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ASSESSMENT OF BIOLOGICAL ACTIVITY AND ELEMENT ANALYSIS OF *PSYLLIOSTACHYS SPICATA* (WILLD.) NEVSKI

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ABSTRACT

This study was conducted to determinethe antioxidant activity, total phenolic and flavonoid contents and also detection of phenolic compounds and element composition of *Psylliostachys spicata* by extraction of two different solvents (ethanol 80% andpure water).18 different phenolic compounds and 19 elements were determined from the *P. spicata* which collected from the Akdoğmuş salt area of Siirt province (Turkey).Total phenolic content was determined as 30.65 mg $mL^{-1}$and 6.39 mg mL^{-1}in ethanol and water extracts, respectively. The total flavonoid content was found as73.29 mg $mL^{-1}$and 19.89mg mL^{-1}in ethanol and water extract. The antioxidant activity wasdetermined by DPPH assay. The inhibition rates in 1mg mL^{-1}concentration in ethanol and water extracts were determined as 93.91% and 41.73%, respectively. The obtained IC50 value was 0.058 mg $mL^{-1}$for ethanol and 1.196 mg $mL^{-1}$for water extract. The FRAP assay of *P. spicata* was determined as 774.440 mM mL^{-1}mM mL^{-1}in the ethanol and 74.074 mM mL^{-1}in the water extract. According to the results of element analysis,*P. spicata* has high antioxidant capacity and potential to be used as an accumulator plant for halophytic areas in the future.

Key words: Total phenolic-flavonoid, phenolic compound, element analysis, *Psylliostachys spicata,* Turkey.

INTRODUCTION

People have used plants for the treatment of different diseases throughout human history. According to the reports of World Health Organization (WHO), the number of herbal drugs is approximately 1900. The number of studies investigating the use of plants and spices as a natural antioxidant source is constantly increasing (Damien Dorman *et al*., 1995; Tomaino *et al*. 2005). Phenolic substances which are polyphenolic components found in all parts of plants, are considered as the most important groups of natural antioxidants (Gray, 1978; Moure *et al.,* 2001). Flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids are the most common herbal phenolic antioxidants.

Medicinal plants are natural source of pharmacologically active compounds which have been used against many diseases (Vital *et al.,*2010)and have important biological effects on humans (Njume*et al.,*2009). Chemicalcompound,derivedfrom plants such as flavonoids, alkaloids, terpenoids, tannins, berberins, quinines and emetines are widely used in curing the infectious diseases (Tariq *et al.*,2011). The number of microorganisms resistant to more than one antibiotic has increased significantly due to infections caused by microbes and the struggle against them becomes more difficult every day. Yarnell and Abascal (2004) recommended using medicinal plants as alternatives to drugs, and some traditional plants are currently being used as antimicrobials.

The therapeutic effect of plants depends on coexistence of a large number of chemical compounds. That is why combating microorganisms using plants is more efficientthan that of a single antibiotic (Nazri *et al.*2011). In many developed countries, a large proportion of the population use traditional herbal remedies that are commonly used for treatment. *Psylliostachys spicata* is a member of the Plumbaginaceae family with many medicinal aromatic properties. The plants belonging to the family of Plumbaginaceae are spread all over the world, especially Irano-Turanian and the Mediterranean region.Most members of this family are halophytes which can survive in extreme environmental conditions. The Plumbaginaceae represents with 28 genera and about 650 species around the world (Kubitzki and Kadereit,2004; Lledó *et al.,*2005).There are 84 taxa of the Plumbaginaceae family in Turkey (Güner *et al.* 2012; Celep*et al*.,2016).The first record of Psylliostachys genus in Turkey was from salty lands of Siirt province (Celep *et al*.,2016). The aim of this study was to determine the antioxidant effect, total phenolic and flavonoid contents, phenolic compounds and elemental composition of *Psylliostachysspicata* by 2 different solvent (80% ethanol and pure water).

MATERIALS AND METHODS

Plant material and preparation of extracts: *Psylliostachys spicata* species were collected from salty lands in Siirt province(Turkey Akdoğmuş salt area; Registration number: MeF 2357). The collected plant samples were dried, powdered and then stored in a jar for laboratory analysis. Ethanol and water extracts were

prepared by shaking 3 g of dry powdered plant material in separate containers with 30 mL of ethanol (80%) and 30 mL of water for 24 hours at room temperature. The extracts were filtered then the extracts were transferred into tared flasks and dried at 37 ºC in an oven. Stock concentrations were adjusted to 20 $mgmL^{-1}$ by addition of ethanol (80 %) and water solution.

Total phenolic content: Folin-Ciocalteu (FCR) method was used for phenolic compound content. This method relies on the reaction of phenolic compounds with Folin–Ciocalteu and phenolic compounds were determined with the measurements the visible section of the spectrum(Slinkard and Singleton 1977). An aliquot (0.2mL) of extracts and Folin–Ciocalteureagent (1 mL)were added to a volumetric flask. After incubation for 6 min. at room temperature, 1 mL of 7% Na_2CO_3solution was added and the mixture was shaken. The absorbance of mixed solution was measured at 760 nm with a UV mini-1240 Spectrophotometer. The same procedure was repeated withGallic acid solutions (8-200 μgmL^{-1}). All samples were analyzed in 3 replicates Phenolic compound analysis by LC-MS/MS was performed usingthe method described by Ertaş *et al.* (2014) and Ertas *et al.* (2015).

Total flavonoid content: Total flavonoid content was measured by aluminum chloride ($AlCl_3$) colorimetric method(Zhishen*et al.*,1999). An aliquot (0.25 ml) of test samples solution in ethanol and water were mixed with 1.25mL of distilled water and 75 µL of 5% sodium nitrite ($NaNO_2$). After 6 min, 75 µL 10% $AlCl_3$and 0.5mL 1 M sodium hydroxide (NaOH)were added and incubated at room temperature for 30 min. The solutions were mixed well and the absorbance of mixtures was measured at 510 nm. The standard solutionsofcatechin50, 100, 200, 400, 600, 800, 1000 μgmL^{-1}were used to determine flavonoid contents of extracts. All samples were analyzed in 3 replicates.

Antioxidant activity assay: The percentage of antioxidant activity of samples was assessed by using bothfree radical capture activity (2,2-diphenyl-1-picryl-hydrazyl-hydrate; DPPH) assay and Ferric Reducing Antioxidant Power (FRAP) method.

DPPH Assay: The DPPH assay was performed according to the method explained by Villaño *et al.* (2007).A solution of 0.5mL of sample and 2mL of 0.01 mM DPPH (dissolved in methanol) were stirred and incubated for 30 min. in dark covered with aluminum foil. The absorbance of mixture was measured at 517 nm with a UV mini-1240 Spectrophotometer.

Percentage inhibition was calculated by the formula given;

Inhibition% =(Ac– A1)/Ac × 100

Where;Acis thecontrol absorbance and A1 is the sample absorbance values.

The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL) and Acwas determined to be 0.723.

FRAP Activity: The procedure described by Benzie and Strain (1999) was used for FRAP assay. 100 µL sample extract were mixed with 300 µL distilled water and 2 mL FRAP reagent. The mixtures wereincubated for 2 h in dark conditions. The absorbance of mixtures was measured spectrophotometrically at 593 nm.

The FRAP reagent contained 300 mM sodium acetate solution (pH 3.6), 10 mM TPTZ ((2,4,6-tripyridy-s-triazine) in solution of 40 mMHCl and 20 mM ferric chloride solution. (10:1:1). Calibration was prepared with ferrous sulfate standard solution (0.1-1.0 $mgmL^{-1}$). Results were defined as mg Fe^{+2}.

Element analysis (ICP-OES): Plant samples were digested by Berghof Speedwave MWS-3 model microwave. In the microwave acid digestion, 0.5 g dried sample was weighed to a pressure-resistant vessel (volume 100 mL) and 10 mL mixture of HNO_3(2.5 mL) and HCl (7.5 mL) acids. Vessels were closed, placed into the microwave oven and digested at 70-80 W power for 1 h. The acids were removed at evaporation module. The residue was then dissolved in Milli-Q water and the filtrate was diluted to a constant volume. Microwave digestion system setupwas described in Table 1. Inductively Coupled Plasma Optical Emission Spectrometer (Model Optima™ 7000 DV ICP-OES; PerkinElmer, Inc., Shelton, CT, USA) was used to measure the concentrationsof metals in plant samples.The instrumental conditions of ICP-OES were optimized according to Uyan *et al.* (2017).

Table 1 Operating conditions of microwave oven.

	1	**2**
Temperature (^{0}C)	150	190
Pressure (Bar)	50	50
Power (W)	70	80
Ta (min)[a]	5	5
Time (min)[b]	5	1

Note: [a] waiting time at desired temperature; [b] time between two sequential temperatures.

RESULTS AND DISCUSSION

The average of total phenolic contents of *P. spicata* samples were 30.65 mg $mL^{-1}$and 6.39 mg mL^{-1}inethanol extract and water extract, respectively. Thetotal flavonoid contents of *P. spicata* samples were 73.29 mg $mL^{-1}$and 19.89 mg mL^{-1}in ethanol extract and water extract, respectively(Figure 1). The obtained resultsare in agreement with previouslypublished studies. Ethanol extracts were indicated higher values of total

polyphenols, total anthocyanins and antioxidant activity compared with water extracts because ofthe polarity of ethanol. The most extensively used solvents for extracting phenolic compounds are water, ethanol, methanol, acetone, and their water combination with or without acids (Naczk and Shahidi, 2006). Laporniket *et al.* (2005) showed that ethanol is more efficient for releasing anthocyanins and other polyphenols from unipolar cell walls. In our study, phenolic and flavonoid contents in ethanol extracts were higher than water extracts. Therefore, using of ethanol as a solvent in food industry is more common than in water.

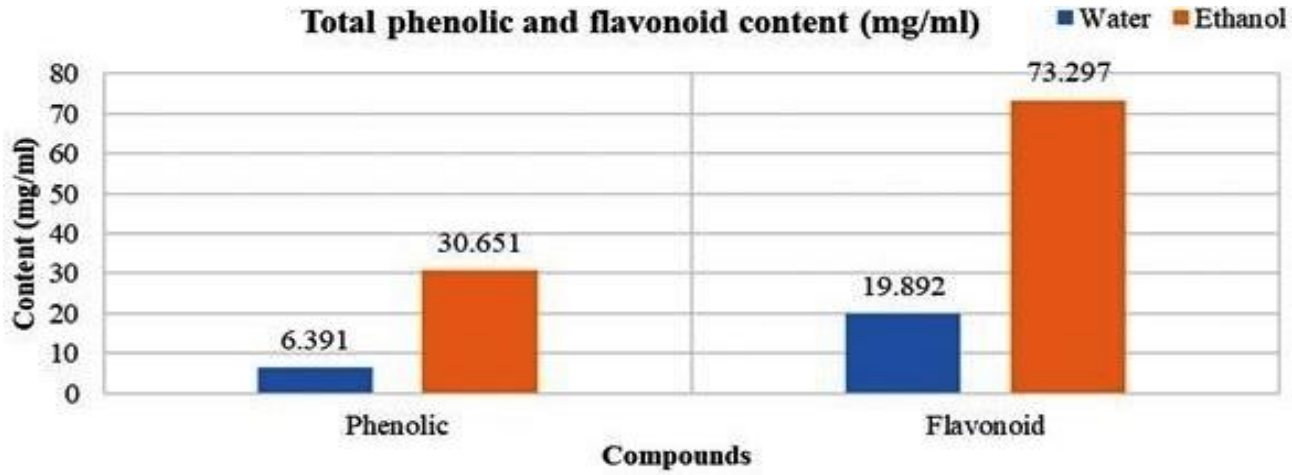

Figure 1. Total phenolic and flavonoid contents of *P. spicata*

The DPPH radical scavenging activities of *P. spicata* showed that the inhibition of 1 mg mL^{-1}concentration of extracts were found 93.91% for ethanol and 41.73% for water extract (Table 2). IC50 valuesof extracts were 0.058 mg $mL^{-1}$for ethanol and 1.196 mg $mL^{-1}$for water extract (Figure 3). The result of the Ferric Reduction Assay Power (FRAP) of the *P. spicata* obtained by the ethanol extract was 774.44 and 74.07 mM $mL^{-1}FeSO_4$, for water extract (Table 2).

In our study, ethanol extracts were indicated more efficient antioxidant activity than water extracts these situations are similar for DPPH and FRAP assay. Comparisons between FRAP and DPPH values of two different extract solvents support our assumption. Our results are in agreement with previous reports suggesting that, a binary solvent system was superior to a mono-solvent system (water or pure ethanol) in the extraction of phenolic compounds in regard to their relative polarity (Boeing*et al.,*2014); Naczk and Shahidi,2004).

Table 2. Result of DPPH and FRAP activity assay.

Solvent	DPPH % Inhibition ($mg.ml^{-1}$)	DPPH IC 50 ($mg.ml^{-1}$)	FRAP Activity ($mM.ml^{-1}$)
Water	41.73	0.05	74.07
Ethanol	93.91	1.19	774.44

Polyphenol oxidase enzyme in water extract was reported that degrading polyphenols which decreases the amounts of phenolic compounds, while polyphenol oxidase is found inactive in ethanol extract (Zhang *et al.,* 2001). Therefore, identification of phenolic compounds in *P. spicata,* were determined by using an ethanol extract only.Eighteen different phenolic compounds were determined by using LC-MS/MS. The highest concentration was determined as Quinic acid (37248. 9 µg analytekg^{-1}extract) and the lowest concentration was as hesperidin (2. 1 µg analytekg^{-1}extract) (Figure 2.). The D (−)-Quinic acid, widely occurring-plant metabolite, is an invaluable chiral material in multistep synthesis. This may suggest that the binding of quinic acid to caffeic acid, increases the antioxidant activity and decreases hydrogen peroxide and DPPH radical scavenging activities of caffeic acid (Asl and Hosseinzadeh, 2008).Malic and tannic acids are also two important pharmacologically active phenolic compounds and detected in *P. spicata*extracts. The results revealed that *P. spicata* can be considered as a strong antioxidantsource.

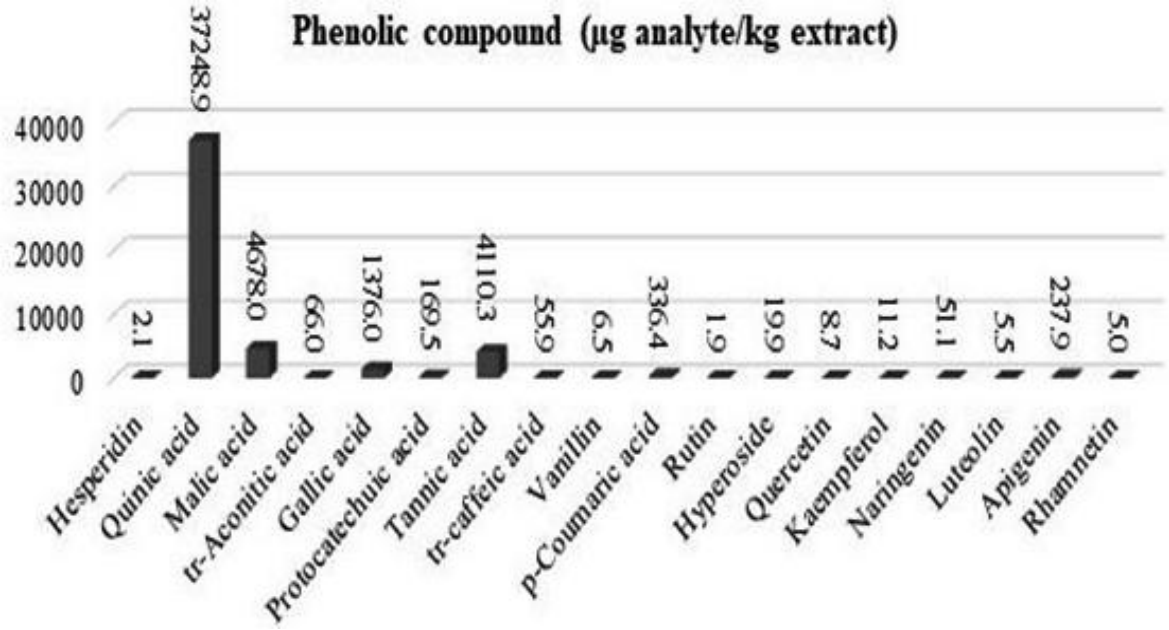

Figure 2. Phenolic compounds of *P. spicata*

The results of current study showed that Cd, Zn, Cr and Pbconcentrations of *P. spicata*are higher and Ni and Cu are lower compared to those reported by World Health Organization(Table 3).

Due to the different characteristicsof halophytes such asmechanism of tolerance to salinity, drought and heavy metal tolerance and higher heavy metal accumulation, halophytes have been used also as accumulator plants. Halophyteshave recently been studied to investigate the potential for removal of toxic substances insoil and water. Heavy metal uptake of halophytes is generally regulated at the root endodermis through modifying uptake from predominantly apoplastic to selective symplastic transport (Dodd and Coupland,1966). The results of elemental analysis of *P. spicata* have shown that this plant can be an accumulator plant for salted areas. Extensive studies have been carried out to determine suitable plants that can accumulate heavy metals in soil and water (Zabłudowska *et al.,* 2009).

Table 3. Heavy metal concentrations of *P. spicata.*

Metals	Permissible valuesfor plants ($mgkg^{-1}$) (WHO (1996)	*P. spicata* ($mgkg^{-1}$)
Cd	0.02	1.59
Zn	0.60	13.18
Cu	10	8.68
Cr	1.30	7.02
Pb	2	7.62
Ni	10	9.47

Elemental composition of *P. spicata* was presented in Table 4. Since *P. spicata* is a halophytic plant, the concentration of Na (13682.12 $mgkg^{-1}$) was much higher as compared to the other elements. Also the concentrations of K, Mg and Fe were also quite high. In contrast, the concentrations of Mo, Be and Sb were detected lower.

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Table 4.Elementel composition ($mg.kg^{-1}$) of *P. spicata.*

	Be	Co	Fe	K	Li	Mg	Mn	Mo	Na	Sb	Se	Ti	V
Elements	1.52	1.85	1409.93	11847.68	5.63	6088.74	48.15	1.46	13682.12	3.25	3.97	27.28	4.04

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