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A Comparative Study on Antioxidant and Antimicrobial Activities of Four *Senecio* L. Species from Turkey

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Abstract: The present study was conducted to investigate total phenolic contents, antioxidant and antimicrobial activities of four *Senecio* L. (Asteraceae). The *in vitro* antioxidant activities of extracts were evaluated with different antioxidant testing systems. Their antioxidant activities were assessed by phosphomolybdenum reduction, DPPH and β-carotene bleaching assays. The extracts exhibited strong antioxidant activity and high scavenging activities against DPPH free radicals. The extracts exhibited weak to moderate β-carotene bleaching activity. The antimicrobial activity of *Senecio* extracts was tested against 15 microorganisms using agar diffusion and broth microdilution assays. The extracts were found to be weak to moderate effective against 8 out of the 15 microorganisms tested with minimal inhibitory concentration (MIC) values ranging from 1.5 to 12.5 mg/ml. In conclusion, it can be concluded that *Senecio* extracts may be considered as natural sources in many industry such as food and pharmacy.

***Keywords**: Senecio;* phenolic content; antioxidant activity; antimicrobial activity

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radicals and hydrogen peroxide, are chemically reactive molecules derived from oxygen. They are produced in cells by different means (Zho et al., 2006). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Unal et al., 2008). ROS may cause damage on carbohydrates, proteins, lipids and DNA (Zho et al., 2006). There is increasing evidence to suggest that various degenerative diseases, such as brain dysfunction, cancer, heart diseases and immune system decline, could be the result of cellular damage caused by free radicals (Lu and Foo, 2001). Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Wang and Ballington, 2007). As well known, many polyphenolic and flavonoid compounds possess antioxidant activities and can react as reducing agent, free radicals quenchers and metal chelating agent (Wu et al., 2006a). At the present time, the most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and *tert*-butyl hydroquinone. However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Ak and Gulcin, 2008). In recent years, multiple resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Zampini et

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al., 2005). Therefore, there is a great interest concerning medicinal plants and herbs have the antioxidant and antimicrobial properties.

Senecio L. is the largest genus in the family Asteraceae (tribe Senecioneae) and includes about 1500 species widespread all over the world (Christov et al., 2002; El-Shazly et al., 2002; Loizzo et al., 2004; Loizzo et al., 2006). In the Turkish flora *Senecio* is represented by 39 species (Uğur at al., 2006). *Senecio* species were used as food and in folk medicine in Mediterranean area in the treatment of wounds and as antiemetic, anti-inflammatory and vasodilator preparations (Conforti et al., 2006 a; Tundis et al., 2006; Loizzo et al., 2004; Loizzo et al., 2006). Moreover, some studies reported the cytotoxic activity of some species of *Senecio* (Conforti et al., 2006 a). Pyrrolizidine alkaloids, chalcones and flavonoids have characterized from *Senecio* species (Conforti et al., 2006 a; Loizzo et al., 2004). A current search of literature revealed that there were only limited research on the potential antioxidant and antimicrobial activities of some *Senecio* species belonging to Turkish flora (Uğur et al., 2006, Uzun et al., 2004; Albayrak et al., 2008; Albayrak et al., 2014), although many studies indicated antibacterial and antifungal activities of compounds from *Senecio* species (Pérez et al., 1999; Kiprono et al., 2000; El-Shazly et al., 2002). In view of these reports and in continuation of our previous works in the biological activity of *Senecio* species, we have investigated the total phenolic content, *in vitro* antimicrobial and antioxidant activities of extracts from four *Senecio* species (*S. racemosus*, *S. nemorensis*, *S. fluviatilis*, *S. pseudo-orientalis*).

2. Materials and Methods

Plant materials

1. Collection information of the four *Senecio* species, which are individually numbered, is listed below:
2. 1. *S. fluviatilis* Wallr. Erzincan: Between Tercan and Aşkale, Turkey (39^{o}48'14"K–40^{o}31'24"D) 1550 m, 31.07.2006 (Aksoy 2074).
3. 2. *S. nemorensis* L. Artvin: Between Şavşat and Ardahan, (41^{o}13'67"K–42^{o}31'25"D) 2350 m, 19.08.2006 (Aksoy 2074).
4. 3. *S. pseudo-orientalis* Schischkin Between Erzurum and Bayburt, (40^{o}01'58"K–40^{o}30'97"D) 2425 m, 20.07.2006 (Aksoy 2045).
5. 4. *S. racemosus* (Bieb.) DC. Erzurum ring road, (39^{o}57'57"K–41^{o}16'67"D) 1800 m, 02.07.2006 (Aksoy 2041).

Voucher specimens identified by Dr. Ahmet Aksoy and have been deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey.

Preparation of the plant extracts

Collected plant materials were dried at room temperature**.** Aerial parts of plants were ground to fine powder. Ground herbs (10 g) were extracted in a Soxhlet extractor with 100 ml methanol (50 °C for 6 h). The extract was concentrated by using rotary evaporator (Rotavator, T<40 °C) under vacuum to get crude extracts. Dried extracts were stored at 4 $^{\circ}$C until use.

Determination of total phenolic content

The total phenolic amount in the extracts has been assessed with the Folin–Ciocalteau total phenols photometric assay (Singleton & Rossi, 1995). 40 µl of methanol extract (1 mg/ml) were taken in test tubes. 2.4 ml of distilled water, 200 µl of Folin-Ciocalteu reagent, 600 µl sodium carbonate (20%) and 760 µl distilled water were added respectively. The tubes were mixed and allowed to stand at the dark for 2 hours. The absorbance was measured at 765

nm against a blank, which contained 40 µl of methanol in place of extract. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of methanol extract.

Evaluation of total antioxidant capacity by phosphomolybdenum method

The antioxidant power of the extracts has been assessed with the phosphomolybdenum reduction assay according to Prieto et al. (1999). 0.4 ml of methanol extract (1 mg/ml) was mixed with 4 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 0.4 ml methanol was mixed with 4 ml of the reagent. The samples were incubated in water bath at 95 ºC for 90 min. After the samples had cooled to room temperature, the absorbance of the green phosphomolybdenum complex was measured at 695 nm. The antioxidant capacity of the extracts was expressed as the ascorbic acid equivalents (mg AAE /g of methanol extract).

DPPH free radical scavenging activity

The procedure of Lee et al. (1998) has been adopted for the evaluation of free radical scavenging capacity of the studied plant extracts. The 50 µl of extract dilution at the concentration range of 0.1-2 mg/ml was mixed with 450 µl of Tris-HCl buffer, pH = 7.4 and 1 ml of the methanol DPPH solution (0.1 mM). The absorbance has been measured at 517 nm after standing at room temperature for 30 min. The control contained 50 µl of methanol in place of extract. The butylated hydroxytoluene (BHT) was used as a positive control. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the free radical scavenging activity, as percentage inhibition (I %) of DPPH radical.

$$\text{Percentage inhibition (I \%)} = [(A_C\text{-}A_S)/ A_C] \times 100$$

Where $A_{C:}$ absorbance of the control after 30 min; A_S: absorbance of the test sample after 30 min. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. The assay was carried out in triplicate.

β-Carotene bleaching activity assay

The extracts' ability to inhibit the bleaching of the β -carotene–linoleic acid emulsion was determined (Cao et al. 2009). β -Carotene (10 mg) was dissolved in 10 ml of chloroform ($CHCl_3$). An aliquot (0.2 ml) of this solution was added to a boiling flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator at 40 ºC for 5 min. Distilled water (50 ml) was slowly added to the residue and mixed vigorously to emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the extract solution. The test emulsion was incubated in a water bath at 50 ºC for 2 h, at which point the absorbance was measured at 470 nm. In the negative control, the extracts were substituted with an equal volume of ethanol. BHT was used as positive control.

$$\text{\% Inhibition} = [(At–Ct)/(Co–Ct)] \times 100$$

Where At and Ct are the absorbances measured for the test sample and control, respectively, after incubation for 2 h, and Co is the absorbance values for the control measured at zero time during the incubation.

Antimicrobial activity

The fifteen microorganisms which contain thirteen bacteria and two yeasts were used as test organisms: *Aeromonas hydrophila* ATCC 7965, *Bacillus brevis* FMC 3, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella*

pneumoniae ATCC 27736, *Mycobacterium smegmatis RUT*, *Morganella morganii*, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRLE 4463, *Staphylococcus aureus* ATCC 29213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223 and *Saccharomyces cerevisiae* BC 5461.

These microorganisms were supplied by the department of Food Engineering, Erciyes University, Kayseri, Turkey. Test yeast namely *C. albicans* and *Y. enterocolitica* were grown in malt extract and nutrient broths at 25 ^{o}C for 18 h, respectively. The other microorganisms were grown in nutrient broth at 35 ^{o}C for 18 h. All test microorganisms in nutrient broth or malt extract broth were enumerated by using the serial dilution method. Their final cell concentrations were 10^{6}-10^{7} cfu/ml. The agar well diffusion method (Sagdic et al., 2006) was used to detect antimicrobial activity. 250 µl of each microorganism was added into a flask containing 25 ml sterile nutrient agar or malt extract agar at 45 ^{o}C and poured into Petri dishes (9 cm diameter). Then, the agars were allowed to solidify at 4 ^{o}C for 1 h. The holes were made in the agar using sterile cork borers (Ø = 6 mm). The extracts (50 µl) were prepared at 1.0-10.0% concentrations in absolute methanol and were applied to the holes using a pipettor and absolute methanol without herb extract was used as a control. *Y. enterocolitica* and *C. albicans* was incubated at 25 ^{o}C for 14-24 h in the inverted position. The other microorganisms were incubated at 35 ^{o}C for 18-24 h. At the end of the period, inhibition zones which formed on the medium were measured in millimeters (mm). Tetracycline (10 mg/ml) (Sigma T3258-56) standard antibiotics was used as positive control.

Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) values were also studied for the microorganisms, which were determined as sensitive to the extracts in the agar well diffusion assay. The inocula of the bacterial strains were prepared from 18 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. First of all, the extracts were dissolved in dimethylsulfoxide (DMSO). Then they were diluted to the highest concentration (50 mg/ml) to be tested. Next, serial two-fold dilutions were made of the extracts in a concentration range of 0.75 to 50 mg/ml in 10 ml sterile test tubes containing nutrient broth. The MIC values of the extracts against bacterial strains were determined based on a micro-well dilution method (Sokmen et al. 2004). In brief, 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inocula into each well. Then, 100 µl of their serial dilutions was transferred into six consecutive wells. The last well, containing 195 µl of nutrient broth without compound and 5 µl of the inocula on each strip, was used as a negative control. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 37 ^{o}C for 24 h. The MIC was defined as the lowest concentration of the sample that prevented visible growth.

Statistical analyses

Data from the experiments were subjected for the analysis of variance (ANOVA) using SPSS (2001) for Windows. Percentage data were transformed using arcsine $\sqrt{x}$ before ANOVA. Means were separated at the 5% significance level by the least significant difference (LSD) test. Bivariate correlations were analyzed by Pearson's test using SPSS 10.0 for Windows.

3. Results and Discussion

The extract yields of the extracts obtained from four *Senecio* species with methanol varied from 15.14% to 25.44% (w/w) (Table 1). The amount of total phenolic contents, measured by Folin–Ciocalteu method, ranged from 32.25 to 139.43 mg GAE/ g dry weight.

The statistical differences among total phenolic contents of the extracts were important ($P< 0.05$). Among the four *Senecio* species, the amounts of total phenolic content decrease in the following order: *S. racemosus* > *S. nemorensis* > *S. fluviatilis* > *S. pseudo-orientalis*. The statistical differences among the total phenolic contents of *S. racemosus* and *S. nemorensis* were not found. Their total phenolic contents were 139.43 and 136.05 mg GAE/ g dry weight, respectively (Table 1).

Total antioxidant activity, measured by the phosphomolybdenum method, ranged from 122.96 to 171.25 mg AAE/ g dry weight. There were statistical differences among the total antioxidant activities of the extracts tested ($P<0.05$). The highest level of antioxidant activity was found in *S. nemorensis*, while the lowest was in *S. pseudo-orientalis* (Table 1). Total antioxidant activity decreases in the order: *S. nemorensis*> *S. racemosus*> *S. fluviatilis* > *S. pseudo-orientalis*.

Table 1. The yields, total phenolic contents, total antioxidant, β-carotene bleaching activities and IC_{50} values of *Senecio* methanolic extracts

Senecio species	Yield (%)	Total Phenolic Content (mg GAE/ g extract)	Total Antioxidant Activity (mg AAE/ g extract)	β-Carotene Bleaching Activity	DPPH IC_{50} (µg /ml)
S. fluviatilis	15.14	$60.65 \pm 4.6^{b*}$	135.62 ± 0.8^{c}	54.80 ± 1.5^{a}	61.31^{b}
S. nemorensis	18.15	136.05 ± 1.2^{a}	171.25 ± 1.8^{a}	21.79 ± 0.1^{c}	18.81^{d}
S. pseudo-orientalis	21.69	32.25 ± 0.0^{c}	122.96 ± 3.6^{d}	17.75 ± 0.8^{d}	70.80^{a}
S. racemosus	25.44	139.43 ± 1.5^{a}	142.03 ± 0.5^{b}	25.47 ± 0.0^{b}	25.40^{c}

*: In each column, means of three independent experiments (± SD) with different superscript letters are significantly different ($p < 0.05$). Total phenolic activity expressed as gallic acid equivalent (GAE), total antioxidant activity expressed as ascorbic acid equivalent (AAE).

DPPH molecule that contains a stable free radical has been widely used to evaluate the radical scavenging ability of antioxidants. DPPH radical scavenging activities of four *Senecio* species are shown in Table 1 and Fig 1. The extracts exhibited a concentration-dependent DPPH radical scavenging activity. The Figure 1 shows that *S. nemorensis* and *S. racemosus* were excellent DPPH radical scavengers, with 92.58% and 87.60% inhibition rate, respectively. *S. nemorensis* is significantly better than BHT (92.15%) at 66.6 µg/ml concentration. On the other hand, *S. fluviatilis* and *S. pseudo-orientalis* showed only moderate activities with 54.41% and 48.01% inhibition rate, respectively. *S. nemorensis* showed significantly stronger activity with IC_{50}= 18.81 µg/ml than other tested *Senecio* species (Table 1). *S. nemorensis* showed the highest antioxidant activity in both phosphomolybdenum and DPPH methods with the second highest total phenolic content. Later, *S. racemosus* showed the second highest antioxidant activity in both phosphomolybdenum and DPPH methods with the highest total phenolic content while the antioxidant activity was weak at the β-Carotene bleaching method (25.47%) (Table 1).

β-Carotene bleaching activity method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β -carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al., 2004). The β-carotene bleaching inhibition rates of *Senecio* extracts were evaluated in comparison with BHT and are shown in Table 1. Result showed that all extracts had lower antioxidant activities than had standard BHT. The inhibition rates of the extracts ranged from lowest of 17.75% to the highest of 54.80%, while BHT showed 93.75% inhibition. The highest antioxidant activity among the extracts was observed in *S. fluviatilis* whereas *S. pseudo-orientalis* had the lowest antioxidant activity. The orders of the

antioxidant activity are as follow: BHT > *S. fluviatilis* > *S. racemosus*> *S. nemorensis*> *S. pseudo-orientalis*.

Inhibition of microbial growth was investigated using agar diffusion and microdulition methods. The results are given Table 2 and Table 3. As can be seen from the Tables 2-3, all of the extracts showed weak to moderate the antibacterial activity against most bacteria. Inhibition zone diameters varied from 7.0 to 11.0 mm. The solvents used for control did not show any activity. *A. hydrophila, P. mirabilis, P. aeruginosa, B. brevis, B. subtilis, M. smegmatis* and *S. aureus* were the most sensitive bacteria to the extracts. All of the extracts did not exhibit antibacterial activity against *E. coli, K. pneumonia, M. morganii, S. typhimurium, Y. enterocolitica* among the Gram (-) bacteria. The minimum inhibition concentration values (MICs) of the extracts showing inhibition zones were also determined (Table 3). MIC values were in the range of 1.5-12.5 mg/ml. Among the Gram (-) bacteria, *P. mirabilis* was only inhibited by *S. fluviatilis* and *S. pseudo-orientalis* with 12.5 mg/ml MIC value. Among the Gram (+) bacteria, *B. cereus* was only inhibited by *S. pseudo-orientalis* with 3.13 mg/ml MIC value, while *S. aureus* was only inhibited by *S. fluviatilis* and *S. racemosus* with 12.5 and 1.5 mg/ml MIC values, respectively. All *Senecio* extracts did not exert any activity against yeasts tested (*C. albicans* and *S. cerevisiae*).

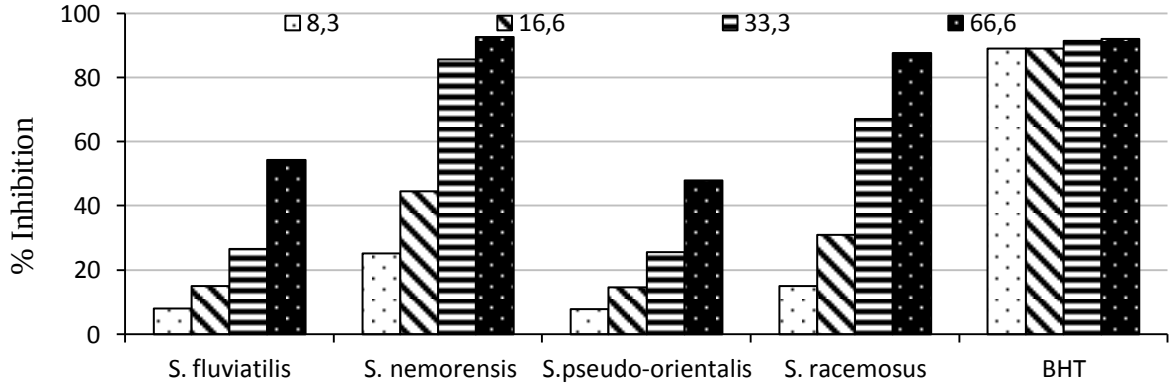

Figure 1. %DPPH inhibition values of four *Senecio* species at different concentration (µg/ml)

Statistically, Pearson correlation showed there was positive correlations between total phenolic content and total antioxidant activity (r= 0.788). The negative correlation was also found for DPPH radical scavenging activity with total phenolic content (r= -0.987). However, there was no correlation between total phenolic content and β-carotene bleaching activity (r= -0.227). There was not any relationship between total antioxidant activity and DPPH radical scavenging activity with β-carotene bleaching activity (r= -0.157 and r= 0.300, respectively). But, correlation between total antioxidant activity and DPPH radical scavenging activity is significant at the 0.01 level (r= -0.853). Similar results were reported by Maisarah et al., (2013) who found positive correlations between total phenolic content and DPPH radical scavenging assay, while no correlation between total phenolic content and β-carotene bleaching activity.

Table 2. Antimicrobial activities of four *Senecio* species

Microorganisms	Extracts (% Concentrations)																Tetracycline
	S. fluviatilis				*S. nemorensis*				*S. pseudo-orientalis*				*S. racemosus*				
	10.0	5.0	2.5	1.0	10.0	5.0	2.5	1.0	10.0	5.0	2.5	1.0	10.0	5.0	2.5	1.0	10.0 mg/ml
Gram (-)																	
A. hydrophila	9.0*	8.0	7.5	7.0	9.0	8.5	8.0	7.5	9.0	8.0	8.0	7.0	10.0	8.5	8.0	7.0	25.0
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.0
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25.0
M. morganii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.0
P. mirabilis	7.0	-	-	-	-	-	-	-	9.0	8.0	7.5	7.0	-	-	-	-	21.0
P. aeruginosa	9.0	8.5	8.0	7.0	9.0	8.5	7.0	6.5	9.5	8.5	8.0	7.5	8.0	7.0	-	-	23.0
S. typhimurium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.0
Y. enterocolitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29.0
Gram (+)																	
B. brevis	8.5	8.0	7.5	7.0	8.5	8.0	7.5	7.0	9.0	8.0	7.5	-	8.0	7.5	7.0	-	35.0
B. cereus	-	-	-	-	-	-	-	-	9.0	-	-	-	-	-	-	-	33.0
B. subtilis	11.0	8.0	7.0	-	10.0	8.0	-	-	10.0	7.5	-	-	-	-	-	-	30.0
M. smegmatis	9.5	8.5	8.0	7.5	9.0	8.0	8.0	7.5	10.0	9.0	8.5	8.0	10.0	8.5	8.0	7.5	17.0
S. aureus	7.5	7.0	-	-	-	-	-	-	-	-	-	-	9.0	7.0	-	-	22.0
Yeasts																	
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*: inhibition zones include diameter of hole (6.0 mm). Sample amount 50 µL. -: Not active

Table 3. MICs values (mg/ml) of four *Senecio* species

	S. fluviatilis	*S. nemorensis*	*S. pseudo-orientalis*	*S. racemosus*	Tetracycline (µg/ml)
Gram (-)					
A. hydrophila	3.13	6.25	12.5	6.25	<7.8
P. mirabilis	12.5	-	12.5	-	31.3
P. aeruginosa	3.13	6.25	12.5	6.25	15.6
Gram (+)					
B. brevis	3.13	6.25	6.25	3.13	<7.8
B. cereus	-	-	3.13	-	62.5
B. subtilis	12.5	12.5	12.5	-	<7.8
M. smegmatis	3.13	6.25	6.25	6.25	125.0
S. aureus	12.5	-	-	1.5	125.0

-: Not active

To the best of our knowledge, the total phenolic contents, *in vitro* antioxidant and antimicrobial activities of the four *Senecio* species tested in this study have not been reported before. The new alkaloids were identified and their biological activities were tested by Christov et al. (2002). The antioxidant activity of the methanolic extract and fractions of *S. gibbosus* subsp. *gibbosus* was assessed by DPPH radical scavenging assay and showed that both methanolic extract and ethyl acetate fraction demonstrated high activity with IC_{50} of 0.022 and 0.010 mg/ml respectively (Conforti et al., 2006 a). These values are very lower than results obtained from our study. In our study, IC_{50} values of four *Senecio* species ranged from 18.81 to 70.80 µg/ml. In other study, same author and co-workers (Conforti et al., 2006 b) reported that the ethyl acetate extracts of *S. inaequidens* and *S. vulgaris* possessed the highest radical scavenging activity with 61.60% and 44.57% of inhibition, respectively, at a concentration of 0.312 mg/ml. The aqueous extracts of *S. scanakns* exhibited the high potency in inhibiting lipid peroxidation (Liu et al., 2000). In a study on total phenolic contents and antioxidant activities of methanol extracts of six *Senecio* species growing in the Black Sea region of Turkey (*S. pandurifolius*, *S. trapezuntinus*, *S. integrifolius* subsp. *aucheri*, *S. hypochionaeus* var. *argaeus*, *S. hypochionaeus* var. *ilkasiensis* and *S. lorentii*), the total phenolic contents and antioxidant properties were found as 19.54- 81.78 mg GAE/g dry extract and 70.07-165.21 mg AAE/g dry extract, respectively. *S. hypochionaeus* var. *ilkasiensis* extract showed also maximum activity with an IC_{50} of 15.94 µg/ ml in DPPH assay (Albayrak et al., 2008). In another study, same authors reported that the total phenolic contents of the extracts were found to be highest in *S. cilicius* and *S. mollis* extracts (117.45 and 113.40 mg GAE/g, respectively) among the nine *Senecio* species growing in Turkey. *S. salsuginea* showed the strongest free radical scavenging activity with IC_{50} = 26.23 µg/ ml and *S. mollis* showed the highest antioxidant capacity in the phosphomolybdenum method (434.48 mg AAE/ g) (Albayrak et al., 2014).

An antifungal and antibacterial active compound from *S. lyratus* methanol extract was isolated and identified by Kiprono et al. (2000). Loizzo et al., (2004) reported that MIC values of *S. vulgaris* methanol extract against the Gram (+) bacteria, *B. subtilis* and *S. aureus* were 0.5 and 0.125 mg/ml, respectively, while *S. inaequidens* showed no antimicrobial activity against those organisms (MIC > 1 mg/ml). In the same study, it has been reported that Gram (-) bacteria (*E. coli* and *P. aeruginosa*) were unaffected by the methanol extracts of both *S. inaequidens* and *S. vulgaris*. These findings are in accordance with the results obtained our study. The MIC value of *S. samnitum* methanol extract against *S. aureus* was found as 500 µg/ ml (Loizzo et al., 2006). This value is very lower that than of the extracts tested in our study. MIC values of the methanol extract of *S. leucanthemifolius* against *B. subtilis*, *S. aureus* and *P. aeruginosa* were found as >1 µg/ ml (Tundis et al., 2006). Our results are in

agreement with those reported by Wu et al. (2006b) in that all the compounds isolated from *S. cannabifolius* showed antibacterial activities against *S. aureus* and *B. subtilis* but not *E. coli*. Although many *Senecio* species have been the subject of several studies, only a limited number have been devoted to biological activity analysis of *Senecio* species belonging to Turkish flora (Uzun et al., 2004; Uğur et al., 2006, Albayrak et al., 2008; Albayrak et al., 2014). Uğur et al. (2006) reported that the strains of *Stenotrophomonas maltophilia*, which are important pathogens, were inhibited by some extracts of *S. sandrasicus*. The antimicrobial activity of *S. vulgaris* collected from Turkey against *E. coli* (14 mm inhibition zone and MIC = 156.3 μg/ml) had been reported previously (Uzun et al., 2004). In our previous study, methanol extracts of six *Senecio* growing in the Black Sea region of Turkey showed antimicrobial activities against thirteen bacteria and two yeast strains. On the contrary to our present study results, *K. pneumoniae* was found as the most sensitive bacteria to the all extracts examined while similarly, *E. coli* and *C. albicans* were found as the most resistant one (Albayrak et al., 2008). Similarly, in continuation of our previous works on bioactivity of *Senecio* species, the methanol extracts of nine *Senecio* species growing in Turkey exerted promising antibacterial activity against most of the test bacteria (MIC = 6.25-12.5 mg/ml), but no activity was observed against *C. albicans*. In the present study, MICs of four *Senecio* species were found ranged from 1.5 to 12.5 mg/ml.

4. Conclusion

The results reported at the present study can be considered as the first information on the total phenolic contents, antioxidant and antimicrobial activities of methanol extracts obtained from four *Senecio* species belonging to Turkish flora. The results reported here may also contribute to the knowledge about the biological properties of other *Senecio* species. The results of this study showed that all *Senecio* species tested were rich in phenolic contents and have potent antioxidant activity measured by different methods such as DPPH scavenging, reduce Mo(VI) to Mo(V) and inhibition of β-carotene bleaching assay. A dose response relationship was observed for all samples. The samples possessed comparatively weak to moderate antibacterial activities. All of the *Senecio* species tested could be a good source of natural antioxidant and antibacterial agents. Based on a results concerning the positive correlation existed between antioxidant activity and total phenolic contents, phenolic compounds were more likely to be responsible for antioxidant and antibacterial activity observed in extracts. Therefore, more scientific works could be done regarding the qualitative and quantitative analysis of major individual phenolics in the *Senecio* species tested.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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