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Contributed Paper

Antimicrobial and Antioxidant Activity of Various Solvent Extracts of *Salsola stenoptera* Wagenitz and *Petrosimonia nigdeensis* Aellen (Chenopodiaceae) Plants

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ABSTRACT

This study was conducted to examine the in vitro antimicrobial and antioxidant activities of various extracts (ethanol, methanol, n-hexane, dicholoromethane and water) prepared from the stem and leaf + fruit of *Salsola stenoptera* Wagenitz and *Petrosimonia nigdeensis* Aellen (Chenopodiaceae) plants. The antimicrobial activities of the plant extracts were tested according to agar well diffusion and microdilution broth methods. The extracts showed a strong antimicrobial activity and a broad antimicrobial spectrum for all tested microorganisms. Minimal bactericidal (MBC) or fungicidal (MFC) concentrations for microorganisms which were sensitive to the stem and fruit + leaf extracts of *S. stenoptera and P. nigdeensis* in the agar well diffusion assay were in the range of 5.63-180.00 mg/mL and 11.25-180.00 mg/mL, respectively. The extracts were also subjected to screening for their possible antioxidant activity with scavenging activity of DPPH radicals, hydrogen peroxide scavenging activity and chelating ability on ferrous ions. All tested extracts showed varying degrees of efficacy in each assay in a dose-dependent manner. In general, polar extracts exhibited stronger activities than non-polar extracts. The ethanol and n-hexane extracts of *S. stenoptera* and *P. nigdeensis* stem showed the highest scavenging abilitiy on DPPH radicals with the IC_{50} values of 29.84 μg/mL and 83.56 μg/mL, respectively. The total phenolic and flavonoid contents were also determined. The highest gallic acid equivalents (GAE) and quercetin equivalents (QE) values, for both plants, were 44.78 μg GAE/mg and 66.69 μg QE/mg, respectively. The results indicate that both plant species may have health benefits as sources of natural antioxidants and antimicrobial agents for use in functional foods and/or in pharmaceutical industry.

Keywords: stem, leaf + fruit, antibacterial activity, antifungal activity, phenolics, flavonoids

1. INTRODUCTION

Chenopodiaceae comprises ca. 110 genera and ca. 1700 species worldwide [1]. The members of Chenopodiaceae are mostly adapted for arid to semiarid and/or saline habitats. The family has a cosmopolitan distribution, and comprises of herbs or shrubs, rarely small-trees or lianas [2].

The presence of various alkaloids, flavonols, flavonoids and triterpenoid saponins has been reported in the Chenopodiaceae, particularly in the tribe Salsoleae family. One of the largest and economically very important is the almost cosmopolitan genus *Salsola*. The geographical distribution of *Petrosimonia* includes Kazakhstan, Azerbaijan, Bulgaria, China, Turkey and Europe. The number of endemic taxa of *Salsola* and *Petrosimonia* was determined to be 18 and 3, 4 and 1 tax, respectively [3, 4]. *Salsola stenoptera* Wagenitz and *Petrosimonia nigdeensis* Aellen are endemic plants of Aksaray province and grow in the salty soils close to the Tuz Lake in Turkey.

Illnesses resulting from consumption of food containing pathogenic bacteria and/or their toxins are major problem in public health [5]. Synthetic antimicrobial subtances can sometimes cause adverse effects including hypersensitivity, allergic reaction and immunity suppression [6]. Due to the increasing consumer demand for more natural foods, the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics. Natural bioactive compounds isolated from plants are considered as promising alternatives for a wide range of industrial applications [7]. Therefore, there has been a growing interest in research concerning alternative natural antimicrobial agents in the form of extracts and essential oils from various species of edible and medicinal plants, herbs, and spices that are relatively less damaging to human health [8].

Antioxidants play important roles in the scavenging of free radicals and/or chain breaking of the oxidation reactions both in vivo and in vitro [9]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used as potential inhibitors of lipid peroxidation and thereby stabilizing fat-containing food-stuffs. However, due to their unstable and highly volatile nature, questions have been raised about safety as they exhibit carcinogenic effects in living organism, by enlarging liver size and increasing microsomal enzyme activity [10]. Due to these limitations, there is an increasing interest in finding natural bioactive compound with antioxidants activity capable of inhibiting free radical reactions, retarding oxidative rancidity of lipids, protecting the human body from diseases, and preserving foods from spoilage [11].

The purpose of this study was to evaluate some endemic plants of Aksaray province as new potential sources of natural antimicrobial and antioxidant compounds. Extracts of *S. stenoptera* and *P. nigdeensis* prepared with different solvents were subjected to screening for their possible antioxidant and antimicrobial activities.

2. MATERIAL AND METHODS

2.1 Plant Material

Salsola stenoptera plants (Karaman 2501 & Teksen) were collected during the flowering and fruiting stage in September- October 2010, about 50 km from Aksaray to Şereflikoçhisar and salty steppe, from Aksaray in Turkey. *Petrosimonia nigdeensis* plants (Karaman 2504 & Teksen) were collected during the flowering and fruiting stage in August- September 2010, in areas

between Sultanhani and Aksaray, 1 km to Sultanhani and salty steppe, from Aksaray in Turkey. The authenticated specimens of the plants were deposited at the herbarium of Biology Department, Faculty of Arts and Sciences, Aksaray University.

2.2 Preparation of The Extracts

Collected plants were washed under running tap water for 15 min to remove sand and any adhering dirt, and then rinsed thoroughly with double distilled water for 3 to 4 times. The stem and fruit + leaf were separated manually and left to dry in an enclosed room (25-28°C) for approximately two weeks. Dried plant samples were ground using a heavy duty grinder (Waring, USA). Fifteen grams of the dried and powdered plant materials (stem and fruit + leaf) were separetaly extracted with ethanol (E), methanol (M), n-hexane (H), dicholoromethane (DCM) and water (W) with using Soxhlet apparatus for 24 h. The extracts were filtered and concentrated under vacuum with using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) and stored in the dark at 4°C until used within a maximum period of one week. Ethanol, methanol, dimethyl sulfoxide (DMSO) and water were used to dissolve the extracts for the following experiments.

2.3 Antimicrobial Activity

2.3.1 Microbial strains

Antibacterial activity of various extracts of different plant parts of *S. stenoptera* and *P. nigdeensis* was investigated against 13 microorganisms. Seven Gram negative bacteria were *Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* Mu:57 and *Yersinia enterocolitica* NCTC 11175. Four Gram positive bacteria were *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863 and *Micrococcus luteus* NRRL B-4375. Two yeasts, *Saccharomyces cerevisiae and Candida albicans* ATCC 10231, were also studied. All strains were stored at -20°C in the appropriate medium containing 10% glycerol and regenerated twice before use in the evalution.

2.3.2 Inhibitory effect with the agar well diffusion method

The determination of the inhibitory effect of the extracts on the test bacteria was carried out with agar well diffusion method [12]. Bacterial cultures were grown at 37 °C for 24 h in Nutrient Broth (NB). *Listeria monocytogenes* ATCC 7644 was cultured in Tryptic Soy Broth. Fungi were cultured in YPD (Yeast Extract Peptone Dextrose) medium at 30°C for 24 h. The culture suspensions were adjusted with comparing with 0.5 McFarland standart turbidity. Petri dishes containing 20 mL of agar medium were inoculated with 200 µL of the culture suspension. The wells (7.0 mm in diameter) were made and the extracts were added to wells (100 µL) and the same volumes (100 µL) of solvents were used as controls. Then, the inoculated plates were incubated at 37°C for 24 h for bacterial strains and at 30°C for 48 h for yeasts. After incubation, the diameter of the inhibition zone was measured with a calliper. The measurements were done basically from the edge of the zone to the edge of the well. All the experiments were carried out in triplicate and mean and average and standard deviation (SD) were calculated for the inhibition zone diameters.

2.3.3 Determination of minimal bactericidal (MBC) and fungicidal (MFC) concentration

Minimum inhibitory concentrations (MICs) of extracts were tested with a 2-fold serial dilution method with some modifications [13]. The MBC and MFC values were studied for the the microorganisms, being sensitive to the extracts in the agar well diffusion assay. The test samples were added to growth broth medium to get a final concentration of 360.00 mg/mL, and serially diluted to reach 180.00, 90.00, 45.00, 22.5, 11.25, 5.63 and 2.82 mg/mL. The final volume in each tube was 100 μL. 2.5 μL of standardized suspension of each tested microorganism were transferred into each tube. The inoculum of microorganisms was prepared using 12 h cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. A positive control (containing 2.5 μL inoculum and 100 μLgrowth medium) and a negative control (containing 2.5 μL of extract, 100 μL growth medium without inoculum) were included on each microtube. The contents of the tubes were mixed by pipetting, and they were incubated for 24 h. The lowest concentration of the tube which did not show any visible growth was considered as the MIC. However, the tested plant extracts in the study were colored, the visible growth could not be observed and so, 5 μL samples from all tubes were plated on solid growth medium. The MBC and MFC were recorded as the lowest concentration of the extract that did not permit any visible bacteria and fungal colony growth on the appropriate agar plate after the period of incubation [13]. So, the concentrations of the extracts that prevent the growth of a microorganism on the solid media were evaluated as MBC or MFC values in this study.

2.4 Determination of Antioxidant Activity

2.4.1 DPPH radical scavenging activity

Radical-scavenging ability was determined by a spectrophotometric method based on the reduction of a ethanol solution of DPPH (2, 2-diphenyl-1-picryl hydrazyl) using the method of Blois [14]. The extracts were dissolved in absolute ethanol (1 mg/mL) and the aliquots of 10, 50, 100 and 150 mL of each extract were transferred into test tubes. Same concentrations of BHT were used as positive control. Then, total volume was adjusted to 1.5 mL with ethanol. Finally, 0.5 mL of 0.1 mM solution of DPPH radical in ethanol was added to each of the test tubes, and the mixture was shaken vigorously. After 30 min incubation at room temperature in the dark, the decrease in absorbance was measured at 517 nm against ethanol as a blank by a spectrophotometer (UV-Vis Spectrophotometer HITACHI U-2000). The radical scavenging activity was calculated from the following equation:

$$\text{DPPH Scavenging activity (\%)} = [\,(A_{Control} - A_{Sample}) / A_{Control}\,] \times 100$$

where $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of plant extracts/standard antioxidant i.e., BHT. The IC_{50} value, extract concentration providing 50% inhibition, was calculated from the graph of percentage inhibition against extract concentration.

2.4.2 Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. [15] with slight modifications. A solution of

hydrogen peroxide (43 mM) was prepared in 0.1 M phosphate buffer (pH 7.4). 30 μg/mL concentration of the each extract in ethanol was added to a solution of 43 mM H_2O_2 (130 μL). Then, total volume was adjusted to 1.0 mL with 0.1 M phosphate buffer (840 μL). The absorbance of hydrogen peroxide at 230 nm was determined after 30 min against a blank solution containing phosphate buffer without hydrogen peroxide. BHA, BHT, α-tocopherol, ascorbic acid and trolox were used as positive controls. The percentage of scavenging activity of hydrogen peroxide was calculated using the following equation.

$$\% \text{ Scavenging } H_2O_2 = [(Abs_{control} - Abs_{sample})/Abs_{control}] 100$$

where $Abs_{control}$ is the absorbance of the control reaction, and Abs_{sample} is the absorbance in the presence of the test compound.

2.4.3 Chelating ability on ferrous ions

The chelating of ferrous ions by the extracts and standards was estimated according to the method of Dinis et al. [16] with some modifications. Briefly, 30 μg/mL of each extract in ethanol (30 μL) was added to a solution of 2 mM $FeCl_2$ (25 μL). Then, total volume was adjusted to 1.9 mL with ethanol. The reaction was initiated by the addition of 5 mM ferrozine (0.1 mL), and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm (UV-Vis Spectrophotometer HITACHI U-2000). The positive controls used were BHA, BHT, ascorbic acid, α-tocopherol, trolox and EDTA (ethylenediaminetetraacetic acid). The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following equation:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] 100$$

where $Abs_{control}$ is the absorbance of the control reaction, and Abs_{sample} is the absorbance in the presence of the test compound.

2.5 Determination of Total Antioxidant Contents

2.5.1 Determination of total phenolics

The concentration of total phenolic compounds in *S. stenoptera* and *P. nigdeensis* extracts was estimated with the Folin-Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi [17] with some modification as described below, using gallic acid as the standard phenolic compound. Briefly, 1 mL solution of extract containing 1mg extract in ethanol was mixed with 22 mL distilled water. 0.5 mL of Folin-Ciocalteu reagent was added, and the content of the flask mixed thoroughly. After 3 min, 1.5 mL of 2% Na_2CO_3 solution was added to the mixture. After incubation for 2 h at room temperature, the absorbance against blank was measured at 760 nm. Quantitative measurements were performed, based on a standard calibration curve of six points: 50, 100, 150, 200, 250, 300, 350 mg/mL of gallic acid in ethanol. The total phenolic content was expressed as μg gallic acid equivalents (GAE)/mg dry extracts. All samples were analyzed in triplicates and the average values were calculated.

2.5.2 Determination of total flavonoids

The measurement of total flavonoid content of the *S. stenoptera* and *P. nigdeensis* extracts was based on the method described by Park et al. [18] with slight modifications.

1 mL of the extract solution containing 1 mg extract was added to a test tube containing 0.1 mL of aluminum nitrate (10%), 0.1 mL of aqueous potassium acetate (1 M), and 4.1 mL of ethanol. After incubation for 40 min at room temperature, the absorbance was read spectrophotometrically at 415 nm using a HITACHI, U-2000 spectrophotometer. A calibration curve of quercetin was prepared, and flavonoid contents were determined from the linear regression equation of the calibration curve. The total flavonoid content was expressed as μg quercetin equivalents (QE)/mg of dry extracts. All extracts were analyzed in triplicate and the average values were calculated.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The results of the extraction yields of the *S. stenoptera* and *P. nigdeensis* are presented in Table 1. The highest yield was obtained from water followed by methanol, ethanol, DCM and n-hexane extraction for the stem and fruit + leaf of plants. The fruit + leaf yield was higher than the stem extracts. These variations in the yields were due to used different parts of the plant in the extraction.

Table 1. The yield of *S. stenoptera* and *P. nigdeensis* extracts.

		% Extract Yield*				
Plant	Part	Methanol	Ethanol	Water	n-Hexane	DCM
S. stenoptera	stem	9.55	3.06	15.19	0.58	1.06
	fruit+leaf	20.39	10.99	55.66	3.32	3.71
P. nigdeensis	stem	13.67	5.48	29.02	0.68	1.48
	fruit+leaf	18.35	7.88	35.52	1.76	2.57

*Yield (%) = (*W1* * 100) / *W2* (*W1* was the weight of extract after evaporation of solvent and *W2* was the dry weight of the sample)

The antimicrobial activity of *S. stenoptera* and *P. nigdeensis* extracts (obtained by ethanol, methanol, n-hexane, DCM and water), was evaluated against a set of 13 microorganisms. The results of *S. stenoptera* extracts are given in Table 2 and indicate that, the highest inhibitory activity was observed against *S. cerevisiae* (16.06 mm, DCM extract) and *E. coli* O157:H7 (9.98 mm, n-hexane extract) by stem and fruit+leaf part, respectively. The weakest inhibitory activity was against *L. monocytogenes* ATCC 7644 (3.17 mm, ethanol extract of stem) and *S. cerevisiae* (3.03 mm ethanol extract of fruit+leaf) by ethanol extract of the stem and fruit+leaf part, respectively. As a result, the inhibition zones of *S. stenoptera* stem and fruit+leaf extracts, against all test microorganisms were in the range of 3.03-16.06 mm.

The antimicrobial activities of *P. nigdeensis* extracts showed different values of inhibition zones on the test microorganisms (Table 3). The highest inhibitory activity was observed against *S. cerevisiae* (14.56 mm, DCM extract of stem) and *E. coli* O157:H7 (12.38 mm, n-hexane extract of fruit+leaf). The weakest inhibitory activity was determined against *S. cerevisiae* by methanol extracts stem and fruit+leaf part of *P. nigdeensis*, 3.19 mm and 3.31 mm, respectively. *Salsola stenoptera* and *P. nigdeensis* DCM stem extracts showed antimicrobial activity against all tested microorganisms except for *L. monocytogenes* ATCC 7644 and *Y. enterocolitica* NCTC 11175.

The MBC and MFC values for microorganisms which were sensitive to the

stem and fruit+leaf extracts of *S. stenoptera* in the agar well diffusion assay were in the range of 5.63-180.00 mg/mL and 22.50-180.00 mg/mL, respectively (Table 2). The highest inhibitory activity for the DCM stem extract was against *S. cerevisiae* which showed a lower MFC (22.50 mg/mL), the lowest inhibitory activity for the ethanol extract from fruit+leaf extract was against *S. cerevisiae* which showed a higher MFC (45.00 mg/mL) when compared to other studied extracts. Water extracts with low inhibitory activity, had the highest MBC (180.00 mg/mL) values for both parts of the plant. DCM stem extract showed the lowest MBC (5.63 mg/mL) value against *B.cereus* RSKK 863. MBC or MFC values for microorganisms which were sensitive to the stem and fruit+leaf extracts of *P. nigdeensis* were in the range of 11.25-45.00 mg/mL except for fruit+leaf-water extract (180.00 mg/mL) (Table 3). As a result, the highest MBC values obtained on tested microorganisms were the water extracts of *S. stenoptera* and *P. nigdeensis*.

The data for the inhibition zones (mm) and MBC and MFC values of *S. stenoptera* and *P. nigdeensis* extracts on the microorganisms indicated that the aqueous extract had ower or no effect against microorganisms tested when compared with other solvents extracts (Table 2 and 3). Several researchers have generally reported that the aqueous extracts of plants do not have much antibacterial activity [19-22]. The results of our study confirm the previous studies, indicating that water is not a suitable solvent for extraction of antibacterial compounds from plants when compared to other solvents [23-26].

Any part of the plant may contain active antimicrobial compounds but many reports show that plants leaves possess high antimicrobial activity than other parts [27, 28]. However, the present study suggested that stems of *S. stenoptera* and *P. nigdeensis* possess stronger antimicrobial activities than the fruit+leaf parts. As can be seen from the Table 2 and 3, *S. stenoptera* and *P. nigdeensis* extracts showed various degrees of antimicrobial activity depending on the tested microorganisms, polarity of the extraction solvent and parts of the plant.

Table 2. Antimicrobial activity of *S. stenoptera* extracts.

Org.	Diameter of zone of inhibition(mm)[a]										MBC[b] or MFC[c] (mg/mL)									
	stem extracts					fruit + leaf extracts					sem extracts					fruit + leaf extracts				
	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME
1	-	-	-	-	9.24±0	-	-	-	7.48±0	6.02±0.62	-	-	-	-	45.00	-	-	-	90.00	45.00
2	-	4.05±0.07	-	-	7.74±0	-	-	-	5.10±0.03	-	-	45.00	-	-	45.00	-	-	-	45.00	-
3	-	-	-	-	6.98±0	-	-	-	-	-	-	-	-	-	11.25	-	-	-	-	-
4	-	3.17±0.01	3.72±0	7.22±0	-	-	-	3.06±0	-	-	-	45.00	180.00	90.00	-	-	-	180	-	-
5	3.87±0.47	-	-	-	12.72±0	-	-	-	9.98±0.42	9.04±0.37	45.00	-	-	-	45.00	-	-	-	45.00	90.00
6	-	5.50±0.59	-	-	8.34±0	-	-	-	5.06±0	-	-	11.25	-	-	5.63	-	-	-	22.50	-
7	6.06±0.54	7.01±1.32	-	-	13.32±0	-	-	-	8.88±2.09	5.77±0.49	22.50	22.50	-	-	22.50	-	-	-	45.00	22.50
8	-	-	-	-	5.22±0	-	-	-	-	-	-	-	-	-	22.50	-	-	-	-	-
9	-	-	-	-	14.32±0	-	-	-	6.43±0.44	4.71±0.81	-	-	-	-	22.50	-	-	-	22.50	45.00
10	-	-	-	-	-	-	-	-	8.26±0	-	-	-	-	-	-	-	-	-	45.00	-

Table 2. Continued.

Org.	Diameter of zone of inhibition(mm)[a] stem extracts					fruit + leaf extracts					MBC[b] or MFC[c] (mg/mL) sem extracts					fruit + leaf extracts				
	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME
11	-	-	-	-	9.84±0	-	-	-	8.06±0.62	6.30±0.96	-	-	-	-	45.00	-	-	-	45.00	45.00
12	-	3.45±0.44	-	7.62±0	16.06±0	-	3.03±0.33	-	4.34±0	5.08±0.34	-	22.50	-	22.50	22.50	-	45.00	-	45.00	45.00
13	-	-	-	-	7.28±0	-	-	-	-	-	-	-	-	-	45.00	-	-	-	-	-

1: *E. coli* ATCC 11229, 2: *E.coli* ATCC 35218, 3: *S. aureus* ATCC 25923, 4: *L. monocytogenes* ATCC 7644, 5: *E. coli* O157:H7, 6: *B.cereus* RSKK 863, 7: *P. aeruginosa* ATCC 27853, 8: *M. luteus* NRRL B-4375, 9: *S. sonnei* Mu:57, 10: *Y. enterocolitica* NCTC 11175, 11: *C. albicans* ATCC 10231, 12: *S.cerevisiae* (grape isolate), 13: *S. enteritidis* ATCC 13076

[a]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of three seperate experiments.

[b]: Minimal Bactericidal Concentration (MBC)

[c]: Minimal Fungicidal Concentration (MFC)

ME: Methanol Extract, EE: Ethanol Extract, WE: Water Extract, HE: n-Hexane Extract, DCME: Dichloromethane Extract

Table 3. Antimicrobial activity of *P. nigdeensis* extracts.

Org.	Diameter of zone of inhibition(mm)[a] stem extracts					fruit + leaf extracts					MBC[b] or MFC[c] (mg/mL) sem extracts					fruit + leaf extracts				
	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME	ME	EE	WE	HE	DCME
1	-	-	-	-	9.22±0.01	-	-	-	-	-	-	-	-	-	45.00	-	-	-	-	-
2	-	3.62±0	-	-	5.70±0.01	-	-	6.38±0.28	-	-	-	45.00	-	-	45.00	-	-	180.00	-	-
3	-	-	-	-	6.38±0.01	-	-	-	-	-	-	-	-	-	11.25	-	-	-	-	-
4	-	-	-	6.27±0.01	-	-	-	-	-	-	-	-	-	22.50	-	-	-	-	-	-
5	-	-	-	5.87±0.27	11.44±0	4.06±0.31	-	-	12.38±1.65	-	-	-	-	45.00	45.00	45.00	-	-	45.00	-
6	5.57±0.01	4.52±0.01	-	6.01±1.32	8.34±0.01	-	-	-	-	6.30±0.01	22.50	45.00	-	22.50	22.50	-	-	-	-	45.00
7	-	6.83±0.30	-	9.22±0.06	11.60±0	-	3.85±0.38	-	8.85±0.30	11.16±0.25	-	45.00	-	45.00	45.00	-	11.25	-	22.50	45.00
8	-	-	-	6.22±0	5.58±0	-	-	-	-	-	-	-	-	22.50	45.00	-	-	-	-	-
9	-	-	-	6.75±0.27	14.40±0.01	-	-	-	6.97±0.07	-	-	-	-	45.00	45.00	-	-	-	22.50	-
10	-	-	-	-	-	-	-	-	11.52±1.61	8.54±0	-	-	-	-	-	-	-	-	22.50	22.50
11	3.68±0	3.89±0.33	-	-	13.36±1.44	-	-	-	6.18±0	4.30±0.01	45.00	22.50	-	-	11.25	-	-	-	45.00	22.50
12	3.19±0.64	4.37±0.18	-	14.47±1.97	14.56±0.01	3.31±0.78	-	-	-	6.18±0.01	45.00	22.50	-	22.50	11.25	45.00	-	-	-	11.25
13	-	-	-	-	5.22±0.01	-	-	-	-	8.50±0.01	-	-	-	-	11.25	-	-	-	-	45.00

1: *E. coli* ATCC 11229, 2: *E.coli* ATCC 35218, 3: *S. aureus* ATCC 25923, 4: *L. monocytogenes* ATCC 7644, 5: *E. coli* O157:H7, 6: *B.cereus* RSKK 863, 7: *P. aeruginosa* ATCC 27853, 8: *M. luteus* NRRL B-4375, 9: *S. sonnei* Mu:57, 10: *Y. enterocolitica*NCTC 11175, 11: *C. albicans* ATCC 10231, 12: *S.cerevisiae* (grape isolate), 13: *S. enteritidis* ATCC 13076

[a]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD of three seperate experiments.

[b]: Minimal Bactericidal Concentration (MBC)

[c]: Minimal Fungicidal Concentration (MFC)

ME: Methanol Extract, EE: Ethanol Extract, WE: Water Extract, HE: n-Hexane Extract, DCME: Dichloromethane Extract

3.2 Antioxidant Activity

3.2.1 Free radical scavenging activity

DPPH assays have been used by many researchers to determine free radical scavenging activity of antioxidant compounds [29, 30]. All the extracts obtained by different solvents of varying polarities were subjected to screening for their possible radical-scavenging ability. DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds and crude extracts of plants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [31]. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical-scavenging ability of specific compounds or extracts [32]. The free radical DPPH possesses a characteristic absorption at 517 nm, which decreases significantly on exposure to radical-scavengers. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract. The results are expressed as percentage (%) of inhibition exhibited by the plant extracts and the standard antioxidant i.e., BHT (Figure 1).

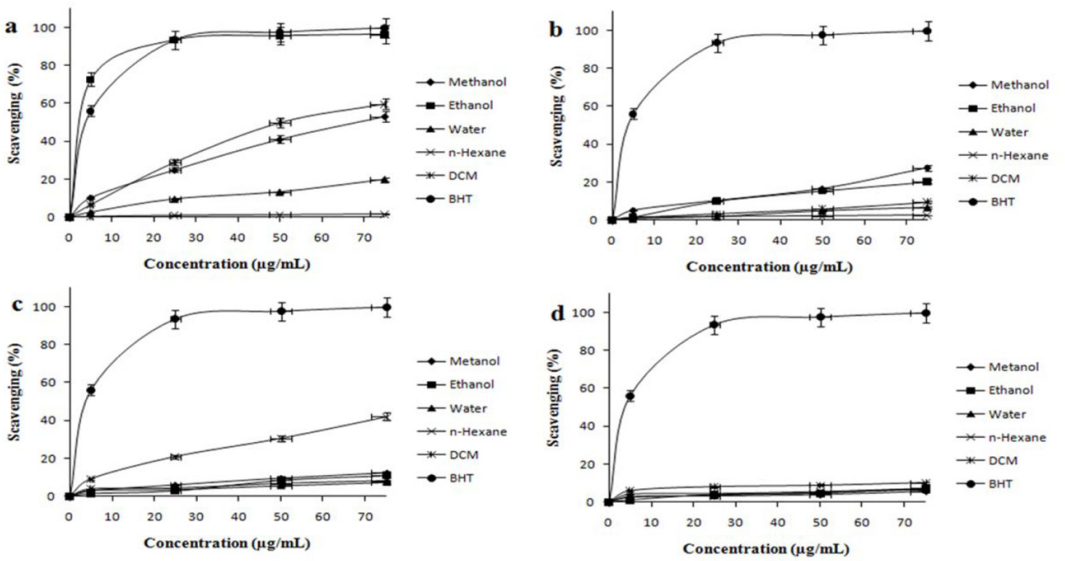

Figure 1. DPPH radical scavenging activities (%) of *S. stenoptera* stem (a) and fruit + leaf (b), and *P. nigdeensis* stem (c) and fruit + leaf (d).

The investigation showed that the radical scavenging activity increased with the increase in the concentration of all the extracts or standart antioxidant (at concentrations ranging from 5 to 75 µg/mL). As seen in Figure 1, different solvent extracts of stem of *S. stenoptera* and *P. nigdeensis* plants showed higher radical scavenging activity than the fruit + leaf parts. Especially, the ethanol extract of stem part of *S. stenoptera* and n-hexane extract of stem part of *P. nigdeensis* showed the highest DPPH scavenging activities. On the other hand, DPPH radical scavenging activity of the investigated extracts slightly differs depending on the solvent used. For example, ethanol extract of stem part of *S. stenoptera* exhibited the highest scavenging activity, whereas n-hexane extract had the lowest scavenging activity. Generally, the higher ability of ethanolic

extracts could be due to more hydrogen-donating componets extracted by ethanol. N-hexane extracts of *S. stenoptera* showed the least antioxidant, which can be explained by the presence of no-scavenger molecules like pigments (chlorophyll) or wax. The IC_{50} values of *S. stenoptera* and *P. nigdeensis* extracts were determined from the graph of the percentage inhibition against extract concentration. Lower IC_{50} value indicates a higher DPPH free radical scavenging activity. The ethanol extract of stem of *S. stenoptera* and the n-hexane extract of stem of *P. nigdeensis* showed the highest scavenging abilitiy on DPPH radicals with the IC_{50} values of 29.84 μg/mL and 83.56 μg/mL, respectively. Since BHT is a strong synthetic antioxidant, it showed the lowest IC_{50} value of 29.33 μg/mL. This result is in correlation with data found by Liu et al. [33], who revealed that the three of the seven flavonoids, namely luteolin, chrysin, and quercetin 3-O-β-D-glucopyranoside isolated from the ethyl acetate fraction of the crude ethanol extract of the aerial parts of *Halostachys caspica* (Chenopodiaceae) showed IC_{50} values of 25.75, 36.67 and 82.55 μg/mL, respectively. In addition, Tundis et al. [34] reported that alkaloid extract obtained from *S. oppositifolia* showed activity with an IC_{50} value of 16.3 μg/mL, while *S. soda* and *S. tragus* extracts exhibited an IC_{50} value of 24.3 μg/mL and 26.2 μg/mL, respectively. The IC_{50} value for curcumin was found to be 34.86 μg/mL [35].

3.2.2 Hydrogen peroxide scavenging activity

Hydrogen peroxide can attack many cellular energy-producing systems. H_2O_2 itself is not very reactive but becomes toxic to cells when it give rise to hydroxyl radical within the cells. Therefore, removing hydrogen peroxide is very important for protection of cells. The ability of BHA, BHT, Vitamin C, a-tocopherol, trolox and extracts of *S. stenoptera* and of *P. nigdeensis* to scavenge hydrogen peroxide are shown in Figure 2.

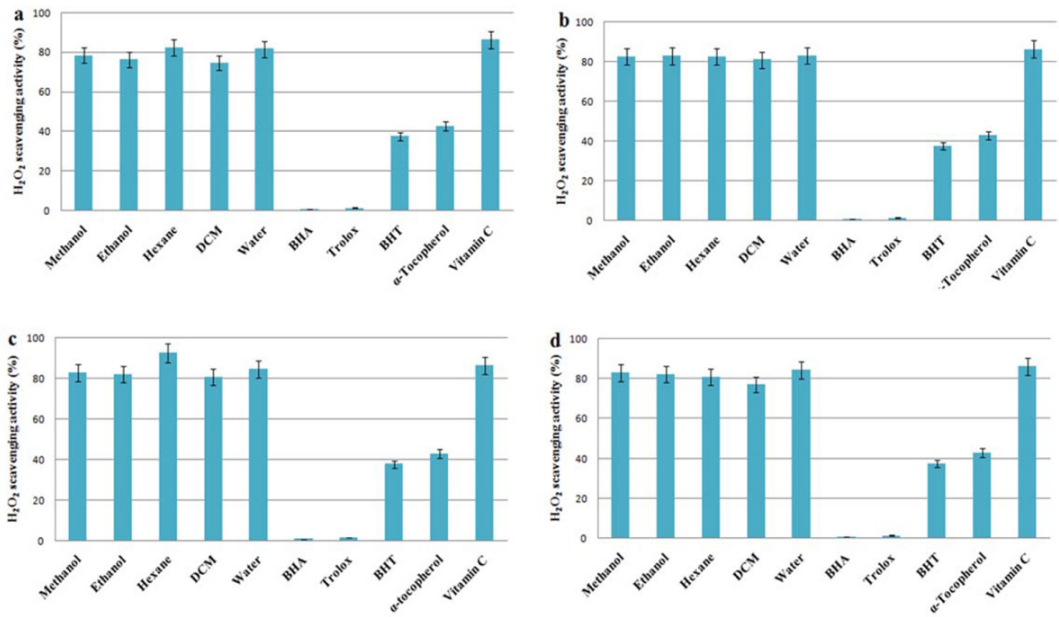

Figure 2. Hydrogen peroxide scavenging activity of different solvent plant extracts and standard antioxidants at 30 μg/mL concentration. *S. stenoptera* stem (a) and fruit + leaf (b), and *P. nigdeensis* stem (c) and fruit + leaf (d).

The percentages of hydrogen peroxide scavenging capacity of the standarts i.e., BHA, trolox, BHT, α-tocopherol, and Vitamin C were 0.73%, 1.55%, 37.60%, 42.85% and 86.17%, respectively, at the 30 μg/mL concentration. All the extracts of *S. stenoptera* and of *P. nigdeensis* showed significant hydrogen peroxide scavenging activity. For example, the percentages of hydrogen peroxide scavenging ability of methanol, ethanol, n-hexane, DCM, and water extracts of fruit + leaf part of *S. stenoptera* were found as 82.42%, 82.77%, 82.57%, 80.83% and 82.84%, respectively, at the 30 μg/mL concentration. On the other hand, among the extracts, n-hexane extract of stem part of *S. stenoptera* exhibited the highest scavenging activity on hydrogen peroxide with a value of 92.97%. These results showed that extracts of *S. stenoptera* and of *P. nigdeensis* had a strong hydrogen peroxide scavenging activities. For example, Ak and Gülçin [35] reported that hydrogen peroxide scavenging activity of curcumin at 15 μg/mL is 28.40%.

3.2.3 Ferrous metal ions chelating activity

The ferrous ion chelating activities of the extracts of *S. stenoptera* and of *P. nigdeensis*, and the standart antioxidants i.e., BHA, BHT, Vitamin C, a-tocopherol, trolox and EDTA are shown in Figure 3. Among the transition metals, iron is known to be the most important lipid oxidation prooxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\times$) [36]. Therefore, metal chelating activity is significant, since it reduces the concentration of the catalysing transition metal in lipid peroxidation. Chelating activities of the extracts were determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, complex formation is disrupted, resulting in a reduction in the red colour of the complex. Measurement of colour reduction therefore allows estimation of the metal chelating activity of the coexisting chelator. Lower absorbance indicates higher metal-chelating activity [37].

Ferrous ion chelating effects of *S. stenoptera* and *P. nigdeensis* stem extracts were compared to those of BHA, BHT, α-tocopherol, trolox, Vitamin C and EDTA. The percentages of metal scavenging capacity of the standart antioxidants i.e., α-tocopherol, trolox, BHT, BHA, Vitamin C and EDTA were found as 8.33%, 11.36%, 30.30%, 40.91%, 60.61% and 84.09%, respectively, at the 15 μg/mL concentration. The formation of Fe^{2+} -ferrozine complex is not completed in the presence of extracts which indicate that the extracts chelate the ferrous ion. All the studied extracts had a strong chelating effect on ferrous ions. But, significant differences in chelating activity were observed among the extracts. For example, extracts of *P. nigdeensis* have higher metal chelating ability than *S. stenoptera*. *Petrosimonia nigdeensis* fruit + leaf part extracts exhibited the highest ferrous ion chelating ability. The percentages of metal chelating capacity of methanol, ethanol, n-hexane, DCM, and water extracts of *P. nigdeensis* were found as 98.88%, 99.15%, 99.19%, 99.15% and 98.65%, respectively, at the 15 μg/mL concentration. The ferrous metal ions chelating ability of these extracts decreased in the order of n-hexane > etanol ≈ DCM > methanol > water. The metal chelating capacity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Figure 3 shows that all the extracts of *S. stenoptera* and

P. nigdeensis demonstrate a marked capacity for iron binding, suggesting that their action as lipid peroxidation protector. Ak and Gülçin [35] reported that at 15 μg/mL concentration, curcumin exhibited 56.7% chelation of ferrous ion.

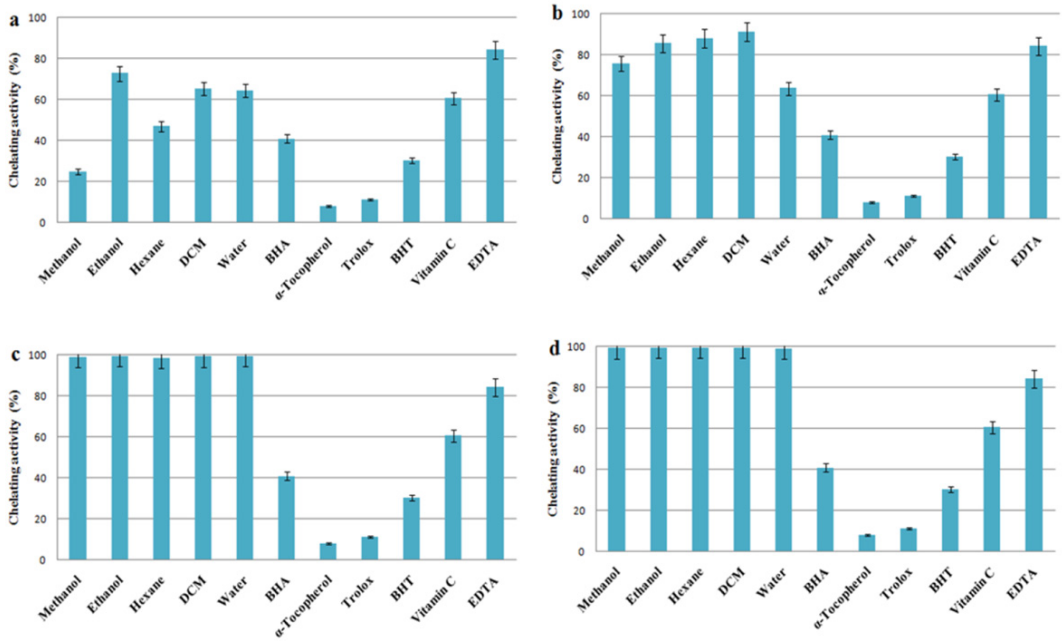

Figure 3. Ferrous ion (Fe^{+2}) chelating activity of different solvent plant extracts and standard antioxidants at 15 μg/mL concentration. *S. stenoptera* stem (a) and fruit + leaf (b), and *P. nigdeensis* stem (c) and fruit + leaf (d).

3.3 Total Phenolic and Flavonoid Contents

Phenolic and flavonoid compounds scavenge radicals and inhibit the chain initiation or break the chain propagation. Phenolic contents are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, it is important to consider the effect of the total phenolic content on the antioxidant activity of *S. stenoptera* and *P. nigdeensis* extracts. Moreover, flavonoids have been shown to exhibit the antioxidative, antiviral, antimicrobial and anti-platelet activities [38]. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure [39]. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [40, 41]. Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; (2) scavenging ROS; and (3) upregulation or protection of antioxidant defenses [39, 42]. The results in Table 4 show that all the extracts had high phenolic and flavonoid contents. The total phenolic and flavonoid contents values significantly differ for methanol, ethanol, water, n-hexane and DCM extracts.

Table 4. Amount of total phenolic and flavonoid contents of *S. stenoptera* and *P. nigdeensis* extracts.

		Antioxidant Components		ME	EE	WE	HE	DCME
S. stenoptera	stem	Phenolics	(μgGAE/mg extract)	44.78± 0.72	33.81± 0.16	30.06± 0.24	27.42± 0.02	34.22± 0.08
		Flavonoids	(μgQE/mg extract)	61.51± 0.07	34.53± 0.07	42.83± 0.20	5.76± 0	29.06± 0.07
	fruit + leaf	Phenolics	(μgGAE/mg extract)	30.06± 0.56	42.97± 0.96	27.00± 0.08	27.42± 0	31.58± 0.16
		Flavonoids	(μgQE/mg extract)	24.91± 0.20	36.79± 0.20	28.59± 0.13	19.72± 0.13	63.68± 0.13
P. nigdeensis	stem	Phenolics	(μgGAE/mg extract)	29.39± 0.02	30.19± 0.16	25.33± 0.08	28.94± 0.08	40.89± 0.08
		Flavonoids	(μgQE/mg extract)	40.85± 0.13	36.32± 0.13	2.26± 0.07	27.64± 0	57.55± 0.07
	fruit + leaf	Phenolics	(μgGAE/mg extract)	27.83± 0.08	28.11± 0.56	26.44± 0.08	29.36± 0	28.39± 0.40
		Flavonoids	(μgQE/mg extract)	38.21± 0.13	32.55± 0	29.25± 0.33	66.69± 0.13	45.57± 0.01

ME: Methanol Extract, EE: Ethanol Extract, WE: Water Extract, HE: n-Hexane Extract, DCME: Dicholoromethane Extract

The amount of total phenolics in extracts was determined with the Folin - Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as gallic acid equivalents (GAE) The total phenolic contents of stem extracts of *S. stenoptera* varied from 27.42 to 44.78 mg GAE/mg extract and that of fruit + leaf extracts varied from 27.00 to 42.97 mg GAE/ mg extract. On the other hand, the total phenolic contents of stem extracts of *P. nigdeensis* were between 25.33 and 40.88 mg GAE/mg extract and that of fruit + leaf extracts were between 26.44 and 29.36 mg GAE/mg extract.

The result of total flavonoid contents of the extracts of *S. stenoptera* and *P. nigdeensis* is given in Table 4. Total flavonoid contents of the extracts were determined by using the aluminium chloride colorimetric assay method and expressed as quercetin equivalents. The total flavonoid contents of stem extracts of *S. stenoptera* varied from 5.76 to 61.51 μg QE/mg extract and that of fruit + leaf extracts varied from 19.72 to 63.69 μg QE/mg extract. On the other hand, the total flavonoid contents of stem extracts of *P. nigdeensis* were between 2.26 and 57.55 μg QE/mg extract and that of fruit + leaf extracts were between 29.25 and 66.69 μg QE/mg extract. Bursal and Gülçin [37] found the total content of flavonoids in lyophilized aqueous extract of propolis as 8.15 quercetin equivalents.

The methanol extract of *S. stenoptera* stem part showed the highest concentration of total phenolics. On the other hand, the extract that had the lowest concentration in total phenolics was the aqueous extract of *S. stenoptera* fruit + leaf part. The extract

that had the highest concentration in total flavonoids is the DCM extract of *S. stenoptera* fruit+leaf part, while the n-hexane extract of *S. stenoptera* stem part showed the lowest concentration of total flavonoids.

The extract that had the highest concentration in total flavonoids is the n-hexane extract of fruit+leaf part of *P. nigdeensis*, while the aqueous extract of stem part of *P. nigdeensis* showed the lowest concentration of total flavonoids. The DCM extract of *S. stenoptera* fruit+leaf part showed the highest concentration of total flavonoids. On the other hand, the extract that had the lowest concentration in total flavonoids is the n-hexane extract of stem of *S. stenoptera.* The variation may be due environmental conditions, which can modify the constituents of the plant and the polarity of the solvent used for extraction.

4. CONCLUSIONS

The results demonstrated antimicrobial and antioxidative activities of various solvent extracts of *S. stenoptera* and *P. nigdeensis* plants of Aksaray province in Turkey. Ethanol (semi polar) and n-hexane (non-polar) extracts of *S. stenoptera and P. nigdeensis* stem showed the highest scavenging abilitiy on DPPH radicals. This difference could be explained by the chemical nature of flavonoids and phenolics that the each extract contains. Ethanol is a polar solvent known to extract a broad range of molecules including glycoside, sugar and weakly polar compounds. On the other hand, n-hexane is a apolar solvent that extracts hydrophobic compounds like aglycone and long carbon chain ones. As it is well known that antioxidant acivities are structure dependent. It might be possible that the types of flavonoid and phenolic compounds present in ethanol or n-hexane extracts of *S. stenoptera and P. nigdeensis* stem are appropriate for these functional activities. Present study has established that phytochemicals present in *S. stenoptera and P. nigdeensis* extracts have capability to quench free radicals generated in the system, iron binding and scavenging of hydrogen peroxide. Therefore *S. stenoptera and P. nigdeensis* could be considered promising source of bioactive compounds for food and/or pharmaceutical industry.

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