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ORIGINAL ARTICLE

A Comparative Study on the *in vitro* Antioxidant and Antimicrobial Potentials of Three Endemic *Ononis* L. Species from Turkey

Türkiye'den Üç Endemik *Ononis* L. Türünün *in vitro* Antioksidan ve Antimikrobiyal Potansiyelleri Üzerine Karşılaştırmalı Bir Çalışma

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ABSTRACT

Objectives: In this study, the antioxidant capacity, antimicrobial activity and phenolic contents of aerial parts and roots extracts of three endemic *Ononis* L. (Leguminosae) species (*O. sessilifolia* Bornm., *O. basiadnata* Hub. & Mor., *O. macrosperma* Hub. & Mor.) were investigated for the first time.

Materials and Methods: The phenolic contents of the extracts [water and ethanol (EtOH)] and fractions [dichloromethane, EtOAc and *n*-butanol] were determined using Folin-Ciocalteu's phenol reagent. Also, their antioxidant capacities were studied using qualitative $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhdrazyl radical) and TBA assays. The antimicrobial activity of these extracts and fractions compared with standard antibiotics were studied using disc diffusion assays against various Gram-positive and Gram-negative bacteria and fungi.

Results: The total phenolic contents of the water extracts were found to range between 14.78-80.33 mg/g, and the EtOH extracts ranged from 67.19-145.33 mg/g. EtOAc fractions of the three species were rich in terms of total phenolic contents when compared with other extracts (242.56-620.89 mg/g). The most significant results in the TBA assays were obtained in EtOH extracts of *O. macrosperma* (IC_{50}=0.13±0.17 µg/mL), *O. sessilifolia* (IC_{50}=1.41±0.58 µg/mL) and root (IC_{50}=1.96±0.39 µg/mL).

Conclusion: EtOAc fractions rich in phenolic content were also found to be the most effective in antioxidant activity assays. Although all water extracts had no antimicrobial activity, EtOH extracts and *n*-butanol fractions showed generally moderate activity against bacteria. Some EtOAc fractions except for *O. sessilifolia* showed less activity against *Escherichia coli*, *Staphylococcus aureus*, *MRSA* and *Candida albicans*.

Key words: *Ononis sessilifolia*, *Ononis basiadnata*, *Ononis macrosperma*, antioxidant capacity, antimicrobial activity, endemic

ÖZ

Amaç: Bu çalışmada üç endemik *Ononis* L. (Leguminosae) türünün (*O. sessilifolia* Bornm., *O. basiadnata* Hub. & Mor., *O. macrosperma* Hub. & Mor.) antimikrobiyal aktivite, antioksidan kapasite ve fenolik içerikleri ilk kez araştırılmıştır.

Gereç ve Yöntemler: Ekstrelerin [su ve etanol (EtOH)] ve fraksiyonların [diklorometan, etil astetat (EtOAc) ve *n*-butanol] fenolik içerikleri Folin-Ciocalteu'nun fenol reaktifi kullanılarak belirlendi. Ayrıca, antioksidan kapasiteleri kalitatif $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhdrazyl radical) ve TBA deneyleri ile çalışıldı. Ekstre ve fraksiyonların antimikrobiyal aktivitesi disk difüzyon tekniği kullanılarak standart antibiyotiklere kıyaslanarak çeşitli Gram-pozitif, Gram-negatif ve mantara karşı çalışıldı.

Bulgular: Su ekstrelerinin toplam fenol içerikleri 14.78-80.33 mg/g aralığında değişirken, EtOH ekstreleri 67.19-145.33 mg/g aralığında bulunmuştur. Üç türün EtOAc fraksiyonları, diğer ekstrelerle (242.56-620.89 mg/g) karşılaştırıldığında toplam fenol içeriği bakımından zengindir. TBA metodundaki en önemli sonuçlar, *O. macrosperma* herba (IC_{50}=0.13±0.17 µg/mL), *O. sessilifolia* herba (IC_{50}=1.41±0.58 µg/mL) ve kök (IC_{50}=1.96±0,39 µg/mL) EtOH ekstraktlarında elde edildi.

Sonuç: Fenolik içerik bakımından zengin olan elilasetat fraksiyonları, antioksidan aktivite deneylerinde de en yüksek etkili olarak tespit edilmiştir. Su ekstrelerinin antimikrobiyal aktivitesi olmamasına rağmen, türlerin EtOH ekstreleri ve *n*-butanol fraksiyonları, bakterilere karşı genellikle orta düzeyde etkinlik gösterdi. *O. sessilifolia* dışında bazı EtOAc fraksiyonları, *Escherichia coli*, *Staphylococcus aureus*, *MRSA* ve *Candida albicans*'a karşı daha az aktivite gösterdi.

Anahtar kelimeler: *Ononis sessilifolia*, *Ononis basiadnata*, *Ononis macrosperma*, antioksidan kapasite, antimikrobiyal aktivite, endemik

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INTRODUCTION

Antioxidants have been recognized as potential therapeutics for preventing different human diseases (e.g., cancer, aging, cardiovascular diseases, asthma, acute central nervous system injury, neurodegenerative disease, and malaria).[1,2] Medicinal plants and herbs have played an important role in the health care of ancient and modern cultures. They are promising as natural antioxidant sources.[3,4] The number of studies on new natural antioxidants with plant origin is increasing.[5] *Ononis* L. genus (Leguminosae) is represented by 18 species, 4 of which are endemic to Turkey.[6] The *Ononis* species has various pharmacologic properties, such as antioxidant, aperient, diuretic, antimicrobial, analgesic, antiviral, cytotoxic, anti-inflammatory and anti-diarrheal activities. In Turkish folk medicine, *O. spinosa* L. has been used for the urinary tract, kidney stones, inflammatory diseases, wounds healing, and skin disorders.[1,7-9] Süntar et al.[10] reported that water and ethanolic extracts of the herb *O. macrosperma* demonstrated the highest activity in both wound models and anti-inflammatory activity. In previous studies, some *Ononis* species were found to contain different component groups such as isoflavone, triterpene, sterol, pterocarpane, and resorcinol derivatives, flavonoids, isocoumarins, and hydroxycinnamic acids.[1,11-13] A $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhdrazyl radical) radical is a stable radical with maximum absorbance at 517 nm, and when reduced to hydrazine derivatives by electron and hydrogen atom transfer from substances with antioxidant properties, its absorbance is decreased.[14] Lipid peroxidation is a chain reaction that causes the deterioration of biologic systems, and is the accumulative effect of reactive oxygen species. Reactive free radicals start the reaction by the deletion of allylic hydrogen atoms from the methylene group of unsaturated fatty acids.[15] Thiobarbituric acid (TBA) and DPPH methods have been used to evaluate the antioxidant capacities of the plant extracts/component.[16] Medicinal plants represent potential sources of natural antioxidant and antimicrobial agents for food and medicinal purposes. The purpose of this investigation was to study antioxidant activities using TBA and DPPH [thin layer chromatography (TLC) screening], determine phenolic contents using spectrophotometry, and the antimicrobial activity with disc diffusion assays of herb and root extracts of three *Ononis* species. This is the first study on the antioxidant capacity and antimicrobial activities of the three *Ononis* species.

MATERIALS AND METHODS

Plant materials

Ononis species were gathered from different provinces of Turkey in their natural habitats. Voucher specimens were stored in the Herbarium of the Faculty of Pharmacy at the University of Ankara, Turkey (AEF). *O. sessilifolia* was collected from the Çamardı county of Niğde in June 2007 (AEF 23979); *O. basiadnata* was collected from the Gülnar county of İçel in June 2007 (AEF 23968); and *O. macrosperma* was collected from the Elmalı county of Antalya in May 2008 (AEF 24698).

Extraction of plants

The herb and root of the *O. basiadnata* and *O. sessilifolia* and the herb of *O. macrosperma* were powdered and then 50 g of the herbs and roots were macerated separately with 500 mL of water for 5 h at 60°C. Afterwards, the water extracts were filtered, frozen, and lyophilized. One hundred grams of plant material was macerated with ethanol (EtOH) for 5 h at 50°C. The extracts were then filtered and evaporated until dry. Ethanolic extracts were dispersed in methanol: water (1:9), partitioned with dichloromethane (DCM), ethyl acetate (EtOAc) and *n*-butanol (BuOH). Afterward, the fractions were evaporated until dry.

Determination of total phenolic content

The total phenolic contents of extracts were evaluated using the Folin-Ciocalteu assay as gallic acid equivalents (GAE).[17]

Antioxidant capacities of extracts

DPPH test

The antioxidant capacity of extracts (EtOH and water extracts; DCM, EtOAc and BuOH fractions) were evaluated with rapid TLC screening.[18]

TBA test (measurement of malondialdehyde value)

The amount of malondialdehyde formed in the reaction mixture was determined using the TBA reagent spectrophotometrically.[18]

Antimicrobial activity

Test microorganisms

The test microorganisms used in the experiment were Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (*MRSA*) (clinical isolate), *Bacillus subtilis* ATCC 25923); Gram-negative bacteria (*Escherichia coli* ATCC 25922) and fungus: (*Candida albicans* ATCC 10231).

Ampicillin sulbactam (20 µg), ciprofloxacin (5 µg), fluconazole (25 µg) and cefotaxime (30 µg) were used as control drugs. ATCC strains were obtained from the culture collection of the Refik Saydam Health Institution of Health Ministry, Ankara.

Media

Mueller-Hinton agar (Difco, Detroit, MI, USA) was used for bacteria, and MHA supplemented with 2% glucose and 0.5 µg/mL methylene blue was used for *C. albicans*.

Disc diffusion assay

Antimicrobial activities of the extracts and fractions were evaluated by using the disc diffusion technique.[19,20]

RESULTS

Phenolic compounds are known as a main class of active compounds determined by Folin-Ciocalteu assay. The highest total phenolic contents of all extracts and fractions of the three species were determined in the EtOAc fractions. The results of total phenolic contents of extracts and fractions are shown in Table 1.

When all DCM fractions and EtOAc fractions, excluding *O. basiadnata* root, were compared using propyl gallate, they were

Table 1. Total phenolic contents of the extracts and fractions of *Ononis* species

Species	Total phenolic contents ($mg_{gallic\ acid}/g_{extr}$) ± SD*				
	Water extracts	EtOH extracts	DCM fraction	EtOAc fraction	BuOH fraction
O. sessilifolia herb (OSH)	37.19±1.58	132.01±4.16	208.11±3.93	413.67±5.50	124.77±0.79
O. sessilifolia root (OSR)	14.78±0.79	145.33±1.57	221.44±2.36	327.08±0.79	131.44±2.36
O. basiadnata herb (OBH)	80.33±0.79	111.44±0.79	105.33±4.16	620.89±12.57	89.77±4.71
O. basiadnata root (OBR)	20.15±0.64	91.17±5.55	119.22±2.36	242.56±4.84	88.11±0.79
O. macrosperma herb (OMH)	46.17±3.78	67.19±0.64	63.67±4.21	467.03±3.93	156.44±4.71

*SD: Standard deviation, EtOH: Ethanol, DCM: Dichloromethane, EtOAc: Ethyl acetate, BuOH: n-butanol

Table 2. Antioxidant capacities of extracts and fractions of *Ononis* species in TBA test

Species	IC_{50} value (µg/mL) ± SD*				
	Water ext.	EtOH ext.	DCM frac.	EtOAc frac.	BuOH frac.
OSH	NE*	1.41±0.58	14.38±1.32	51.18±3.31	NE
OSR	>1000±8.55	1.96±0.39	26.03±0.24	146.35±2.73	NE
OBH	>1000±3.11	24.19±2.21	49.73±1.41	532.01±5.58	NE
OBR	>1000±7.99	15.57±0.95	26.06±1.19	3.10±1.14	138.93±2.26
OMH	NE	0.13±0.17	>1000±2.85	61.56±3.61	570.41±7.14
Propyl gallate	3.72±1.6				

*Non-effective; SD: Standard deviation, OSH: *O. sessilifolia herb*, OSR: *O. sessilifolia root*, OBH: *O. basiadnata herb*, OBR: *O. basiadnata root*, OMH: *O. macrosperma herb*, EtOH: Ethanol, DCM: Dichloromethane, EtOAc: Ethyl acetate, BuOH: n-butanol

Table 3. Results of the antimicrobial activity of *O. sessilifolia* herb (OSH) and root (OSR) extracts (inhibition zones in mm)

Extracts/Drugs OSA	*S. aureus* ATCC 25923	*MRSA* isolate	*B. subtilis* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
OSH EtOH ext.	10	12	-	7	13
OSR EtOH ext.	-	5	-	-	11
OSH DCM	-	-	-	-	-
OSR DCM	-	-	-	-	-
OSH EtOAc	-	-	-	15	-
OSR EtOAc	-	-	-	-	-
OSH BuOH	11	15	15	17	12
OSR BuOH	14	11	10	16	17
OSH water ext.	-	-	-	-	-
OSR water ext.	-	-	-	-	-

OSH: *O. sessilifolia* herb, OSR: *O. sessilifolia* root, EtOH: Ethanol, DCM: Dichloromethane, EtOAc: Ethyl acetate, BuOH: *n*-butanol

shown to have a high radical scavenging effect with qualitative DPPH. Yellow zones on a purple ground were marked for the DCM and EtOAc fractions of all species. In DPPH test, EtOH extracts were generally active from the water extract, especially the root and herb extracts of *O. sessilifolia* (Figure 1).

The most significant results in the TBA method were obtained in EtOH extracts of *O. macrosperma* herb (OMH) (IC_{50}=0.13±0.17 µg/mL), *O. sessilifolia* herb (IC_{50}=1.41±0.58 µg/mL) and root

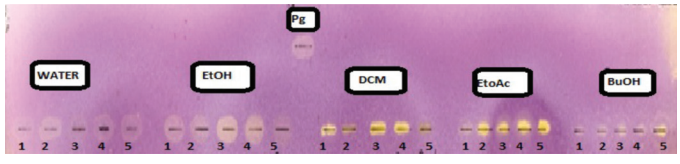

Figure 1. Antioxidant capacity by qualitative DPPH test on TLC of *Ononis* species

1: *O. basiadnata* root extract, 2: *O. basiadnata* herb extract, 3. *O. sessilifolia* root extract, 4: *O. sessilifolia* herb extract, 5: *O. macrosperma* herb extract, Pg: Propyl gallate

(IC_{50}=1.96±0.39 µg/mL) (Table 2). A survey of the published literature shows that the antioxidant activity of *O. sessilifolia*, *O. basiadnata* and *O. macrosperma* has not been subjected to research so far.

In our antimicrobial activity studies, the extracts and fractions of three endemic *Ononis* species were examined against various bacteria and fungi. First, EtOH extracts were prepared, and then extracted with DCM, EtOAc and BuOH. The antimicrobial activity of the water extract was also examined. Water extracts of herb and root parts had no antimicrobial activity against Gram (-), Gram (+) bacteria and yeast. All of the BuOH extracts showed moderate activity compared with the standards. Some EtOAc fractions also demonstrated less activity against *E. coli*, *S. aureus*, *MRSA* and *C. albicans*. Apart from OMH, other DCM extracts showed no activity against Gram (+), Gram (-) bacteria and fungi. All EtOH extracts showed less activity against some bacteria. In addition to this, they showed moderate activity against *C. albicans* according to fluconazole (Table 3, 4, 5 ,6).

DISCUSSION

In the literature, there are some studies on the antioxidant activities and phenolic contents of other *Ononis* species. Leaf methanolic extract of *O. natrix* has significant total phenolic content (51 mg GAE/g DW) and flavonoid content (14.76 CE/g DW).[21] The antioxidant activity and total phenolic contents of *O. natrix* used in folk medicine in Jordanian were identified as follows: according to antioxidant capacity results, the aqueous extract has 82.0±1.5 µmol TE/g, methanolic extract has 76.7±2.0 µmol TE/g dry weight; in total phenolic content, the aqueous extract has 16.9±0.4 mg GAE/g, methanolic extract has 21.1±0.7 mg GAE/g dry weight.[9] In another study, *O. spinosa* root infusion was evaluated in both DPPH inhibition (20.5±0.8%) and total phenolic content 3.09±0.01 mg GAE/g extract.[22] Although the ethanolic extract of *O. spinosa* indicated concentration-dependent superoxide anion radical scavenging capacity (IC_{50}=1.35 mg/mL), the extract showed no concentration-dependent inhibitory effect on lipid peroxidation.[23] Unlike the present study, in our lipid peroxidation experiment, significant results were obtained (Table 2).

Table 4. Results of the antimicrobial activity of *O. basiadnata* herb (OBH) and root (OBR) extracts (inhibition zones in mm)

Extracts/Drugs OBA	*S. aureus* ATCC 25923	*MRSA* isolate	*B. subtilis* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
OBH EtOH ext.	11	-	12	10	11
OBR EtOH ext.	10	7	-	14	10
OBH DCM	-	-	-	-	-
OBR DCM	-	-	-	-	-
OBH EtOAc	10	11	-	-	7
OBR EtOAc	-	-	-	14	-
OBH BuOH	12	12	14	14	-
OBR BuOH	11	14	17	17	14
OBH water ext.	-	-	-	-	-
OBR water ext.	-	-	-	-	-

OSR: *O. sessilifolia* root, OBH: *O. basiadnata* herb, OBR: *O. basiadnata* root, OMH: *O. macrosperma* herb, EtOH: Ethanol, DCM: Dichloromethane, EtOAc: Ethyl acetate, BuOH: *n*-butanol

Table 5. Results of the antimicrobial activity of *O. macrosperma* herb (OMH) extracts (inhibition zones in mm)

Extracts/ Drugs OMH	*S. aureus* ATCC 25923	*MRSA* İsolate	*B. subtilis* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
EtOH ext.	10	9	-	-	16
DCM frac.	12	-	-	7	-
EtOAc fract.	-	10	-	-	-
BuOH frac.	12	15	-	16	13
Water ext.	-	-	-	-	-

OMH: *O. macrosperma* herb, EtOH: Ethanol, DCM: Dichloromethane, EtOAc: Ethyl acetate, BuOH: *n*-butanol

Table 6. Inhibition zones of standard antibiotics (inhibition zones in mm)

Reference Substances	*S. aureus* ATCC 25923	*MRSA* isolate	*B. subtilis* ATCC 25923	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
Ampicillin sulbactam	30	-	21	-	-
Ciprofloxacin	-	27	-	-	-
Fluconazole	-	-	-	-	32
Cefotaxime	-	-	-	25	-

The present study shows that the three *Ononis* species contained phenolic compounds, which inhibit the oxidation of lipids by donating hydrogen atoms to scavenge free radicals.[24] Phenolic compounds have been shown to be more effective antioxidants than vitamin A and C.[25] Our results showed that there seemed to be good compatibility between the phenolic content and antioxidant capacity of the extracts because the EtOAc fractions with a higher phenolic content showed higher DPPH radical scavenging capacity.

According to previous studies, the BuOH extracts of *O. spinosa* (4 mg/disc) had moderate antifungal activity against *Aspergillus flavus*, *Fusarium moniliforme* and *C. albicans* in comparison with miconazole nitrate at 40 µg/disc. Petroleum benzene, EtOH, and water extracts showed high activity against Gram (+) and Gram (-) bacteria.[26] In another study, EtOH extract of *O. spinosa* demonstrated significant activity against Gram-positive (*E. coli* and *P. aeruginosa*), Gram-negative (*S. aureus*) bacteria and fungi (*C. albicans*).[27] In our study, similar results were obtained, EtOH extracts and BuOH fractions of all species showed generally high activity against Gram (+), Gram negative (-) bacteria and *C. albicans*.

CONCLUSION

Further studies are being conducted to determine the characterization and identification of active components responsible for the antioxidant and antimicrobial activities. Natural products are commonly a source for active compounds that have important potential for developing new therapeutic agents. *Ononis* species can be introduced as new plant source for antioxidant and antimicrobial agents.

Conflict of Interest: No conflict of interest was declared by the authors.

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