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

INTRODUCTION

Antioxidants have been recognized as potential therapeutics for preventing different human diseases (cancer, aging, cardiovascular diseases, asthma, acute CNS injury, neurodegenerative disease and malaria etc.) which are occured tissue damage developing of free radicals (1,2). Medicinal plants and herbs play a 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 day by day (5).

Ononis L. genus (Leguminosae) is represented by 18 species and 4 of them are endemic to Turkey (6). The *Ononis* species have various pharmacological activities, 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. (2011) reported that water and ethanolic extracts of *O. macrosperma* herb demonstrated the highest activity in both wound models and anti-inflammatory activity (10). In previous studies, some *Ononis* species were contained different component groups such as isoflavone, triterpene, sterol, pterocarpane and resorcinol derivatives, flavonoids, isocoumarins, and hydroxycinnamic acids (1,11-13).

A DPPH$^{\bullet}$ radical is, a stable radical with maximum absorbance at 517 nm, 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 biological systems, and is the accumulative effect of reactive oxygen species. Reactive free radicals start the reaction by the deletion of allylic hydrogen atom from the methylene group of unsaturated fatty acids (15). The TBA and DPPH methods have been used to evaluate the antioxidant capacities of the plant extracts/component (16).

Medicinal plants represent as potential sources of natural antioxidant and antimicrobial agents for food and medicinal purposes. The purpose of this investigation was to study antioxidant activities with the TBA and DPPH (TLC screening method), determinated to phenolic contents by spectrophotometry and the antimicrobial activity with disc diffusion technique, of herb and

root extracts of three *Ononis* species. It 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 are stored in the Herbarium of the Faculty of Pharmacy at the University of Ankara, Turkey (AEF). *O. sessilifolia* was collected from Çamardı county of Niğde, in June 2007 (AEF 23979); *O. basiadnata* was collected from the Gülnar county of İçel, in June 2007 (AEF 23968); *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 herb of *O. macrosperma* were powdered and then 50 g of the herbs and roots separately were macerated with 500 mL of water for 5 h at 60 °C. Afterwards, the water extracts were filtered, frozen and lyophilized. 100 g of plant material was macerated with ethanol for 5 h at 50 °C. Then, the extracts were filtered and evaporated upto dryness. Ethanolic extracts were dispersed in methanol: water (1:9), partitionated with dichloromethane (DCM), ethyl acetat (EtOAc) and *n*-buthanol (BuOH). After, the fractions were evaporated to dryness.

Determination of Total Phenolic Content

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

Antioxidant Capacities of Extracts

DPPH test

Antioxidant capacity of extracts (EtOH and water extracts; DCM, EtOAc and BuOH fractions) were evaluated with a rapid thin layer chromatography (TLC) screening method (18).

TBA test (Measurement of MDA Value)

The amount of malondialdehyde (MDA) formed in the reaction mixture was determined by the thiobarbituric acid (TBA) reagent spectrophotometrically (18).

Antimicrobial Activity

Test Microorganisms

Test microorganisms used in the experiment are 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), flucanozol (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 (GMB) was used for *C. albicans*.

Disc Diffusion Method

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

RESULTS AND DISCUSSION

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

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

When all DCM fractions and EtOAc fractions exclude from *O. basiadnata* root compared with propyl gallate, they were shown to have high radical scavenging effect with qualitative DPPH method. If we are to explain, 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 water extract, especially root and herb extracts of *O. sessilifolia* (Figure 1).

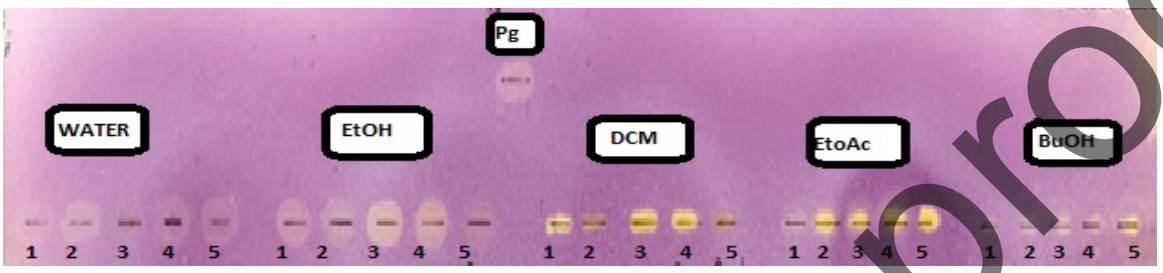

Figure 1. Antioxidant capacity by qualitative DPPH test on TLC of *Ononis* species. 1: *O. basiadnata* root extract, 2: *O. basiadnata* herb extract, 3: *O. sessilifolia* root extract, 4: *O. sessilifolia* herb extract, 5: *O. macrosperma* herb extract, Pg: Propyl gallate.

The most significant results in the TBA method were obtained in EtOH extracts of *O. macrosperma* herb (IC_{50}= 0.13 ± 0.17 µg/mL), *O. sessilifolia* herb (IC_{50}= 1.41 ± 0.58 µg/mL) and root (IC_{50}= 1.96 ± 0.39 µg/mL) (Table 2). A survey of the published literatures showed that antioxidant activity of *O. sessilifolia*, *O. basiadnata* and *O. macrosperma* has not been subjected performed to research so far.

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				

*Noneffective; SD: standard deviation

OSH: *O. sessilifolia* herb, OSR: *O. sessilifolia* root, OBH: *O. basiadnata* herb, OBR: *O. basiadnata* root, OMH: *O. macrosperma* herb.

In the literature, there are some studies on antioxidant activities and phenolic contents of the other *Ononis* species. Leaf methanolic extract of *O. natrix* has significant total phenolic content (51 mg GAE / g DW) and flavonoid (14.76 CE/g DW) (21). Antioxidant activity and total phenolic contents of *O. natrix* used in folk medicine in Jordanian were identified as follows: According to antioxidant capacity results, aqueous extract has 82.0 ± 1.5, methanolic extract has 76.7 ± 2.0 µmol TE/g dry weight; in total phenolic content, aqueous extract has16.9 ± 0.4, 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). While ethanolic extract of *O. spinosa* indicated concentration-dependent superoxide anion radical scavenging capacity (IC_{50}= 1.35 mg/mL), the extract did not show concentration-dependent inhibitory effect on lipid peroxidation (23). Unlike the present study, in our lipid peroxidation experimental was obtained significant results (Table 2).

The present study reported that three *Ononis* species contain phenolic compounds which are inhibited the oxidation of lipids by donating hydrogen atoms to scavenge free radicals (24). Phenolic compounds have been exhibited to be more effective antioxidants than vitamin A and C (25). Our results showed that there seemed to be good compatible between the phenolic content and antioxidant capacity of the extracts since EtOAc fractions with higher phenolic content showed higher DPPH radical scavenging capacity.

In antimicrobial activity studies, extracts and fractions of three endemic *Ononis* species were examined against various bacteria and fungi. Firstly, EtOH extracts were prepared, and then extracted with DCM, EtOAc and BuOH. Antimicrobial activity of the water extract was also examined. Water extracts of herb and root parts had not antimicrobial activity against Gram (-), Gram (+) bacteria and yeast. All of the BuOH extracts showed moderate activity than that of the standarts. Some EtOAc fractions also demonstrated less activity against *E. coli*, *S. aureus*, MRSA and *C. albicans*. Exclude from *O. macrosperma* herb (OMH), other DCM extracts showed no activity Gram (+), Gram (-) and fungi. All EtOH extracts showed less activity against some bacteria. In addition to this, they showed moderate activity against *C. albicans* according to flucanozole (Table 3-6).

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, OBH: *O. basiadnata* herb, OBR: *O. basiadnata* root, OMH: *O. macrosperma* herb.

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.	-	-	-	-	-

OSH: *O. sessilifolia* herb, OSR: *O. sessilifolia* root, OBH: *O. basiadnata* herb, OBR: *O. basiadnata* root, OMH: *O. macrosperma* herb.

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**.**

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	-

According to the previous studies, the *n*-butanol 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 benzine, ethanol and water

extracts showed high activity against Gram (+) and Gram (-) bacteria (26). In another study, ethanol extract of *O. spinosa* demonstrated significant activity against Gram positive (*E. coli* and *P. aeruginosa*), Gram negative (*S. aureus*) and fungi (*C. albicans*) (27). In our study, similar results were obtained that ethanol extracts and *n*-buthanol fractions of all species showed generally high activity against Gram (+), Gram negative (-) bacteria and *C. albicans*.

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

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