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ORIGINAL PAPER

Biological activities and chemical composition of *Senecio vernalis* growing in the lakes region of Turkey

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Abstract

Senecio vernalis Waldst. et Kit. is an annual herbaceous plant that has phytotherapeutical importance. Its aerial parts have been extensively used for healing wounds in traditional medicine. In this study, *S. vernalis* samples collected from the lakes region (Burdur) were examined for the characterization of their phenolic contents, chemical composition and their biological activities. The total phenolic contents were determined by HPLC analysis methods. The chemical composition analysis of the samples was performed using gas chromatography–mass spectrometry (GC–MS). Antimicrobial screening of the flower–leaf extracts was determined by the agar disk diffusion method (inhibition zone) and agar well diffusion method (MIC). In the tests, seven bacteria and one yeast known as food pathogens were used. The antioxidant activities of the flower extracts were assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The ethanolic extracts of leaf–flower belonging to *S. vernalis* had antibacterial activity against four bacteria. The MIC values of the ethanolic extracts of leaf–flower were 13,000 µg/ml against *Escherichia coli* ATCC11229 and *Yersinia enterocolitica* NCTC11174, respectively. The methanolic extracts of the flower samples had a 78% DPPH radical scavenging capacity. The total phenolic content was equivalent to 4.73 mg/ml gallic acid. Furthermore, seven components were found in the methanolic extract of the leaves of *S. vernalis*. The results showed that the various extracts of *S. vernalis* have both antimicrobial and antioxidant activities and consist of sesquiterpenes and sesquiterpenoids, fatty acids and alkaloids.

Keywords 1,1-diphenyl-2-picrylhydrazyl · Antimicrobial activity · Chemical composition · *Senecio vernalis* · Total phenolic content

Introduction

Foodborne microorganisms which include a wide range of bacteria, viruses, parasites, fungal and marine pathogens, and which are a part of biological features of our surroundings, cause some gastrointestinal infectious diseases such as listeriosis, yersiniosis, salmonellosis and some serious complications such as mental retardation, hemolytic uremic syndrome, reactive arthritis and Guillain–Barré syndrome (Archer and Kvenberg 1985; Mossel 1988). Such foodborne diseases are important issues of environmental public health (Gutiérrez-Larraínzar et al. 2012; Lake and Barker 2018). According to the World Health Organization (WHO)'s report in 2010, there are 600 million diseases, and food pathogens are responsible for 420,000 related deaths (Kirk et al. 2015; WHO 2015). In addition to these health impacts, they also impose economic burden on the healthcare and medical systems, food processing and supplying systems (Buzby and Roberts 1997) and cause agricultural product losses (Yeni et al. 2016).

It is widely known that plants have especially been used for treating diseases and preserving foods since ancient times. In recent years, these natural sources have received great attention because of their antimicrobial properties

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(Faydaoğlu and Sürücüoğlu 2013) which come from their rich antioxidant contents (Gutiérrez-Larraínzar et al. 2012). The plant-based antioxidants play an important role in reducing the risk of diseases such as cancer, cardiovascular diseases, Alzheimer's disease, and in macular degeneration by minimizing the damage of free radicals on cells (Johnson et al. 2017). The antioxidant characteristics of the plant extracts are due to their phenolic compounds (Preethi et al. 2010). Plant phenolics are the most common secondary metabolites in whole plant organs, which give taste and color to the plant. They have attracted a great deal of scientific attention in recent years because of their potential antioxidant characteristics, especially in the realm of medicinal treatment and preventive medicine (Dai and Mumper 2010). Moreover, studies on natural antioxidants, which are alternatives to synthetic antioxidants utilized to prevent the spoilage of foods and extend shelf-life, lead to an increase in the interest in plants and phytochemicals (Tawaha et al. 2007). Because of their antioxidant and antimicrobial properties, volatile oils extracted from plants are extensively used in various areas, such as traditional medicine, pharmaceuticals and food technology. In this respect, the characterization of various antioxidant and antimicrobial potentials of different medicinal plants, which have been used for hundreds of years for different purposes, is of great importance (Sengul et al. 2009; Bagci and Kilic 2012; Idrissi et al. 2015).

Senecio L. is the largest and most complex genus in the *Asteraceae* family (Hamzaoğlu et al. 2009; Albayrak et al. 2017), with more than 1500 species worldwide. In *a Checklist of the Flora of Turkey (Vascular Plants)*, the updated list of the genus was represented by 31 taxa (Budak 2012). Many species belonging to this genus have been subjected to numerous investigations because of rich secondary metabolites they possess. There are also several studies in folk medicine on the biological activities of *Senecio* species (Rose 1972; Mogoşanu et al. 2009; Yang et al. 2011; Albayrak et al. 2014), especially their aerial parts, which have been used for their antiemetic, antidiarrheal and anti-inflammatory properties. However, note that there are some studies reporting that antimicrobial activity may vary according to the species of plant, composition of its components, ecological/environmental factors and methods used in the study (Marino et al. 2001; Tongnuanchan and Benjakul 2014).

Senecio vernalis Waldst. et Kit. is an herbaceous annual species of *Senecio*. The species, also known as groundsel, is native to Australia, Eurasia, North and South America, and Northern Africa (Mogoşanu et al. 2009). The plant, commonly found in the Turkish flora, usually grows in sandy and waste places, fields and rocky slopes at heights ranging from 0 to 3000 m (Davis 1975).

The aim of the study is to contribute to developing new antimicrobial agents which will be used against the foodborne pathogens that cause some environmental public health issues. This study is the first to be conducted on specimens from the Burdur region, although there are some studies on chemical composition and antimicrobial properties of *S. vernalis* (Tosun et al. 2004; Usta et al. 2009; Bagci and Kilic 2012). In addition, this study differs from others in terms of giving place to biological activities and working on the different parts of the plant. Also, our study is the first in terms of using and comparing the different methods in determining the antioxidant activity of *S. vernalis*.

Materials and methods

Plant material

Senecio vernalis samples were collected during the flowering season in 2015, 10 km south of Burdur (37°41′27.36″N; 030°20′42.66″E) and at an elevation of 1189 m asl (above sea level). The samples were identified according to the *Flora of Turkey and East Aegean Islands* (Davis 1975). The specimens were stored under refrigeration conditions (4 °C) in the MAKU Biology Department, Botanical Research Lab (Voucher no: Balpinar-1501).

Microorganisms

In this study, eight organisms were used in total, and all of them were food pathogens. Those pathogenic strains were as follows: *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Salmonella* typhimurium RSKK19, *Enterococcus faecalis* ATCC8093, *Escherichia coli* ATCC11229, *Listeria monocytogenes* ATCC7644, *Yersinia enterocolitica* NCTC11174 and *Candida albicans* RSKK02029. They were obtained from ATCC (American Type Culture Collection, USA), RSKK (Refik Saydam National Type Culture Collection, Turkey) or NCTC (North Central Texas College, USA). Bacteria were obtained commercially from culture collections. All of the microorganisms were used in logarithmic phase and as vegetative cells. All of the cultures were studied aseptically in laminar flow cabin. Bacteria were grown at 37 °C to 24 h; yeast was grown at 25 °C to 24 h.

Extraction process

Air-dried plant organs were shredded in a blender. The fine powder was stored away from sunlight at 4 °C until analysis. Dried samples of 40 g each (Seles) were placed in a Soxhlet apparatus (Isotex) and were separately extracted in methanol and ethanol solvents (250 ml) for 4–8 h. The plant extracts were evaporated under a fume hood and transferred into sterilized falcon tubes containing their own solvents. The concentrations of the extracts were set to 200 mg/ml, and they were stored in a refrigerator until further analysis.

Both the methanolic and ethanolic extracts were used in the antimicrobial studies; however, only the methanolic extracts were used in chemical composition studies.

HPLC analysis of phenolic compounds in the extracts

The HPLC analysis (described by Caponio et al. 1999) that was slightly modified was used to determine the total phenols. Phenolic components were identified at 278 nm wavelength and 0.8 ml/min flow velocity. Detector was a diode-array detector (DAD); column oven was CTO-10Avp. Autosampler was SIL-10AD vp. An Agilent Eclipse XDB C-18 (250 × 4.6 mm) 5-µm column was used, and its temperature was 30 °C. The separation was executed by using a gradient program with a two-solvent system. (Solution A was 3% acetic acid, and solution B was methanol.) Injection volume was 20 µl.

The preparation of gallic acid as the standard

Gallic acid was dissolved in methanol, and the stock solution (1000 ppm) was prepared. The solution was diluted to various concentrations and maintained at − 18 °C during analysis. The calibration curve was drawn based on the amount of gallic acid (concentration). Pure solvents were used in all HPLC analyses.

GC–MS analysis

In the GC–MS analysis, a Shimadzu GC-2010 gas chromatograph equipped with an MS-QP2010 spectrometer (Shimadzu Corporation, Kyoto, Japan) was used. All the analyses were performed using the following conditions: Column, Rxi-5Sil MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek, Bellefonte, PA, USA); temperature program, from 60 °C (1 min) to 280 °C at 2 °C/min; injection temperature, 280 °C; carrier gas, He; flow, 1 ml/min; injection mode, split (10:1); MS temperature, 280 °C. In the analysis, the methanolic extract was used alone because methanol and hexane had not formed a homogeneous mixture (Drake 2017).

In vitro antimicrobial activity assay

Antimicrobial activity was measured using the methods of disk diffusion. Ethanol and methanol were used as organic solvents. The bacteria were cultivated in Mueller-Hinton agar plates (MHA, Merck), and the yeast was in Sabouraud Dextrose Agar plates (SDA, Merck) (Bauer et al. 1966). The bacteria were incubated at 37 °C, and the yeast at 30 °C for 24 h. The turbidity of the active cultures was adjusted to 0.5 McFarland (1.5 × 108 cfu/ml), and then they were inoculated in the plates (0.1 ml) in aseptic conditions. The extracts (45 µl from 200 mg/ml concentration) were ingrained into empty disks (6 mm) and were placed on the plate surface. The antimicrobial activity was determined in mm scale by measuring the inhibition zones formed after the incubation. Ethanol and methanol were defined as negative control groups, while the antibiotics tetracycline (30 µg), nystatin (100 µg) and ampicillin (10 µg) were positive control groups. All tests were done in triplicate, and the mean of the obtained values was presented.

Determination of minimal inhibitory concentration

The minimum inhibitory concentration (MIC) values of the flower–leaf extracts were determined by the broth dilution method. The active culture concentrations were set at 0.5 McFarland, and all experiments were conducted in 2 ml Mueller-Hinton broth. Serial dilutions of 13,000, 6500, 3250, 1625 and 812.5 µg/ml were prepared, and the same amounts of active cultures (100 µl) were inoculated into each of them (CLSI 2003, CLSI 2006). The samples were incubated at 37 °C for 24 h, and then their MIC values were determined.

Non-enzymatic antioxidant assay

Antioxidant activity was determined using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (0.004 g) with methanol solution (100 ml). The flower extracts (0.1 ml) were added to the DPPH solution prepared in methanol (2.9 ml), and that mixture was kept in a dark place during the incubation period (30 min). Next, the DPPH absorbance of the extracts was measured at a wavelength of 515 nm in an UV–Vis spectrophotometer (Optizen POP, Korea). The percentage of DPPH radical scavenging activity of the samples was determined by means of a formula (Brand-Williams et al. 1995). The methanol DPPH solution was used as the control. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma) was used as a reference standard. The results obtained for antioxidant capacity are given in mM Trolox equivalents (TE)/g dry mass.

The other method used for screening the non-enzymatic antioxidant activities was 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay. Main stock solution included 7 mM ABTS+ solution and 2.45 mM potassium persulfate solution. The absorbances were measured at a wavelength of 734 nm by a UV–visible spectrophotometer (Shimadzu UV–1201 V, Japan). Trolox was used as a standard. The results are given in mM Trolox equivalents (TE)/g dry mass (Re et al. 1999).

Results and discussion

The analysis of total phenol in *S. vernalis* was performed using the HPLC technique. A total of 23 phenolic standards were used. Retention times of the components and the

HPLC chromatogram of the samples are shown in Table 1 and Fig. 1, respectively.

One of the phenolic compounds found in the leaves extracts was gallic acid. In the analysis of total phenolic content in the methanolic extract of the leaves of *S. vernalis*, the total amount was specified as gallic acid equivalents. The amount in the extracts was determined using HPLC method and was 4.73±0.014 (mg/ml extract). The results are given in Table 2.

The chemical composition of the methanolic leaf extract of *S. vernalis* was analyzed by GC–MS, and seven components were identified (see Table 3). In these components, palmitic acid TMS derivative had the highest percentage (29.30%) and neophytadiene had the second-highest percentage (19.65%). The components in order according to percentage are as follows: palmitic acid TMS der. > neophytadiene > ethyl palmitate > ethyl 9,12-octadecadienoate > senecionine > caryophyllene oxide > intergerrimine. The retention times and the percentage of the components forming the chemical composition are listed in Table 3 and are illustrated in the chromatogram in Fig. 2.

The ethanolic and methanolic extracts of the aboveground organs of *S. vernalis* were analyzed using the agar disk diffusion method for determining antimicrobial activity. The ethanolic extracts of leaves exhibited activity (7 mm) against *S. aureus* RSKK2392, *Salmonella* typhimurium RSKK19 and *E. coli* ATCC11229. However, the ethanolic extracts of flowers only exhibited activity against *Y. enterocolitica* NCTC11174 (7 mm). No antimicrobial activity in the methanolic extracts of the leaf–flower of *S. vernalis* was observed (see Table 4).

MIC was applied as an alternative antimicrobial test. To determine the minimum inhibitory concentrations of the extracts, the concentrations 13,000, 6500, 3250, 1625 and 812.5 µg/ml were tested. Accordingly, the MIC value was measured as 13,000 µg/ml in the ethanolic extracts of the leaves against *E. coli* ATCC11229 and in the ethanolic extracts of the flowers against *Y. enterocolitica* NCTC11174 (see Table 5).

The non-enzymatic antioxidant activities of the plant extracts were determined according to the DPPH and ABTS method. In the analysis, DPPH methanolic solution was used as a control and Trolox as the standard antioxidant. The highest DPPH capacity of the methanol extracts of the flowers was 78.4%, and its Trolox equivalent was 2.2 mM/g DW (see Table 6). The ABTS scavenging capacity of the methanol extracts of the flowers is 43.2%, and the Trolox equivalent is 1.4 mM/g DW (see Table 6).

In this study, the leaf and flower extracts of *S. vernalis* were analyzed and seven components and their chemical composition were determined. Among the components, palmitic acid TMS der. was the major component and neophytadiene was the second (see Table 3). Bagci and Kilic (2012) identified 39 chemical components in their analyses of *S. vernalis*, in which caryophyllene oxide was the second major component, following β-phellandrene. In their GC–MS analysis of the extracts obtained from *S. vernalis*, Nori-Shargh et al. (2008) reported 13 components in total; among them, spathulenol was the most abundant. Mogoşanu et al. (2009) examined the extracts of *S. vernalis* and *S. jacobaea* and

Table 1 HPLC analysis of the methanolic extracts of *Senecio vernalis* leaves

GP	
Time	Value (B%)
0.01	Start
0.10	7
20	28
28	25
35	30
50	30
60	33
62	42
70	50
73	70
75	80
80	100
81	7
90	Stop
PS	RT
Gallic acid	5.2
Protocatechuic acid	8.9
Catechin	12.4
p-Hydroxybenzoic acid	14.2
Chlorogenic acid	15.1
Caffeic acid	18.1
Epicatechin	19.5
Syringic acid	20.6
Vanillic acid	21.8
p-Coumaric acid	25.4
Ferulic acid	29.1
Sinapinic acid	30.4
Benzoic acid	37.5
o-Coumaric acid	42.4
Rutin	44.9
Hesperidin	52.5
Rosmarinic acid	58.2
Eriodictyol	60.7
Cinnamic acid	68.8
Quercetin	72
Luteolin	74.9
Campherol	77.1
Apigenin	77.5

GP gradient program, *PS* phenolic standards, *RT* retention time

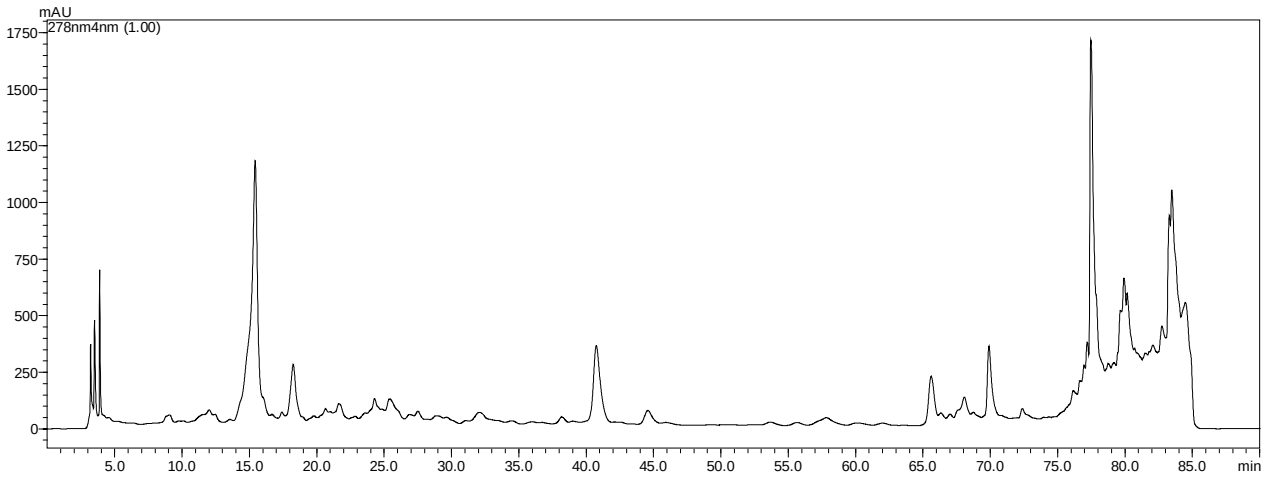

Fig. 1 HPLC chromatogram of *Senecio vernalis*

Table 2 Total phenolic content for the methanolic leaf extract of *Senecio vernalis*

Plant	GAE (mg/ml extract)
Senecio vernalis	4.73 ± 0.014

GAE gallic acid equivalent

Table 3 GC–MS chemical composition analysis of the methanolic leaf extract of *Senecio vernalis*

Peak	Retention time	Name	Area	Area%
1	42.577	Caryophyllene oxide	40,448	7.94
2	57.498	Neophytadiene	100,128	19.65
3	65.517	Ethyl palmitate	77,227	15.16
4	68.097	Palmitic acid, TMS derivative	149,285	29.30
5	73.236	Ethyl 9,12-octadecadienoate	54,408	10.68
6	80.151	Senecionine	52,207	10.25
7	80.490	Intergerrimine	35,815	7.03
Total			509,518	100.00

defined seven compounds that were completely different from the components found in this study. El-Shazly et al. (2002) determined 37 components in the extracts of *Senecio aegyptius*. The results of this work have shown that *S. vernalis* generally contains sesquiterpenes and sesquiterpenoids, fatty acids and alkaloids (see Table 3). The extracts of *Senecio platyphyllus*, on the other hand, consist of monoterpene, sesquiterpene, beta-pinene and (E)-caryophyllene (Usta et al. 2009).

In this study, the total amount of phenolic content was 4.73 mg/ml gallic acid equivalents (see Table 1). Albayrak et al. (2008) posited the total phenolic amount was in the range of 19.54 and 81.78 mg GAE/g in their study on six different *Senecio* species growing in the Black Sea region. Albayrak et al. (2014) discovered the highest amount of phenolic substances (117.45 mg GAE/g) in their analysis of the extracts of *Senecio cilicius*. The phenolic components in this study were completely different from the compounds found in this study. It appears these results are more inclusive than those of the present study. In Albayrak et al. (2015) four different *Senecio* species were examined, in which the total content of phenolics was relatively higher than that of the present study. A possible reason for this difference is that the active components are affected by factors such as harvest season, preparation methods and location, including altitude and climate (Fogden and Neuberger 2003).

This study demonstrated that the ethanolic extracts of leaves of *S. vernalis* exhibited antibacterial activity (7 mm) against *S. aureus*, *Salmonella* typhimurium and *E. coli*, and the ethanolic extracts of the flowers had activity (7 mm) only against *Y. enterocolitica* (see Table 4). The values that Kahriman et al. (2011) reported were 7, 8 and 0 mm zones against *E. coli*; 18, 15 and 15 mm zones against *S. aureus;* 15, 15 and 10 mm zones against *C. albicans*; 15, 12 and 11 mm zones against *Bacillus cereus*; 10, 12 and 10 mm zones against *E. faecalis* in the flower, leave and stem, respectively. However, they found no activity against *Yersinia pseudotuberculosis*. Albayrak et al. (2014) reported the presence of activity zones for *Yersinia* sp. (9 mm), *Senecio inops* (9 mm) and *S. olympicus* (7 mm), but did not report any antibacterial activity against *E. coli, S. aureus* or *S.* typhimurium. This indicated that the results in this study were more inclusive than their results. El-Shazly et al. (2002) investigated the antimicrobial activity of the extracts of *S. aegyptius* leaves and flowers and detected 7 and 8 mm inhibition zones for *E. coli*; 3 and 3 mm zones for *S. aureus*; 7 and 9 mm zones for *B. subtilis*; and 16 and 20 mm zones for

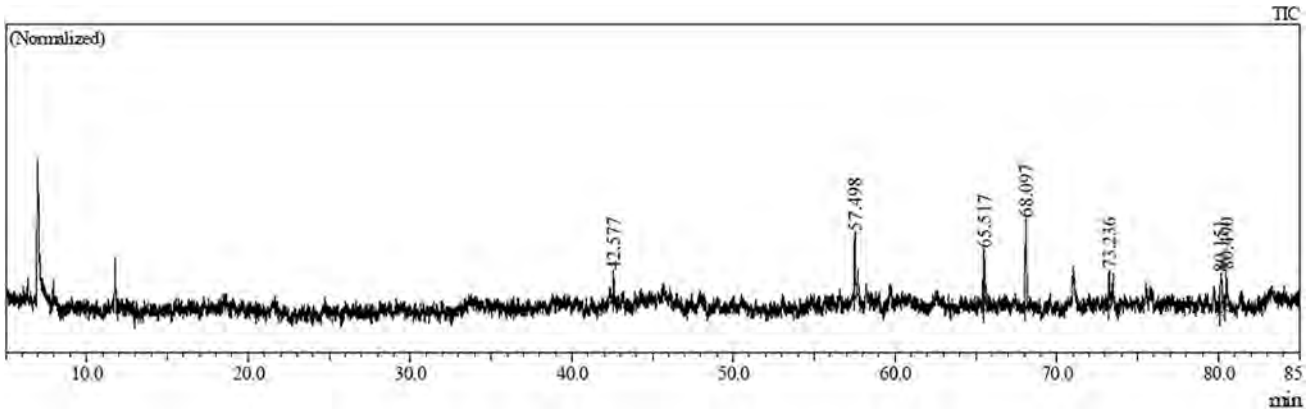

Fig. 2 GC/MS chromatogram of the methanolic extract of *Senecio vernalis* leaves

Table 4 Antimicrobial activities of *Senecio vernalis* against foodborne pathogens

Microorganisms	Inhibition zone (mm)						
	Extracts of plant parts				Antibiotics		
	Ethanol		Methanol		TE	NS	A
	Leaf	Flower	Leaf	Flower			
Bacillus subtilis RSKK245	(–)	(–)	(–)	(–)	nt	nt	10
Staphylococcus aureus RSKK2392	7	(–)	(–)	(–)	nt	nt	10
Salmonella typhimurium RSKK19	7	(–)	(–)	(–)	14	nt	nt
Enterococcus faecalis ATCC8093	(–)	(–)	(–)	(–)	nt	nt	–
Escherichia coli ATCC11229	7	(–)	(–)	(–)	14	nt	nt
Listeria monocytogenes ATCC7644	(–)	(–)	(–)	(–)	nt	nt	12
Yersinia enterocolitica NCTC11174	(–)	7	(–)	(–)	20	nt	nt
Candida albicans RSKK02029	(–)	(–)	(–)	(–)	nt	7	nt

TE tetracycline, *NS* nystatin, *A* ampicillin, (–) not active, *nt* not tested

Table 5 Minimum inhibitory concentrations of *Senecio vernalis* extracts (µg/ml)

Microorganisms	LE	FE	LM	FM
Staphylococcus aureus RSKK23923	(–)	(nt)	(nt)	(nt)
Salmonella typhimurium RSKK194	(–)	(nt)	(nt)	(nt)
Escherichia coli ATCC11229	13,000	(nt)	(nt)	(nt)
Yersinia enterocolitica NCTC11174	(nt)	13,000	(nt)	(nt)

LE leaf ethanol extract, *FE* flower ethanol extract, *LM* leaf methanol extract, *FM* flower methanol extract, (–) not active, *nt* not tested

Table 6 DPPH and ABTS radical scavenging activities of the methanolic extracts of the flowers of *S. vernalis*

DPPH (%)	78.4
Trolox equivalent (mM/g DW)	2.2
ABTS (%)	43.2
Trolox equivalent (mM/g DW)	1.4

DW dry weight

C. albicans, respectively. Additionally, the results regarding 1,10-epoxyfuranoeremophilane were as follows: 16 mm inhibition zone against *S. aureus,* 25 mm against *B. subtilis* and 29 mm against *C. albicans*. Albayrak et al. (2015) examined four *Senecio* species and found no antimicrobial activity zones against *E. coli, S.* typhimurium or *Y. enterocolitica*, but obtained inhibition zones from *Senecio fluviatilis* (7.5 mm) and *S. racemosus* (9 mm). The differences between the activities in these studies may have been caused by the different geographical environment, age of the plant, different methods for oil isolation, cultivar type and seasonality, among others (Zaidi and Dahiya 2015).

The results of the analyses in this study showed that the 13,000 µg/ml MIC value was measured in the ethanolic extracts of *S. vernalis* leaves against *E. coli* and the ethanolic extracts of the flowers of it against *Y. enterocolitica* (see Table 5). According to Aligiannis et al. (2001), the extracts of this plant are regarded as weak inhibitors against food pathogens. As reported in Albayrak et al. (2014), the MIC value for *Yersinia* was 12.5 mg/ml. These results have

some similarities with the results of this work. In their study on four different species of *Senecio*, Albayrak et al. (2015) determined the MIC value to be 12.5 mg/ml for *S. fluviatilis* and 1.5 mg/ml for *S. racemosus* against *S. aureus*. Overall, the results support those from this study on *S. vernalis*.

Because phenolic compounds are one of the most effective radical scavengers, they may be responsible for the strong antioxidant activities of the *S. vernalis* methanolic extracts together with the other phenolics. Albayrak et al. (2008) report that *S. hypochionaeus* var. *ilkasiensis* exhibited the highest free radical scavenging activity (89.9%) among the species of S. *pandurifolius*, *S. trapezuntinus, S. integrifolius* subsp. *aucheri*, *S. hypochionaeus* var. *argaeus*, *S. hypochionaeus* var. *ilkasiensis* and *S. lorentii*. In this study, the DPPH scavenging capacity of the methanolic extracts of the flowers is 78.4% and the Trolox equivalent is 2.15 mM/g DW (see Table 6). These values show that the results of this work are close to those in the literature.

Conclusion

In all, the study showed that the extracts obtained from *S. vernalis* have both antioxidant and antimicrobial activities and they consist of macro-components, such as sesquiterpenes and sesquiterpenoids, fatty acids and alkaloids. In further studies, examination of the phytochemicals in detail is needed, including isolation of their components, determination of their chemical structures and investigation of their various biological activities in *in vivo* and *in vitro* studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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