Some biological compounds, radical scavenging capacities and antimicrobial activities in the seeds of *Nepeta italica* L. and *Sideritis montana* L. subsp. *montana* from Turkey

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RESUMEN

Compuestos biológicos, capacidad atrapadora de radicales libres y actividad antimicrobiana de las semillas de Nepeta italica L. y Sideritis montana L. subsp. montana de Turquía.

Este estudio ha determinado algunos compuestos biológicos (ácidos grasos, vitaminas liposolubles, esteroles y flavonoides), capacidad atrapadora de radicales libres, y actividades antimicrobianas de las semillas de Nepeta italica L. y Sideritis montana L. subsp. montana. Se encontró que el ácido palmítico (C16:0; 8.54±0.13-3.05±0.04%), ácido oleico (C18:1 n9, 22.41±0.8-18.83±0.1%) y α-linolénico (C18:3 n 3:39.56±0.67-77.04±2.07%) eran mayoritarios en ambas semillas de Nepeta italica L. y Sideritis montana L. subsp. montana. Además, se determinó que tanto Nepeta italica L. como Sideritis montana L. subsp. montana contenían estigmasterol (630.07±1.81µg/g; 80,74±0.71µg/g, respectivamente) y ergosterol (11.01±0.14 µg/g; 161.32±0.63 µg/g respectivamente) y esta última además beta-sitosterol (2.93±0.03µg/g). Además, los resultados muestran que Nepeta italica L. contiene morin (37.79±1.09µg/g), catequina (124.39±2.23µg/g) y naringina (475.96±3.57µg/g) y Sideritis montana L. subsp. montana contiene morin (188.41±2.53µg/g), catequina (64.14±1.86µg/g), naringenina (38.34±1.78 µg/g), como principales flavonoides. También, se determinó que los extractos metanólicos de Nepeta itálica L. y Sideritis montana L. subsp. montana fueron eficaces contra los radicales DPPH. Los resultados obtenidos en el presente estudio mostraron que las vitaminas, los flavonoides y los ácidos grasos de los extractos en las semillas de Nepeta itálica L. y Sideritis montana L. subsp. montana impidieron el crecimiento de los microorganismos utilizados en el ensayo, en diferentes proporciones.

PALABRAS CLAVE: Ácidos grasos – Actividad antimicrobiana – DPPH radical scavenging actividad – Esteroles – Flavonoides – Nepeta italica L. – Sideritis montana L. subsp. Montana – Vitaminas.

SUMMARY

Some Biological Compounds, Radical Scavenging Capacities and Antimicrobial Activities in the seeds of *Nepeta italica* L. and *Sideritis montana* L. subsp. *montana* from Turkey.

This study determined some biological compounds (fatty acid compositions, lipid-soluble vitamins, sterols, flavonoids), radical scavenging capacities and antimicrobial activities in the seeds of *Nepeta italica* L. and *Sideritis montana* L. subsp.

montana. It was found that palmitic acid (C16:0; 8.54±0.13-3.05±0.04%), oleic acid (C18:1 n9, 22.41±0.8-18.83±0.1%) and α-linolenic acid (C18:3 n3;39.56±0.67-77.04±2.07%) were the dominant fatty acids in both Nepeta italica L. and Sideritis montana L. subsp. montana. It was concluded that both Nepeta italica L. and Sideritis montana L. subsp. montana contained stigmasterol (630.07±1.81µg/g, 80.74±0.71µg/g, respectively) and ergosterol (1.11±0.14µg/g, 161.32±0.63µg/g respectively) as well as beta-sitosterol (2.93±0.03 µg/g). The present findings show that Nepeta italica L. contains morin (37.79±1.09µg/g), catechin (124.39±2.23µg/g), naringin (475.96±3.57µg/g) and Sideritis montana L. subsp. montana contains morin (188.41±2.53µg/g), catechin (64.14±1.86µg/g), naringenin (38.34±1.78µg/g) as major flavonoids. It was also determined that methanol extracts of Nepeta italica L. and Sideritis montana L. subsp. montana were most effective against DPPH radicals. The results of the present study show that the vitamins, flavonoids and fatty acid extracts in the seeds of N. italica L. and S. montana L. subsp. montana prevented the growth of the microorganisms used in the tests at different ratios.

KEY-WORDS: Antimicrobial Activity – DPPH radical scavenging activity – Fatty acids – Flavonoids – Nepeta italica L. – Vitamins – Sideritis montana L. subsp. montana – Sterols.

1. INTRODUCTION

Recently, considerable attention has been devoted to medicinal plants because they contain chemical constituents which exhibit antioxidant properties (Miliauskas *et al.*, 2004; Grzegorczyk *et al.*, 2007; Mohamad *et al.*, 2010). Antioxidants are viewed as compounds that protect cells against oxidative stress, which might cause cell damage (Giao *et al.*, 2007). It is reported that medicinal plants contain a wide range of natural compounds such as phenolic acids, flavonoids, terpenoids, vitamins and tannins, which show antioxidant activity (Ai-li and Chang-Hai, 2006; Bouayed *et al.*, 2007). In particular, members of the *Lamiaceae* family have been extensively studied for their antioxidant activity and radical scavenging activity (Erdemoğlu *et al.*, 2006; Barros *et al.*, 2009).

Nepeta and *Sideritis,* which are members of the *Lamiaceae* family, have significant antioxidant capacities (Nakiboğlu *et al.,* 2007; Tepe *et al.,* 2007; Güvenç *et al.,* 2010). The genus *Nepeta* (*Nepetoideae* subfamily) comprises about 400 species, most of

which grow in Central and Southern Europe, North Africa and Central and Southern Asia (Miceli et al., 2005). In Turkey, the genus Nepeta is represented by 44 species, 22 of which are endemic (Davis, 1982; Akpinar et al., 2008). The antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities of Nepeta species have been reported in the literature (Tepe et al., 2007). The other genus, Sideritis L. (Lamioideae subfamily), (Lamiaceae) includes approximately 150 species of annual and perennial plants, distributed mainly in the Mediterranean region (Güvenç et al., 2010). Sideritis is represented by 45 species and 53 taxa, of which 39 species are endemic in Turkey (Davis, 1982; Nakiboğlu et al., 2007). The endemism ratio of the Sideritis species is very high (78%) for Turkey among other Lamiaceae species (Sagdic et al., 2008). Sideritis species are used in folk medicine as anti-inflammatory, anti-ulcer, cytostatic, antimicrobial, flu vaccine and stimulant circulatory agents (Basile et al., 2006). The objectives of the present study are: i) to determine fatty acid compositions, vitamin and sterol contents; ii) to evaluate the flavonoid contents and radical scavenging properties of Nepeta italica and Sideritis montana subsp. montana seeds. In addition, the antimicrobial activities of both species were investigated.

2. MATERIAL AND METHODS

2.1. Chemical agents

All chemicals and reagents were purchased from Sigma-Aldrich.

2.2. Collection of plant materials

In the present study, some biological compounds (fatty acids, sterols, lipid-soluble vitamins, flavonoids), radical scavenging capacities and antimicrobial activities in mature seeds of the *Nepeta italica* L., (B7 Elazığ, Baskil-Bolucuk village, 1580-1600 m) and *Sideritis montana* L. subsp. *montana* (B7 Elazığ, Baskil-Bolucuk village, 1580 m) were examined. *Microbial strain*

A total of 4 bacteria (Escherichia coli ATCC 25922, Klebsiella pneumoniae FMC 5, Staphylococcus aureus COWAN 1, Bacillus megaterium DSM 32), 2 yeasts (Candida albicans FMC 17, Candida glabrata ATCC 66032) and 2 dermatophyte species (Trichophyton sp., Epidermophyton sp.) were used in the present investigation. Microorganisms were provided from the Department of Biology, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.3. Extraction of plant materials

Fatty acid, sterol and vitamin analyses

2 g seed materials for fatty acid, sterol and vitamin analyses were finely ground in a mill and were then

extracted with hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). The lipid extracts were centrifuged at 10.000 g for 5 minutes and filtered. The solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored at-25°C until further analysis. The experiment was repeated three times.

Flavonoid analyses and Radical scavenging capacity

2 g seed material was homogenized in 5 ml 80% methanol. Homogenates were centrifuged at 5000 rpm at $+4^{\circ}$ C. After centrifugation, the supernatant was concentrated by reduced-pressure rotary evaporation. Each extract was re-suspended in dimethyl sulphoxide (DMSO) to produce a stock solution. The experiment was repeated three times.

2.4. Determination of bioactive properties

Fatty Acids Analyses

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter) (Permabound 25, Macherey-Nagel, Germany) using nitrogen as a carrier gas (flow rate 0.8 ml/min.). The temperatures of the column, detector and injection valve were 130-220, 240, and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Chromatographic analysis and quantification of lipid soluble vitamins and sterols

Lipid-soluble vitamins and phytosterols were extracted from the lipid fraction according to the method of Sánchez-Machado (2002) with minor modifications. The extracted lipids of seed material were dissolved in acetonitrile/methanol (75/25 v/v) and 50 µL were injected into the HPLC instrument (Shimadzu, Kyota Japan). The column used was a Supelcosil [™] LC18 (250 x 4.6 mm, 5 μm, Sigma, USA). The mobile phase was acetonitrile/methanol (75/25, v/v) and the elution was performed at a flowrate of 1 ml /min. The temperature of the analytical column was kept at 40°C. Detection was performed at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for δ -tocopherol, vitamin D, α -tocopherol, α -tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K1. Identification of the individual vitamins and phytosterols were performed by frequent comparison with authentic

external standard mixtures analyzed under the same conditions (López-Cervantes et al., 2006). Class Vp 6.1 software assisted in the workup of the data. The results of the analyses were expressed as $\mu g/g$ for samples.

Chromatographic Conditions for Flavonoids

The chromatographic analysis was carried out using a PREVAIL C18 reversed-phase column (15x4.6mm, 5µm, USA); the mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (Zu et al., 2006). This mobile phase was filtered through a 0.45 µm membrane filter (Millipore), then de-aerated ultrasonically prior to use. Catechin (CA), naringin (NA), rutin (RU), resveratrol (RES), myricetin (MYR), morin (MOR), naringenin (NAR), quercetin (QU) and kaempferol (KA) were quantified by DAD separation at 280 nm for CA and NA, 254 nm for RU, MYR, MOR and QU, 306 nm for RES, and 265 nm for KA. Flow rate and injection volume were 1.0 ml/min and 10 µL, respectively. The chromatographic peaks of the extracts were confirmed by comparing their retention times with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25°C.

2.5. Determination of antioxidant properties

Antioxidant assay by DPPH radical scavenging activity

The free radical scavenging effect of the extracts was assessed by the decoloration of a methanolic solution of DPPH[•] according to the method of Liyana- Pathiranan and Shahidi (2005). A solution of 25 mg / L DPPH in methanol was prepared and 4.0 ml of this solution were mixed with 25 and 50 μ L of extract in DMSO. The reaction mixture was left in the dark at room temperature for 30 minutes. Absorption of the blank sample containing the same amount of methanol and DPPH· solution was prepared and measured daily. The absorbance of the mixture was measured spectrophotometrically at 517 nm. 1 μ M quercetin was used as a reference.

The ability to scavenge DPPH radicals was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] x 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract /standard.

2.6. Antimicrobial activity

Antimicrobial tests were carried out by the well agar method using 100 μ L of suspension containing 10⁶ cells / mL of bacteria, 10⁴ cells / mL yeast and cells / mL dermatophyta fungi as per McFarland standard, inoculated into Mueller Hinton Agar (Difco), Malt

Extract Agar (Difco), and Sabouroud Dextrose Agar (Oxoid), respectively. Wells were prepared in the plates with the help of a cork-borer (0. 85 cm). 10 µl of the flavonoids, vitamins and fatty acids in plants were introduced directly into the well. Steril petri dishes (9cm diameter) were placed at 4°C for 2h. Then, the inoculated plates were incubated at 37±0.1°C for 24 h for bacterial strains and also at 25±0.1°C for 72 h for yeast and dermatophyta fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (Collins and Lyne, 1987). Wells injected with methanol and hegzan served as negative controls.The experimental studies were carried out three times.

3. RESULTS AND DISCUSSION

3.1. Bioactive Compounds

Fatty acid compositions, lipide-soluble vitamin and sterol contents in the seeds of Nepeta italica L. and Sideritis montana L. subsp. montana

The dominant fatty acids in both *N. italica* and *S. montana* were palmitic acid (C16:0; 8.54 ± 0.13 - $3.05\pm0.04\%$), oleic acid (C18:1 n 9; 22.41\pm0.8-18.83\pm0.1\%) and α -linolenic acid (C18:3 n 3; 39.56±0.67-77.04±2.07%) (Table 1). Palmitic acid

Table 1								
Fatty acid compositions in the seeds of N. italica								
and <i>S. montana</i> subsp. <i>montana</i>								

Fatty acids (%)	N. italica	S. montana
C16:0	8.54±0.13	3.05±0.04
C17:0	0.23±0.02	-
C18:0	2.81±0.03	-
C20:0	0.21±0.02	0.26±0.03
C22:0	0.45±0.02	-
ΣSFA	12.24±0.04	3.31±0.03
C14:1	0.62±0.03	-
C16:1 n9	0.83±0.02	0.23±0.02
C17:1	0.12±0.02	-
C18:1 n9	22.41±0.80	18.83±0.10
C20:1 n9	0.32±0.02	0.34±0.02
C24:1	0.43±0.01	-
$\Sigma MUFA$	24.73±0.15	19.18±0.05
C16:2	0.63±0.02	-
C18:2 n6	22.8±0.14	0.12±0.01
C22:2	0.33±0.02	-
C18:3 n3	39.56±0.67	77.04±2.07
C20:3 n3	_	0.13±0.02
C22:4	0.32±0.01	_
ΣΡUFA	63.64±0.17	77.29±0.7

(8.54±0.13%) and stearic acids (2.81±0.03%) were the major saturated fatty acids of N. italica. Heptadecanoic acid (C17:0; 0.23±0.02%), arachidic acid (C20:0; 0.21±0.02%) and behenic acid (C22:0; 0.45±0.02%) were the lowest in N. italica. Akpinar et al. (2008) identified three individual saturated fatty acid components from N. italica as: palmitic acid (5.8%), stearic acid (1.4%) and arachidic acid (1.05%). Also, Kilic et al. (2007) determined that the main saturated fatty acids of five Nepeta species as palmitic acid (5.2-6.8%) and stearic acid (2.0-3.7%). In addititon, Palmitic acid (3.05±0.04%) and arachidic acid (0.26±0.03%) were saturated fatty acids of S. montana. Ertan et al. (2001) found that the major saturated fatty acids of 15 Sideritis species were palmitic acid (0.3-9.4%), stearic acid (2.3-4.9%) and arachidic acid (0.1-0.2%).

Oleic acid was the major monounsaturated fatty acid both *N. italica* (22.41±0.8%) and *S. montana* (18.83±0.10%). Akpinar *et al.* (2008) determined that the oleic acid composition of five *Nepeta* species was 10.47-19.85% (that of *N. italica* was 14.22%). In another study, Kiliç *et al.* (2007) showed that the oleic acid contents of five *Nepeta* species were 11.5-19.2%. Ertan *et al.* (2001) found that 15 *Sideritis* species had 12.3-26.5% oleic acid content.

It was indicated that the genera under the subfamily Nepetoideae have been characterized by unsaturated fatty acids and the α -linolenic acid composition was reported as generally over 40% (Cantino and Sanders, 1986; Azcan et al., 2004). In the present study, linolenic acid was the major unsaturated fatty acid in N. italica (39.56±0.67%). Akpınar et al. (2008) determined that the linolenic acid composition of five Nepeta species including N. italica (59.51%) was 48.20-61.62%. Another study, by Kilic et al. (2007) determined that the linolenic acid content of Nepeta species was 49.8-58.5%. However, the fatty acid composition of S. montana showed a difference in the percentage of linolenic acid. It was reported that in the subfamily Lamioideae, the ratio of 18:3 fatty acid is less than 30%, and, in most species, it is less than 5% (Ertan et al., 2001). However, the present study found that the 18:3 n3 fatty acid content for S. montana subsp. montana was 77.04±2.07%. In contrast to these results, Ertan et al. (2001) found that Sideritis species have lowest linolenic acid content (0.8-2.0%).

The linoleic acid composition of *N. italica* was found to be $22.8\pm0.14\%$ in this study. A study

by Akpınar *et al.* (2008) determined that the linoleic acid content of *N. italica* was 17.67% and the linoleic acid content of another four *Nepeta* species was 15.66-22.33%. Another study by Kiliç *et al.* (2007) found that the linoleic acid content of five *Nepeta* species was 10.9-23.5%, with the linoleic acid content of *S. montana* being the lowest (0.13 \pm 0.02%). However, Ertan *et al.* (2001) determined that the linoleic acid composition of *Sideritis* species was 45.4-64.0%.

Sterol and Lipide-soluble vitamin contents in the seeds of Nepeta italica L. and Sideritis montana L. subsp. montana

This study determined the phytosterol constituents of N. italica and S. montana (Table 2). Phytosterols include a wide variety of molecules that are structurally similar to cholesterol; the principal examples are 4-desmethyl sterols, basically, beta-sitosterol, campesterol and stigmasterol (Quilez et al., 2003). The present study concluded that N. italica contains stigmasterol (630.07±1.81 µg/g), and ergosterol (11.01±0.14 µg/g). Hussain et al. (2007) determined that Nepeta suavis has beta-sitosterol and stigmasterol. Also, Klimek and Modnicki (2005) found that Nepeta cataria L. var. citriodora has small amounts of beta-sitosterol. Moreover, in this study it was found that *S. montana* has ergosterol (161.32±0.63 µg/g), stigmasterol (80.74±0.71 μ g/g) and beta-sitosterol (2.93±0.03 μ g/g) contents. Aboutabl et al. (2002) identified stigmasterol and betasitosterol in Sideritis taurica. In addition, the present study found that *N. italica* contains D3 (26.59±0.88 µg/g), K1 (2.74±0.03 μg/g), α-tocopherol (1.0 3±0.03μg/g) and retinol-acetate (0.14±0.02 µg/g), while S. contains D3 (44.41±0.19 μg/g), K1 (8.95±0.03 μg/g), D2 (8.23±0.09 µg/g), K2 (3.23±0.03 µg/g), r-tocopherol (1.03±0.02 μ g/g) and retinol (0.33±0.02 μ g/g) constituents. Demo et al. (1998) indicated that Sideritis syriaca does not contain tocopherol (Table 2). As far as we know, there are no previous studies on the vitamin and phytosterol contents of N. italica and S. montana.

Flavonoid Contents

The flavonoid contents of the methanol extracts of *N. italica* and *S. montana* subsp. *montana* are presented in Table 3. Phenolic compounds, including flavonoids, are very important plant constituents because they exhibit antioxidant activity by inhibiting lipid free radicals or preventing the decomposition

Table 2
Lipid-soluble vitamin and sterol contents in the seeds of <i>N. italica</i> and <i>S. montana</i> subsp. montana

Lipid-soluble vitamins (µg/g)								Sterols (µg/g)			
Species	K2	K1	R-Tocopherol	D2	D3	a-tocopherol	Retinol	Retinol acetate	Ergosterol	Stigmasterol	Beta-sitosterol
N. italica	-	2.74±0.03	-	-	26.59±0.887	1.03±0.03	-	0.14±0.02	11.01±0.14	630.07±1.81	-
S. montana	3.23±0.03	8.95±0.03	1.03±0.02	8.23±0.09	44.41±0.19	3.82±0.06	0.33±0.02	-	161.32±0.63	80.74±0.71	2.93±0.03

Table 3 Flavonoid and radical scavenging capacities in the seeds of *N. italica* and *S. montana* subsp. *montana*

Flavonoids (µg/g)									% inhibiton	
	Myricetin	Morin	Quercetin	Kaempferol	Catechin	Naringin	Naringenin	Resveratrol	25 µl	50 µl
N. italica	-	37.79±1.09	3.53±0.14	3.61±0.12	124.39±2.23	475.96±3.57	0.93±0.03	9.83±0.08	92.54±0.58	92.10±0.65
S. montana	8.81±0.8	188.41±2.53	0.19±0.04	6.23±0.17	64.14±1.86	-	38.34±1.78	3.94±0.04	86.58±0.79	88.05±1.47

of hydroperoxides into free radicals (Montoro et al., 2005; Maisuthisakul et al., 2007). Species of the Lamiaceae family are very rich in polyphenolic compounds (Nakiboğlu et al., 2007). The present findings show that N. italica contains morin (37.79±1.09µg/g), catechin (124.39±2.23µg/g) and naringin (475.96±3.57µg/g) as major flavonoids, while *S. montana* contains morin (188.41±2.53 μ g/g), catechin (64.14±1.86 μ g/g) and naringenin (38.34±1.78µg/g). Myricetin, quercetin, kaempferol and resveratrol were present at low levels or absent in both N. italica and S. montana. Proestos et al. (2006) found that N. cataria contained luteolin (3.6 mg/100 g dry sample). They indicated that guercetin and naringenin were not detected in N. cataria. Miceli et al. (2005) showed that Nepeta sibthorpii contained rutin (0.091 mg/g). Also, Proestos et al. (2006) determined that Origanum dictannus contained 1.3 mg quercetin /100 g dry sample. The results obtained from the present study show that the myricetin content of S. montana (8.81 \pm 0.8 μ g/g) and the kaempferol content of N. italica (3.61±0.12 µg/g) and S. montana $(6.23\pm0.17 \mu g/g)$ were higher than in a previous study conducted on the myricetin and kaempferol contents of Salvia officinalis and Origanum vulgare from the family Lamiaceae (Wojdlo et al,. 2007). However, the results of Wojdylo et al. (2007) showed that the quercetin content of Salvia officinalis(178±1.11 mg/100 g dw) was higher than that of N. italica (3.53±0.14 µg/g) and *S. montana* (0.19±0.04 µg/g) found in the present study. Similarly, Skerget et al. (2005) found that Origanum vulgare had higher myricetin (21.0 mg/kg) and guercetin (219 mg/kg) contents than those of N. italica and S. montana. The kaempferol contents of two species examined in the present study were higher than the results reported by Skerget et al. (2005).

3.2. Antioxidant Activity

Radical scavenging activity by DPPH method

In the present study, the radical-scavenging activities of the methanol extracts of *Nepeta italica* and *Sideritis montana* were determined according to the DPPH radical scavenging test (Table 3). The antioxidant potential of the flavonoid contents of *N. italica* and *S. montana* was examined at two different volumes (25 μ l and 50 μ l). It was determined that the methanol extracts of *N. italica* and *S. montana* seeds were most effective against DPPH radicals. The methanol extracts of *N. italica* and *S. montana* almost completely inhibited

DPPH absorption in both 25 µl (92.54±0.58% inhibition for N. italica and 86.58±0.79% inhibition for S. montana) and 50 µl volumes (92.1±0.65% inhibition for N. italica and 88.05±1.47% inhibition for S. montana) in the present study. We could not find any previous report in the literature dealing with the antioxidant properties of N. italica. Miceli et al. (2005) indicated that Nepeta sibthorpii extract had antioxidant activity. On the other hand, Sagdic et al. (2008) found that the antioxidant activities of Sideritis ozturkii and Sideritis caesarea extracts tested by the DPPH radical scavenging test were 41.68% and 72.47%, respectively at 100 ppm concentration. Similarly, Ozkan et al. (2005) determined that the free radical scavenging activities of Sideritis condensata and Sideritis eryhrantha var. eryhrantha extracts were 72.01% and 71.48%, respectively at 100 ppm concentration. In addition, Koleva et al. (2003) determined that the methanol extracts of Sideritis scardica, Sideritis syriaca and Sideritis montana showed strong radical scavenging activity against DPPH. Similarly, Nakiboglu et al. (2007) found that Sideritis sipylea showed antioxidant activity and Sagdic et al. (2008) suggest that two Sideritis species are a potential source of natural antioxidants. Previous studies reported a high correlation between the antioxidant capacity and phenolic constituents of methanol extracts from medicinal plants (Miceli et al., 2005; Maisuthisakul et al., 2007; Li et al., 2008; Sagdic et al., 2008). The present results show that N. italica and S. montana exhibit antioxidant properties.

3.3. Antimicrobial Activity

The antimicrobial activities of the vitamins, flavonoids and fatty acids in the extracts from the seeds of *N. italica, S. montana*, negative control group and standard antibiotics are reported in Table 4. The experimental results show that the vitamins, flavonoids and fatty acid extracts of two species inhibited the growth of microorganisms used in the test at different ratios. The vitamin extracts of *N. italica* and *S. montana* showed antimicrobial activities against all microorganisms and the vitamin and flavonoid extracts usually have higher effects in comparison to antibiotics.

The vitamin extracts of *N. italica and S. montana* have the highest antimicrobial efficiency (inhibition zone between $9.220.1-33.2\pm0.1$ mm and $13.3\pm0.2-35.1\pm0.3$ mm, respectively). As shown in Table 4, the extracts of flavonoids from *N. italica* showed antibacterial and antifungal activity against the microorganisms tested: *E. coli* (13.3 ± 0.2 mm), *K.*

	Inhibition zone (mm)								
Microorganisms	Vitamins		Flavonoids		Fatty acids		Control		
	N. i	S. m	N. i	S. m	N. i	S. m	Methanol	Hexan	Standart
E. coli	15.1±0.1	15.2±0.1	13.3±0.2	-	15.4±0.6	-	-	15.2±0.5	10.1±0.1**
K. pneumoniae	33.2±0.1	32.3±0.2	28.4±0.2	15.1±0.2	13.0±0.1	13.3±0.2	-	14.3±0.3	9.4±0.3**
S. aureus	30.2±0.3	35.1±0.3	9.2±0.1	-	10.1±0.3	12.2±0.2	-	13.2±0.2	13.2±0.2**
B. megaterium	13.2±0.2	13.3±0.2	28.2±0.2	9.2±0.3	12.3±0.2	10.2±0.3	-	12.2±0.2	9.0±0.3**
C. albicans	18.4±0.4	30.3±0.1	23.3±0.3	13.2±0.1	-	-	-	17.4±0.2	18.1±0.5*
C. glabrata	13.3±0.3	13.3±0.2	15.4±0.2	11.0±0.1	-	-	-	112±0.2	12.2±0.3*
Trichophyton sp.	9.2±0.1	16.3±0.3	19.2±0.2	13.2±0.2	-	-	-	17.1±0.2	NT
Epidermophyton sp.	25.1±0.2	21.2±0.2	10.1±0.1	11.0±0.1	-	-	-	9.2±0.1	NT

 Table 4

 Antimicrobial activities of seed extracts containing vitamins, flavonoids and fatty acids

N.i: *N. italica*, S. m: *S. montana* subsp. *montana*. Standard: *:Nystatin (30 µg/disc), **: Streptomysin sulphate (10 µg/disc), Control : methanol and hexane (10 µl), NT: not tested.

pneumoniae (28.4±0.2 mm), S. aureus (9.2±0.1 mm), B. megaterium (28.2±0.2 mm), C. albicans (23.3±0.3 mm), C. glabrata (15.4±0.2 mm), Trichophyton sp.(19.2±0.2 mm) and Epidermophyton sp. (10.1±0.1mm). However, the flavonoid extracts of S. montana showed activity against K. pneumoniae, B. megaterium, C. albicans, C. glabrata, Trichophyton sp. and Epidermophyton sp. (15.1±0.2 mm, 9.2±0.3 mm, 13.2±0.1 mm, 11.0±0.1 mm, 13.2±0.2 mm, 11.0±0.1 mm inhibition zone, respectively), while it did not show any antimicrobial activity against E. coli and S. aureus. Additionally, the fatty acid extracts from *N. italica* did not show any antifungal activity against C. albicans, C. glabrata, Trichophyton sp. and Epidermophyton sp., but it showed antibacterial activity against E. coli (15.4±0.6 mm), K. pneumoniae (13.0±0.1 mm), S. aureus (10.1±0.3 mm), B. megaterium (12.3±0.2 mm) respectively (Table 4). Similarly, the fatty acid extracts of *S. montana* did not show any antifungal activity. The extracts of this plant showed antibacterial activity against *K. pneumoniae* (13.3±0.2 mm), *S. aureus* (12.2±0.2 mm), *B. megaterium* (10.2±0.3 mm), except for *E. coli* (Table 4).

A previous study reported that the *Sideritis* species could be used as natural antimicrobial and antioxidant agents in food preservation and human health (Sagdic *et al.*, 2008). Several studies suggested that *Nepeta* and *Sideritis* species have antimicrobial and antifungal activities (Diaz *et al.*, 1988; Ezer and Abbasoğlu, 1996; Nostro *et al.*, 2001; Basile *et al.*, 2006). Kursat and Erecevit (2009) suggested that plant extract of *Sideritis montana* has a strong effect against *S. aureus* (8 mm), *B. megaterium* (11 mm), *C. albicans* (13 mm). However, they indicated that



Figure 1 Radical inhibition activities of different volumes of methanol extracts of *N. italica* and *S. montana* seeds by DPPH radicals.

the plant extract of S. montana did not have antibacterial or antifungal effects on other tested microorganisms: P. aeruginosa, K. pneumonia, C. Glabrata, Epidermophyton sp. and Trichophyton sp. Other studies suggested that Nepeta and Sideritis extracts did not have antimicrobial capacity (Hernandez-Perez and Rabal, 2002; Proestos et al., 2006). However, this is the first study to report that the vitamins, flavonoids and fatty acids of S. montana and N. italica possess antibacterial and antifungal activities. In conclusion, it can be argued that the inhibitory effects of both extracts on the growth of tested microorganisms are a significant finding, in view of their potential applications as natural antimicrobial agents in the development of new drugs for the treatment of infectious diseases involving resistant microorganisms.

4. CONCLUSION

Palmitic acid (C16:0; 8.54±0.13-3.05±0.04%), oleic acid (C18:1 n 9; 22.41±0.80-18.83±0.10%) and $\alpha\text{-linolenic}$ acid (C18:3 n 3; 39.56±0.67-77.04±2.07%) were found to be the dominant fatty acids in N. italica and S. montana. It was determined that N. italica has stigmasterol (630.07±1.81 µg/g), ergosterol (11.01±0.14 µg/g) contents and S. montana has ergosterol (161.32±0.63µg/g), stigmasterol (80.74±0.71 µg/g) and beta-sitosterol (2.93±0.03 µg/g) contents. N. italica and S. montana were shown to contain the lowest levels of lipid-soluble vitamins. Furthermore, the present findings show that N. italica contains morin (37.79±1.09µg/g), catechin (124.39±2.23µg/g), naringin (475.96±3.57µg/g) and S. montana contains morin (188.41±2.53 µg/g), catechin (64.14±1.86µg/g) and naringenin (38.34±1.78 µg/g) as the major flavonoids. It was also determined that the methanol extracts of N. italica and S. montana were most effective against DPPH radicals. The methanol extracts of N. italica and S. montana inhibited DPPH absorption at 25 µl (92.54±0.58% inhibition for N. italica and 86.58±0.79% inhibition for S. montana) and 50 µl (92.1±0.65% inhibition for N. italica and 88.05±1.47% inhibition for S. montana) in the present study. According to the present results, N. italica and S. montana exhibit antioxidant properties. The results obtained from the present study show that the vitamin, flavonoid and fatty acid extracts of the two taxon inhibited the growth of the microorganisms tested at different ratios.

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