonate solution (Na_2CO_3) were incubated at room temperature for 2 h. The sample absorbances were read at 760 nm in the spectrophotometer. The amount of the total phenolic contents were calculated as milligrams of the gallic acid equivalents (mg GAE/g extract).

Antioxidant activity

DPPH• radical scavenging activity

The effects of the samples on 1,1-diphenyl-2-pipicryl-hydrazyl (DPPH) radical was estimated according to Uysaland Aktumsek 2 mg / 25 mL DPPH• was prepared in methanol.[2] Diluted samples and DPPH solution was read at 517 nm in the spectrophotometer after a 30 min incubation at room temperature in the dark. The ability to scavenge the DPPH radical was calculated with the following equation:

DPPH• Scavenging Effect % = $[(A_0 - A_1)/A_0] \times 100$

A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} values are concentrations of extracts causing 50% inhibition of DPPH radical. Lower IC_{50} value shows high antioxidant activity.

ABTS•+ radical scavenging activity

ABTS•+ was produced by 7 mM ABTS (2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulfonate) with 2.5 mM sodium persulfate ($Na_2S_2O_8$) and mixture to stand for 12-16 hours in the dark at room temperature. ABTS•+ solution was diluted with ethanol to be absorbance 0.8 to 0.7 at 734 nm in the spectrophotometer. 10 µL sample solution was mixed with 990 µL ABTS•+ solution. The absorbances of samples were read at 734 nm in the spectrophotometer after 30 min incubation at room temperature.[15]

β-carotene / Linoleic acid assay

1 mg β-carotene was dissolved in 1 ml chloroform, 120 mg linoleic acid and 1200 mg Tween 20 were added. Chloroform was removed using rotavapor, the mixture was concentrated with nitrogen for 10 min. Adding 300 ml distilled water was shaken for 10 min. The absorbance of the mixture was measured at 470 nm. Antioxidant activities of the extracts were compared with butylated hydroxytoluene (BHT).[16]

RESULTS

The total phenolic contents

In the present study, hexane, ethyl acetate, methanol, 70% methanol extracts and deodorized waters of two *Ajuga* species were investigated. The total phenolic contents of *A. postii* and *A. relicta* were determined with Folin-Ciocalteu method with equivalent to gallic acid. Orders of the extracts from small to large were hexane extract of *A. relicta*< ethyl acetate extract of *A. relicta*< hexane extract of *A. postii*< ethyl acetate extract of *A. postii*< methanol extract of *A. relicta*< methanol extract of *A. postii*< 70% methanol extract of *A. postii*< 70% methanol extract of *A. relicta*<

deodorized water of *A. relicta*< deodorized water of *A. postii* (Figure 1).

Antioxidant activity

The antioxidant activities of the two *Ajuga* species were determined with DPPH•, ABTS•+ free radical scavenging, and β-carotene/Linoleic acid assays. In DPPH experiment, the results were given as IC_{50} values at tested concentrations and compared with standard gallic acid values. Low IC_{50} values have high antioxidant activities in DPPH• scavenging activity. The orders of the extracts were GA >70% methanol extract of *A. relicta*>deodorized water of *A. postii*>70% methanol extract of *A. postii*>methanol extract of *A. relicta*>hexane extract of *A. postii*>methanol extract of *A. postii*>deodorized water of *A. relicta*>hexane extract of *A. relicta*>ethyl acetate extract of *A. relicta*= ethyl acetate extract of *A. postii* (Figure 2).

ABTS•+ (TEAC) assay was applied to figure ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) free radical scavenging activities of the extracts. In the present paper, Butylated hydroxytoluene (BHT) and gallic acid (GA) were used to control. The orders of the extracts were BHT = GA >70% methanol extract of *A. relicta*>70% methanol extract of *A. postii*>deodorized water of *A. relicta*>methanol extract of *A. relicta*>-methanol extract of *A. postii*>ethyl acetate extract of *A. postii*>hexane extract of *A.postii*>deodorized water of *A. postii*>ethyl acetate extract of *A. relicta*>hexane extract of *A. relicta* (Figure 3).

Linoleic acid is a model lipid for peroxidation. Prevention of the β-carotene fading result from the high antioxidant activity. Antioxidant values of the studied extracts were deodorized water of *A. relicta*<deodorized water of *A. postii*<hexane extract of *A. relicta*<ethyl acetate extract of *A. relicta*<ethyl acetate extract of *A. postii*<70% methanol extract of *A. postii*<methanol extract of *A. postii*<hexane extract of *A. postii*<methanol extract of *A. relicta*<70% methanol extract of *A. relicta*< BHT (Figure 4).

DISCUSSION

The total phenolic contents

The phenols are the secondary metabolites of the plants, are good at scavenging of the free radicals.[1] In the present study, the highest total phenolic contents were determined on deodorized waters of *A. postii* and *A. relicta* (Figure 1). In the other study, extracts of *A. orientalis* L. were investigated for the total phenolic contents and antioxidant activities,[17] the highest phenolic content and antioxidant activities were found in methanol extracts.

In the literature, the total phenolic content of the methanolic extract of *A. iva* L. was reported 26.86 (mg GAE/g extract).[4] In the present study, the results of the phenolic contents of the methanolic extracts were

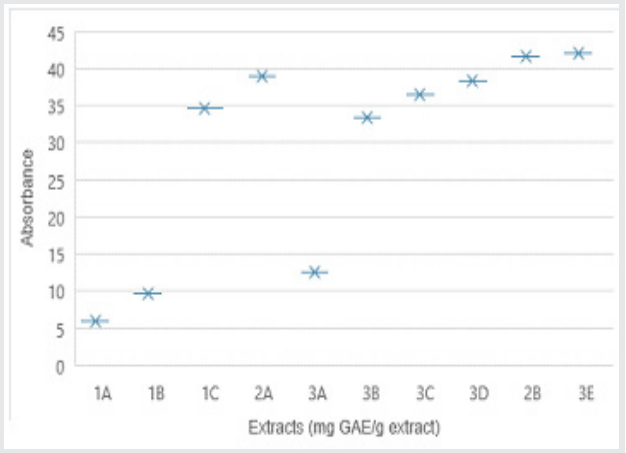


Figure 1: The amount of the total phenolic contents of *Ajuga* extracts.
1A (hexane extract of A. relicta), 1B (ethyl acetate extract of A. relicta), 1C (methanol extract of A. relicta), 2A (70% methanol extract of A. relicta), 3A (hexane extract of A. postii), 3B (ethyl acetate extract of A. postii), 3C (methanol extract of A. postii), 3D (70% methanol extract of A. postii), 2B (deodorized water of A. relicta), 3E (deodorized water of A. postii)

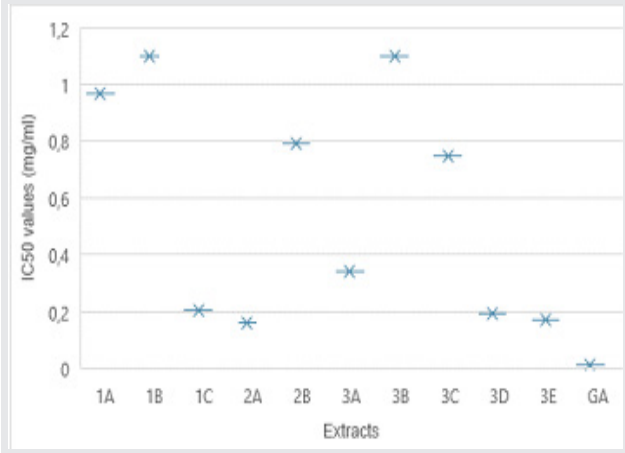


Figure 2: DPPH• free radical scavenging effects of *Ajuga* extracts.
1A (hexane extract of A. relicta), 1B (ethyl acetate extract of A. relicta), 1C (methanol extract of A. relicta), 2A (70% methanol extract of A. relicta), 2B (deodorized water of A. relicta), 3A (hexane extract of A. postii), 3B (ethyl acetate extract of A. postii), 3C (methanol extract of A. postii), 3D (70% methanol extract of A. postii), 3E (deodorized water of A. postii), GA (Gallic acid)

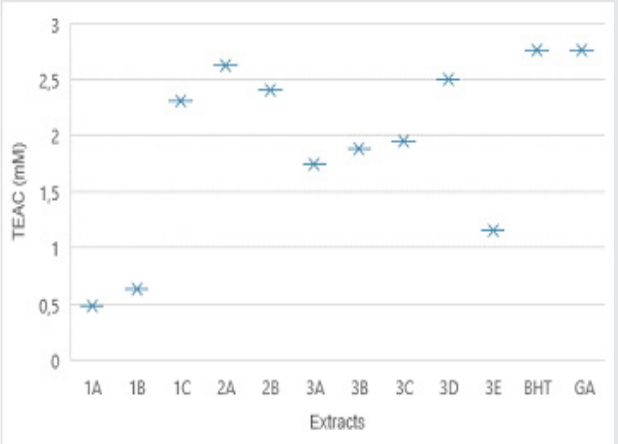


Figure 3: The results of ABTS·+.
1A (hexane extract of A. relicta), 1B (ethyl acetate extract of A. relicta), 1C (methanol extract of A. relicta), 2A (70% methanol extract of A. relicta), 2B (deodorized water of A. relicta), 3A (hexane extract of A. postii), 3B (ethyl acetate extract of A. postii), 3C (methanol extract of A. postii), 3D (70% methanol extract of A. postii), 3E (deodorized water of A. postii), BHT (Butylatedhydroxytoluene), GA (Gallic acid).

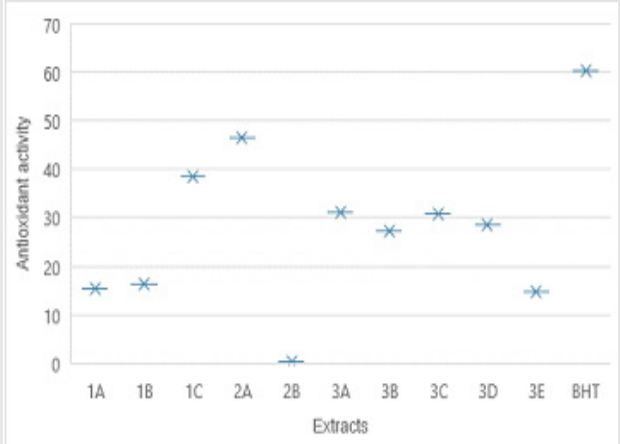


Figure 4: Antioxidant activities of the *Ajuga* extracts in β-carotene-Linoleic acid test.
1A (hexane extract of A. relicta), 1B (ethyl acetate extract of A. relicta), 1C (methanol extract of A. relicta), 2A (70% methanol extract of A. relicta), 2B (deodorized water of A. relicta), 3A (hexane extract of A. postii), 3B (ethyl acetate extract of A. postii), 3C (methanol extract of A. postii), 3D (70% methanol extract of A. postii), 3E (deodorized water of A. postii), BHT (Butylatedhydroxytoluene)

34.6 (*A. relicta*) (mg GAE/g extract) and 36.5 (*A. postii*) (mg GAE/g extract). In the other study, the increase in the antioxidant activity was associated with the higher content of phenolic substances.[1] Thus, the phenolic contents result from biological and environmental conditions.[18]

Antioxidant activity

The plants have complex phytochemical compounds so multiple antioxidant activity assays were performed in the present study. DPPH• method is used for anion radicals and ABTS•+ is used for cation radicals. DPPH• is a stable free radical, the largest absorbance occurs at 517 nm and when faced with antioxidants free radicals scavenged and their absorbances decrease.[19] Scavenging activity of DPPH radical was tested in the earlier study for *A. iva* and found result of IC_{50} of methanolic extract was 1.168 mg/ml and was 0.72 mg/ml for essential oil of *A. pseudoiva* Rob.[4,19] In the present paper, IC_{50} values were 0.205 mg/ml (*A. relicta*) and 0.75 mg/ml (*A. postii*). Hence, studied extracts had high antioxidant activities. In the other study, the water extract of *A. chamaepitys* (L.) Schreber had the highest result.[1] In the present study, 70% methanol extract of *A. relicta* and deodorized water extract of *A. postii* had the highest values.

ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) is the other free radical. In the ABTS•+ (TEAC) scavenging assay, the highest ABTS•+ results were observed in 70% methanol extracts of the two species. In the other study, ABTS•+ radical scavenging capacities of the methanol, water, and chloroform extracts of *A. chamaepitys* were studied, and the highest rate was found in the water extract.[1]

In the β-carotene-Linoleic acid assay, colored β-carotene results from the high antioxidant activity. In the present study, 70% methanol extract of *A. relicta* and hexane extract of *A. postii* had the highest rates in the experiment. In the other study, as the essential oil concentration of *A. pseudoiva* increased, the β-carotene-Linoleic acid activity also increased.[19]

CONCLUSION

To the best of our knowledge, the total phenolic contents and antioxidant activities of *A. postii* and *A. relicta* have not been reported. The results of the study show deodorized water extracts of *A. postii* and *A. relicta* have the highest phenolic contents. In the DPPH• assay, the highest value for *A. postii* is deodorized water extract (3E) and for *A. relicta* is 70% methanol extract. In the TEAC assay, 70% methanol extracts of *A. postii* (3D)and *A. relicta* (2A)have the highest rates and in the Beta-carotene / Linoleic acid inhibition test, the highest values are 70% methanol extract of *A. relicta* (2A) and hexane extract of *A. postii* (3A). In the literature studies, antioxidant activity assays give the similar results with the total phenolic contents of the plants, but in the present study the same results have not been observed. In the present paper, the highest phenolic contents have

been observed in deodorized water extracts of *A. postii* and *A. relicta*, in the antioxidant activity assays 70% methanol extracts of *A. relicta* have the highest rates for all three tests, but different extracts of *A. postii* have the highest values for the three antioxidant assays.

ACKNOWLEDGEMENT

The study has been supported by Anadolu University Scientific Research Committee (Project No. 1501S009). Also, the authors are thankful to Dr. Fatih Göger.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

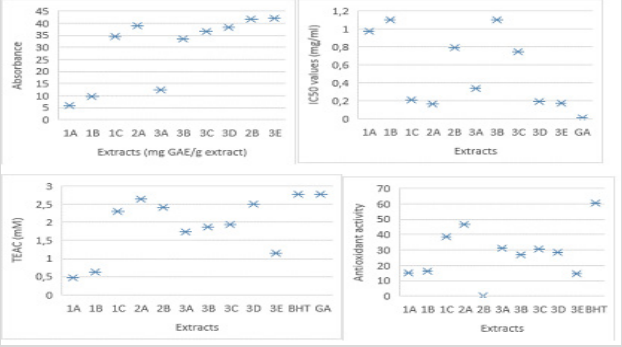
ABBREVIATION USED

µL: Microliter (10^{-6} litre); **ABTS•+:** 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate); **BHT:** Butylated hydroxytoluene; **DPPH•**: 1,1-diphenyl-2-pipicrylhydrazyl; **g:** Gram; **GAE:** Gallic acid equvalent; **h:** Hour; **IC_{50}:** The concentration of an inhibitor where the response (or binding) is reduced by half; **mg:** Milligram; **ml:** Milliliter; **mM**: Millimolar; **nm:** Nanometer; **TEAC**: Trolox equivalent antioxidant capacity; **Tween® 20:** Polyethylene glycol sorbitan monolaurate.

REFERENCES

1. Turkoglu S, Turkoglu I, Kahyaoglu M, Celık S. Determination of antimicrobial and antioxidant activities of Turkish endemic *Ajuga chamaepitys* (L.) Schreber subsp. euphratica P.H. Davis (Lamiaceae). J Med Plants Res. 2010;4:1260-8.
2. Uysal S, Aktumsek A. A phytochemical study on *Potentilla anatolica*: An endemic Turkish plant. Ind Crops Prod. 2015;76:1001-7. https://doi.org/10.1016/j.indcrop.2015.08.017.
3. Trivellini A, Lucchesini M, Maggini R, Mosadegh H, Villamarin TSS, Vernieri P *et al*. Lamiaceae phenols as multifaceted compounds: bioactivity, industrial prospects and role of "positive-stress". Ind Crops Prod. 2016;83:241-54. https://doi.org/10.1016/j.indcrop.2015.12.039.
4. Khaled-Khodja N, Boulekbache-Makhlouf L, Madani K. Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. Ind Crops Prod. 2014;61:41-8. https://doi.org/10.1016/j.indcrop.2014.06.037.
5. Draughon FA. Use of botanicals as biopreservatives in foods. Food Technol. 2004;58:20-8.
6. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol. 1997;58(1):45-54. https://doi.org/10.1016/S0378-8741(97)00077-9.
7. Muthaura CN, Rukunga GM, Chhabra SC, Mungai GM, Njagi ENM. Traditional Phytotherapy of Some Remedies Used in Treatment of Malaria in Meru District of Kenya. S Afr J Bot. 2007;73(3):402-11. https://doi.org/10.1016/j.sajb.2007.03.004.
8. Sarac N, Ugur A. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. EurAsia J Bio Sci. 2007;4:28-37.
9. Güneş F, Özhatay N. An ethnobotanical study from Kars (Eastern) Turkey. Bio Di Con. 2011;4(1):30-41.
10. Keter LK, Mutiso PC. Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya. J Ethno Pharmacol. 2012;139(1):74-80. https://doi.org/10.1016/j.jep.2011.10.014 ; PMid:22020309.
11. Israili ZH, Lyoussi B. Ethnopharmacology of the Plants of Genus Ajuga. Pak J Pharm Sci. 2009;22(4):425-62. PMid:19783524.
12. Gören AC, Zhou BN, Topcu G, Kökdil G, Kingston DGI. DNA Damaging Activities of Methanol Extract of *Ajuga postii* and Iridoid Glucoside Reptoside. Nat Prod Rep. 2005;19(5):457-60. https://doi.org/10.1080/14786410412331272095 ; PMid:15938192.
13. Kökdil G, Topcu G, Goren AC, Voelterd W. Steroids and Terpenoids from *Ajuga relicta*. Z Naturforsch B. 2002;57(8):957-60.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free RadicBiol Med. 1999;26(9):1231-7. https://doi.org/10.1016/S0891-5849(98)00315-3.
15. Karaosmanoğlu H, Soyer F, Ozen B, Tokatlı F. Antimicrobial and Antioxidant Activities of Turkish Extra Virgin Olive Oils. J Agric Food Chem. 2010;58:8238-45. https://doi.org/10.1021/jf1012105 ; PMid:20604567.
16. Göger F, Köse YB, Göger G, Demirci F. Phytochemical characterization of phenolics by LC-MS/MS and biological evaluation of *Ajuga orientalis* from Turkey. Bangladesh J Pharmaco. 2015;10(3):639-44. https://doi.org/10.3329/bjp.v10i3.23500.
17. Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A *et al*. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C R Biol. 2008;331(11):865-73. https://doi.org/10.1016/j.crvi.2008.07.024 ; PMid:18940702.
18. Mansour MB, Balti R, Rabaoui L, Bougatef A, Guerfel M. Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil from south Tunisian *Ajuga pseudoiva* Rob. Lamiaceae. Process Biochem. 2013;48(4):723-9. https://doi.org/10.1016/j.procbio.2013.02.022.

PICTORIAL ABSTRACT

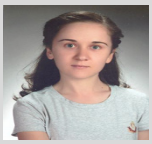


SUMMARY

- The studied Ajuga species are endemic to Turkey.
- The total phenolic contents and antioxidant activities of the species were studied.
- Results of the phenolic contents and antioxidant activities of the species could be used for the pharmacological studies.

About Authors

Dr. Yavuz Bülent Köse: Received his MSc degree in 2001 from Osmangazi University Graduate School of Science. He received his PhD from Anadolu University Graduate School of Science. Now he is Assoc. Prof. Dr. in the Department of Pharmaceutical Botany at Faculty of Pharmacy/Anadolu University. He has published about 40 academic papers in reputed journals.



Emel Sönmez: Has received her MSc from Department of Pharmaceutical Botany at Faculty of Pharmacy/Anadolu University. Now she is pursuing PhD studies in the same department.

Cite this article: Sönmez E, Köse YB. The total phenolic contents and antioxidant activities of endemic species Ajuga postii Briq. and Ajuga relicta P.H.Davis (Lamiaceae) from Turkey. Indian J of Pharmaceutical Education and Research. 2017;51(4):700-5.