SCREENING OF THE ANTIOXIDATIVE AND ANTIMICROBIAL PROPERTIES OF THE VARIOUS EXTRACTS *BERBERIS VULGARIS* L. (*BARBERRY*) LEAVES FROM TURKISH FLORA

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ABSTRACT

Free radicals are highly reactive compounds that play an essential role in many biological processes, both beneficial and deleterious. Detection and quantification of these species is critical to develop a better understanding of normal and pathophysiological functions at the cellular and tissue levels. In this study, the antioxidant activities of *Berberis vulgaris* L. extracts were evaluated by various *in vitro* assays, including DPPH free radical-scavenging, reducing power, ferric thiocyanate and total phenolic contents tests. Antimicrobial activities of various extracts were tested against bacteria strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli ATCC* 35218, *Escherichia coli ATCC* 25922, *Bacillus subtilis* ATCC 29213, *Bacillus cereus* NRLL B3008, *Enterobacter fecalis* ATCC 292112 and *Proteus vulgaris*. The IC_{50} of aqueous extract was 170.12 µg.mL^{-1}. The reducing powers increased with increasing amount of extract sample. Aqueous extract (OD_{700}=0.215) also exhibited the strongest reducing power. Total phenol content was highest in the aqueous extract (108 mg PKE). The aqueous extract had greater ferric thiocyanate than ether extract. The strongest antibacterial activity was observed using the hexane extract of *B. vulgaris* L. against *S.aureus* (zone of inhibition: 25.3±2.1 mm).
KEY WORDS: *Berberis vulgaris* L., Antioxidant activity, Antimicrobial activity.

1.INTRODUCTION

Free radicals are atomic or molecular species with unpaired electrons. They are highly reactive and unstable as compared to similar ions. Free radicals play an important role in many biological processes including metabolic pathways, cell signaling, immune response and a variety of pathophysiological conditions (Ansari,1997). Detection and quantification of these species is critical to decipher cellular pathways and mechanisms to understand disease and function. Free radicals are generated in the biological environment as a result of reactions associated with common biochemical pathways involving oxygen metabolism. Thus, their universal presence and their role as critical mediators of normal and pathophysiology have resulted in considerable development of techniques that can detect these radicals. Reactive oxygen and nitrogen species (RONs) are a family of molecules that include molecular oxygen and its derivatives produced in all aerobic cells (Ansari,1997). The RONs include both radical and non-radical species such as superoxide (O_2•–), nitric oxide (•NO), nitrogen dioxide (•NO_2), carbonate radical (CO_3•–), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), peroxynitrite (PN; ONOO–), peroxynitrous acid (ONOOH; p*K*a 6.2) and hypohalous acids (HOX) (Kuppusamy,2004; Zavoisky,1945; Halpern,1989). RONs, in particular those derived from •NO and O_2•–, have been shown to cause cellular oxidative damage and trigger specific signaling events (Kuppusamy,2004; Zavoisky,1945; He,2001; Ilangovan,2004) that culminate in altered cellular physiology. RONs have also been shown to cause the oxidative degradation/modification of DNA, proteins, carbohydrates and polyunsaturated lipids resulting in oxidative stress (Kuppusamy,1996,2001). Antioxidants, which scavenge free radicals, are known to possess an important role in preventing these free radical-

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induced diseases. Dietary foods contain a wide variety of free radical-scavenging antioxidants; for example, flavonoids and antioxidative vitamins such as ascorbic acid and á-tokoferol (Hanasaki,1994). These compounds are particularly rich in vegetables, fruits and tea. Epidemiological studies have shown that higher intake of fresh vegetables, fruits and tea is associated with lower risk of mortality from cancer and coronary
heart disease (Hertog,1993; Willett,1994). There is currently strong interest in natural antioxidants and their role in human health and nutrition (Aruoma,1994).

Berberis vulgaris L. belonging to the Berberidaceae family. *Barberry* grows in Asia and Europe; The plant is well known in Turkey and it has been used extensively as a medical plant in traditional medicine. The leaves of the plant have been used as food. The plant is a shrub, 1-3 m tall, spiny, with yellow wood and obovate leaves, bearing pendulous yellow flowers succeeded by oblong red berries (Baytop,1963; Davis,1972).

Various parts of the species, *B. vulgaris* L., have been used for the treatment of diarrhea, gallbladder and liver dysfunctions, leishmaniasis, malaria, stomach problems and urinary tract diseases (Baytop,1963). The constituents of this plant include isoquinoline alkaloids, phenolic compounds and triterpenoids (Zagari,1983; Aynehchi,1986). In terms of the biological evaluation of *B. vulgaris* L. extracts and purified constituents, antiarrhythmic, antibabesial, antibacterial, anticholinergic, antihistaminic, antihypertensive, antiinflammatory, antinociceptive and vasodilatory effects have been reported for the extracts or individual constituents of this plant (Fatehi,2005).

Barberry is extensively used as a food additive and its juice is recommended to cure cholecystitis (Zagari,1983; Baytop,1984). Nevertheless, little pharmacological analysis has been performed on barberry. Crude extract of barberry has antihistaminic and anticholinergic activities (Shamsa,1999).

The aim of the present study was to investigate the antimicrobial and antioxidant potential of the aqueous, ethanol, and ether extract of *Berberis vulgaris* L..

2.MATERIALS AND METHODS:

Collection of plant material:

Berberis vulgaris L. were collected at the flowering stage from Erzurum. Collected plant materials were dried in shade and the leaves were seperated from the stem and griund in a grinder with a mesh 2 mm in diameter.

Preparation of the extracts:

Extraction was performed at room temperature. About 200 g of dried, griund plant material were soaked in 2 L ethanol, ether for 5- 7 days. The final extracts were passed through No 1 Whatman filter paper. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40^{o}C and stored at 4^{o}C for further use.

DPPH Radical Scavenging Activity: 1,1-Diphenyl2-picrylhydrazyl (DPPH) radical scavenging capacity.

DPPH radical scavenging capacity was determined according to the Blois method (Blois,1958).

Reducing Power: The reducing power of extracts were quantified by the method of Oyaizu (1986).

Ferric thiocyanate method-total antioxidant activity: The total antioxidant capacities of the extracts were determined according to the ferric thiocyanate method in linoleic acid emulsion (Yen,1995). The percentage inhibition of lipid peroxidation in linoleic emulsion was calculated by following equation: Inhibition of lipid peroxidation (%) = [(control absorbance - sample absorbance)/control absorbance] X 100

Determination of the Amount of Total Phenolic Compounds: The phenolic constituents of the extracts were determined by the literature methods involving the Folin-Ciocalteu reagent and pyrocatechol as standard (Chandler,1983). The phenolic compound content was determined as pyrocatechol equivalents using the following linear equation based on the calibration curve: *A* is the absorbance and *C* is pyrocatechol equivalents (mg)

$A = 0.006C - 0.0192$ $R^2 = 0.999$ **Antimicrobial activities:**

The antimicrobial effect of hexane and ethanol extracts of *B. vulgaris* L. were tested against bacteria strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli ATCC* 35218, *Escherichia coli ATCC* 25922, *Bacillus subtilis* ATCC 29213, *Bacillus cereus* NRLL B-3008, *Enterobacter fecalis* ATCC 292112 and *Proteus vulgaris* .

Agar Well Diffusion: The compound effect was carried out by the agar diffusion method (Kalemba,2003).

3.RESULTS AND DISCUSSION

In the radical scavenging assay, when the DPPH is exposed to antioxidant compounds the purple color of DPPH changed to yellow. The more yellowish color of DPPH observed the greater the antioxidant activity of the compounds tested. The results obtained for DPPH radical scavenging activity of these extracts are summarized in Table 1. Free radical scavenging activity with the IC_{50} value of aqueous, ethanol and ether extract of *B. vulgaris* L. 170.12 $\mu g.mL^{-1}$, 174.42 $\mu g.mL^{-1}$, 183.32 $\mu g.mL^{-1}$ respectively. Lower IC_{50} value indicates higher antioxidant activity. The aqueous, ethanol and ether extracts of *B. vulgaris* L. had lower antioxidant activity than the ascorbic acid (IC_{50}=2.18).

The reducing powers of *B. vulgaris* L. extacts are summarized in Table 2. Ascorbic acid, which is known to be a reducing agent as well as a reductone was used as a standard for the purpose of comparison. The reducing power of aqueous extract of *B.vulgaris* L., the highest among all of the extracts, and it is increased as the amount of extract increased (Figure 1).

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}) (Oyaizu,1986). The results of this research showed that the reducing power of the ether extract(OD_{700}=0.170) was less than all extracts. The highest reducing power was detected for aqueous extract (OD_{700}=0.903) followed by ethanol extract and ether extract (Table 2).

The antioxidant activities of the extracts were compared with the commercial antioxidant BHA by the ferric thiocyanate method, which measures the amount of peroxide generated at the initial stage of linoleic acid emulsion during incubation. Peroxide reacts with ferrous chloride to form ferric chloride, which in turn reacts with ammonium thiocyanate to produce ferric thiocyanate, a reddish pigment(Kim,2010). Low absorbance values indicate high antioxidant activity. Figure 2 shows the changes in absorbance for each sample during 120 h of incubation at 40°C. It was observed that absorbance increases over time and that the autoxidation of linoleic acid emulsion in the control, aqueous, ethanol or ether extracts of *B. vulgaris* L. were accompanied by a rapid increase in the peroxide formation. Significantly lower absorbances were observed for the extracts and BHA, which indicates the extract have greater antioxidant activities. Antioxidant activities increased in the following order: BHT>Aqueous extract>Ether extract. The total phenolic content ranged from 4.89 mg PKE to 108 mg PKE (Table 3). The aqueous extract had higher total phenolic content. Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development (Harborne,1982) and under stress conditions such as infection, wounding and UV radiation, among others (Beckman,2000). The rapid and systematic measurement of phenolic acids and flavonoids is a serious challenge for analytical and food chemists, phytochemists and biochemists because of the inherent structural diversity of the compounds, the dietary and health impact(Stalikas,2007).

Table 4 provides the antimicrobial results obtained using the agar well diffusion method. The highest inhibitory activity was seen against *S.aureus* (zone of inhibition: 25.3±2.1 mm) using the hexane extract of *B.vulgaris* concentration of 100mg/mL. While the weakest activity was demostrated against *P. aeruginosa* ATCC 27853 and *B. cereus* NRLLB-

3008 (zone of inhibition: 11.3± 0.5*) using the ethanol extract of *B.vulgaris* L..

Antioxidative and antimicrobial properties of the various extracts from many plants are of great interest in both academia and the food industry. This result suggests strongly support *B. vulgaris* L. as a possible candidate for the production of antioxidants. This clarify the antioxidant properties of *B. vulgaris* L. and develop natural antioxidant agents, the purification and identification of active compounds will be necessery.

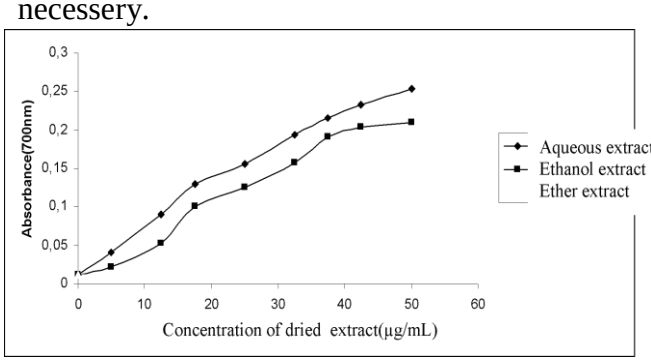

Figure 1. Reducing power of extracts of *B. vulgaris* L.

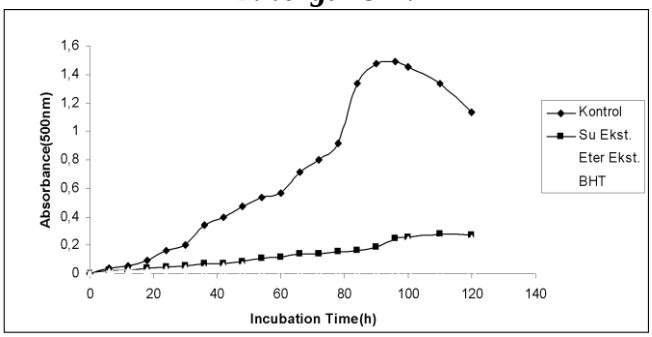

Figure 2. Antioxidant activity of aqueuos, ether extracts *of* *B. vulgaris* L. and BHT. The indicated amounts of dried extract were present in 5 ml of linoleic acid emulsion (0.02 M, P^H 7.0). The control was the linoleic acid emulsion without extract.

Table 1. Free radical scavenging capacities of the extracts measured in DPPH assay

Plant	Extracts	IC_{50}(µg/ mL)
Berberis Vulgaris L.	Aqueous	170.12±0.20
	Ethanol	174.42 ± 0.70
	Ether	183.32±0.40
	Ascorbic acid	2.18 ±0.10

Results are means of three different experiments

Table 2. Comparison of Reducing Powers of Ascorbic Acid and *B.vulgaris* L. Extracts

Sample	Absorbance (700 nm)
Control	0.016±0.003
Ascorbic acid	3.625±0.003
Aqueous extract of *B. vulgaris L.*	0.903±0.010
Ethanol extracts of *B.vulgaris L.*	0.215±0.021
Ether extracts of *B. vulgaris L.*	0.170±0.020

500 ìg of dried extract or ascorbic acid, corresponding to a final concentration of 50 ìg ml^{-1}, was used. Control was test sample without extract or ascorbic acid. High absorbance indicates high reducing power.

Table 3. Amount of Total Phenolic Compounds in *B. vulgaris* L.

250 µg of extract	Absorbance (760 nm)	Pyrocatechol equiv (PKE) (mg)
Control	0.001	
Aqueous extract of *B. vulgaris* L.	0.350	108
Ethanol extracts of *B.vulgaris* L.	0.126	37.6
Ether extracts of *B.vulgaris* L.	0.014	4.89

Table 4. Antimicrobial Activity of Hexane, Ethanol Extracts of *B.vulgaris* L.

Microorganism	Inhibition zone (mm)				
	Hexane extract of *B.vulgaris* (25 mg/mL)	Hexane extract of *B.vulgaris* (100 mg/mL)	EtOH extract of *B.vulgaris* (25 mg/mL)	Control	
				C?	Amp#
	Agar-well D	Agar-well D	Agar-well D		
E. coli ATCC 25922	-	-	-	29± 1.7*	20±0
E. coli ATCC 35218	-	14.6±0.5	12± 0*	R	R
P. aeruginosa ATCC 27853	-	14.3±0.5	11.3± 0.5*	9.3± 0.5*	-
E. fecalis ATCC 292112	-	-	-	25±0	30.3±0.5
B. cereus NRLLB-3008	11.3±0.5	15.3±0.5	12 ± 2*	25.6±1.5	9.6±0.5
B. subtilis ATCC 29213	14.6±2.1	17.3±1.5	12.3± 0.5*	29± 1*	27.3±0.5
S. aureus ATCC 25923	14±1	25.3±2.1	-	29± 1*	36.6± 0.5*
P. vulgaris	-	16±1	-	31± 1*	10.3± 1.2*

C: Chloramphenicol, Amp: Ampicilline, * standard deviation, (-) no inhibition zone

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