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Original Article

Chemical compositions, radical scavenging capacities and antimicrobial activities in seeds of *Satureja hortensis* L. and *Mentha spicata* L. subsp. *spicata* from Turkey

İ. Emre[a], *M. Kurşat*[b]*, *Ö. Yilmaz*[c] and *P. Erecevi*[d]

[a]Department of Primary Education, Faculty of Education, Firat University, 23119, Elazig, Turkey
[b]Department of Biology, Faculty of Sciences and Arts, Bitlis Eren University, 13100, Bitlis, Turkey
[c]Department of Biology, Faculty of Science, Firat University, 23119, Elazig, Turkey
[d]Department of Biology, Faculty of Science, Munzur University, 62000, Tunceli, Turkey
*e-mail: botanikkursat@gmail.com

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Abstract

The present study determined some biological compounds, radical scavenging activity and antimicrobial capacity in seeds of *Satureja hortensis* L. and *Mentha spicata* L. subsp. s*picata*. Alpha-linolenic acid (C18:3 n3) has been found to be the major polyunsaturated fatty acid of *Satureja hortensis* L. (66.24 ± 1.24%) and *Mentha spicata* L. subsp. s*picata* (48.17 ± 1.01%). Linoleic acid (C18:2 n6) is identified as the second major polyunsaturated fatty acid in the present study and oleic acid (C18:1 n9) is determined as the major monounsaturated fatty acid. Current study showed that *Satureja hortensis* L. and *Mentha spicata* L. subsp. s*picata* have low levels of saturated fatty acids. It has been demonstrated that ergosterol (263.1 ± 2.14 µg/g), stigmasterol (39.07 ± 0.91 µg/g) and beta-sitosterol (14.64 ± 0.49 µg/g) have been found in *Mentha spicata* L. subsp. s*picata*, while ergosterol (69.41 ± 1.75 µg/g) and beta-sitosterol (19.81 ± 1.14 µg/g) have been determined in *Satureja hortensis* L. Also, this study determined that *Satureja hortensis* L. and *Mentha spicata* L. subsp. s*picata* have low lipide-soluble vitamin content. Furthermore, it has been found that *Satureja hortensis* L. contains naringenin (612.57 ± 2.57 µg/g), morin (86.97 ± 1.12 µg/g), quercetin (22.87 ± 0.75 µg/g), and kaempferol (20.11 ± 0.94 µg/g) while naringenin (135.91 ± 1.91 µg/g), naringin (61.23 ± 2.15 µg/g) and quercetin (47.51 ± 1.17 µg/g) have been detected as major flavonoids in the seeds of *Mentha spicata* L. subsp. s*picata.* The results of the present study suggest that methanol extracts of *Satureja hortensis* L. and *Mentha spicata* L. subsp. s*picata* have significant free radical scavenging activity. The present results revealed that *Satureja hortensis* L. and *Mentha spicata* L. subsp. s*picata* showed major activity against gram-positive and gram-negative microorganisms, fungi and yeast.

Keywords: antimicrobial activity, biocompounds, *Mentha spicata* L. subsp. *spicata*, radical scavenging activity, *Satureja hortensis* L.

Composições químicas, capacidades radicais eliminadoras e atividades antimicrobianas em sementes de *Satureja hortensis* L. e *Mentha spicata* L. subsp. *spicata* da Turquia

Resumo

O presente estudo determinou alguns compostos biológicos, atividade de eliminação de radicais e capacidade antimicrobiana em sementes de *Satureja hortensis* L. e *Mentha spicata* L. subsp. *spicata*. O ácido alfa-linolênico (C18: 3 n3) foi o principal ácido graxo poliinsaturado de *Satureja hortensis* L. (66,24 ± 1,24%) e *Mentha spicata* L. subsp. *spicata* (48,17 ± 1,01%). O ácido linoléico (C18: 2 n6) é identificado como o segundo principal ácido graxo poliinsaturado no presente estudo e o ácido oleico (C18: 1 n9) é determinado como o principal ácido graxo monoinsaturado. O estudo atual mostrou que *Satureja hortensis* L. e *Mentha spicata* L. subsp. *spicata* tem baixos níveis de ácidos graxos saturados. Foi demonstrado que ergosterol (263,1 ± 2,14 µg/g), estigmasterol (39,07 ± 0,91 µg/g) e beta-sitosterol (14,64 ± 0,49 µg/g) foram encontrados em *Mentha spicata* L. subsp. *spicata*, enquanto o ergosterol (69,41 ± 1,75 µg/g) e beta-sitosterol (19,81 ± 1,14 µg/g) também foram determinados em *Satureja hortensis* L., este estudo determinou que *Satureja hortensis* L. e *Mentha spicata* L. subsp. *spicata* tem baixo teor de vitaminas lipossolúveis. Além disso, verificou-se que *S. hortensis* L. contém naringenina (612,57 ± 2,57 µg/g), morina (86,97 ± 1,12 µg/g), quercetina (22,87 ± 0,75 µg/g) e kaempferol (20,11 ± 0,94 µg/g) enquanto a naringenina (135,91 ± 1,91 µg/g), a naringina (61,23 ± 2,15 µg/g) e a quercetina (47,51 ± 1,17 µg/g) foram detectadas como flavonóides importantes nas sementes de *Mentha spicata* L. subsp. *spicata.* Os resultados do presente estudo sugerem que os extratos metanólicos de *S. hortensis* L. e *Mentha*

spicata L. subsp. *spicata* tem significativa atividade de eliminação de radicais livres. Os presentes resultados revelaram que *Satureja hortensis* L. e *Mentha spicata* L. subsp. *spicata* mostrou atividade importante contra microrganismos gram-positivos e gram-negativos, fungos e leveduras.

Palavras-chave: atividade antimicrobiana, biocompostos, *Mentha spicata* L. subsp. *spicata*, atividade de eliminação radical, *Satureja hortensis* L.

1. Introduction

Medicinal plants include a large variety of substances named phytochemicals that posses antioxidant activity (Giao et al., 2007; Tepe, 2008; Yesiloglu et al., 2013). Typical compounds that exhibit antioxidant activity comprise vitamins, carotenoids and phenolic compounds (Chanwitheesuk et al., 2005). Since synthetic antioxidants may lead to toxicity and carcinogenity interest in natural antioxidants has been rised last years (Pandini et al., 2018). Many herbs, particularly members of *Lamiaceae* family show strong antioxidant activity (Javanmardi et al., 2003).

Satureja, which is from *Lamiaceae*, is represented by 15 species of which the endemism ratio is 33% in Flora of Turkey (Davis, 1982; Gören et al., 2003; Satil and Kaya, 2007). Many members of *Satureja* have aromatic and medicinal characteristics (Eminagaoglu et al., 2007; Abd El Tawab et al., 2014). The leaves, flowers and stems of *Satureja* are used for herbal tea and it has been reported that *Satureja* species posses antimicrobial, antifungal, antiinflammatory (Güllüce et al., 2003; Gören et al., 2003; Boroja et al., 2018). *Mentha,* the other genus studied, distributed thoroughout temperate regions of Eurasia, Australia and South Africa (Gulluce et al., 2007). The genus includes fifteen taxa belonging to eight species in the flora of Turkey (Aksit et al., 2013). Leaves, flowers and the stem of *Mentha* species are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavour (Moreno et al., 2002; Gulluce et al., 2007). It has been reported that the aeiral parts of *Mentha* have tonics, antispasmodic, stomachic and anti-inflammatory effects in the traditional medicine (Benabdallah et al., 2016, 2018).

This is the report about fatty acid, vitamin, sterol, radical scavenging and antimicrobial activity in seeds of *Satureja hortensis* and *Mentha spicata* L. subsp. *spicata.* The goals of present study are i) to detect fatty acid compositions, vitamin and sterol contents; ii) to evaluate flavonoid contents and radical scavening properties in the seeds of *Satureja hortensis* and *Mentha spicata* L. subsp. *spcicata*; iii) in addition, the aim of this research is to investigate the antimicrobial activities of fatty acids, vitamins and flavonoid contents in the seeds which such a study has not been found in the literature.

2. Material and Methods

In the present study, *Satureja hortensis* (Elazığ, Baskil-Bolucuk village, 1580 m) and *Mentha spicata* L. subsp. *spcicata* (Elazığ, Baskil-Bolucuk village, 1580 m) taxa are collected from the natural habitats. The plant materials are deposited in Firat University Herbarium (FUH).

2.1. Extraction of seed oils

Seed materials have been finely ground in a mill and then extracted with hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). The lipid extracts have been centrifuged at 10.000 *g* for 5 minutes and filtered, and the solvent has been then removed on a rotary evaporator at 40 °C. The extracted lipids have been stored under –25 °C until further analysis.

2.2. Fatty acids analyses

2% sulphuric acid (v/v) in methanol has been used to obtain the fatty acid methyle esters in the lipid extracts based on Christie' (1990) method. The methyl esters have been separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 after the fatty acid methyl esters have been treated with n-hexane and. Chromatographical conditions have been done 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 are adjusted to 130-220, 240, and 280 °C, respectively. It has been used authentic standart mixtures to obtain the methyle esters.

2.3. Chromatographic analysis and quantification of lipid soluble vitamins and sterols

Lipide-soluble vitamins and phytosterols have been extracted from the lipid fraction by the method of Sánchez-Machado et al. (2002) with minor modifications. The extracted lipids of seed material have been dissolved in acetonitrile/methanol (75/25 v/v) and have been injected 50 µL to HPLC (Shimadzu, Kyota Japan). The used column is a Supelcosil™ LC18 (250 × 4.6 mm, 5 µm, Sigma, USA) and the mobile phase is acetonitrile/methanol (75/25, v/v). The elution has been performed at a flow-rate of 1 mL/min and the temperature of analytical column is kept constant at 40 °C. the detection has been performed at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for δ-tocopherol, vitamin D, α-tocopherol, α-tocopherol acetate, 265 nm for vitamin K1 and 202 nm for phytosterols (López-Cervantes et al., 2006). *Class Vp 6.1* software assisted at workup of the data. The results of analysis have been expressed as µg/g for samples.

2.4. Flavonoid analysis and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity

2.4.1. Preparation of the extracts

Homogenization of two g seed materials is done in 5 mL 80% methanol. Homogenates have been centrifuged at 5000 rpm at +4 °C. The supernatant is concentrated by

reduced-pressure rotary evaporation after centrifugation. Each extract is re-suspended in dimethyl sulphoxide (DMSO) to produce a reserve solution (Kursat et al., 2011).

2.5. Chromatographic conditions for flavonoids

A PREVAIL C18 reversed-phase column (15 × 4.6 mm, 5 µm, USA) is used to do chromatographic analysis and methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid has been used as the mobile phase (Zu et al., 2006). The mobile phase has been filtered through a 0.45 µm membrane filter (Millipore), then de-aerated ultrasonically prior to use. Catechin (CA), naringin (NA), kaempferol (KA), naringenin (NAR), resveratrol (RES), myricetin (MYR), morin (MOR), quercetin (QU) and rutin (RU) have been measured 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 have been adjusted to 1.0 mL/min and l0 µL, respectively. The chromatographic peaks of the extracts have been evaluated by comparing their retention time with that of the reference standards. All chromatographic operations have been done at a temperature of 25 °C.

2.6. Antioxidant assay by DPPH radical scavenging activity

The free radical scavenging effects of extracts have been measured by the decoloration of a methanolic solution of DPPH$^{\bullet}$ based on the Liyana-Pathirana and Shahidi' (2005) method. A solution of 25 mg/L DPPH in methanol has been solved and 4.0 mL solution is mixed with 50, 100 and 250 µL of extract in DMSO. Then, mixture has been stored in darkness at room temperature for 30 minutes. The absorbance of the mixture has been evaluated spectrophotometrically at 517 nm. 1 µM quercetin is used as a reference (Kursat et al., 2011).

The scavenging capacity of DPPH radicals have been determined by the following Equation 1:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{[(Abs\ control - Abs\ sample)]/}{(Abs\ control)]\ x100} \quad (1)$$

where: Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract /standard (Kursat et al., 2011).

2.7. Antimicrobial activity

2.7.1. Test microorganisms

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.) have been used in the present investigation. Microorganisms have been supplied from the Department of Biology, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.7.2. Antimicrobial activity

Antimicrobial tests have been done by using the well agar method (100 µL of suspension containing 10^6 cells/mL of bacteria, 10^4 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 have been prepared in the plates with the help cork-borer (0.85 cm). 10 µL of the flavonoids, vitamins and fatty acids in the seeds have been added in to the well. Steril petri dishes (9 cm diameter) have been placed at 4 °C for 2h. Then, the inoculated plates have been incubated at 37 ± 0.1 °C at 24 h for bacterial strains and also at 25 ± 0.1 °C at 72 h for yeast and dermatophyta fungi. Antimicrobial activity has been observed by measuring the zone of inhibition against the test organisms (Collins and Lyne, 1987). Wells injected with methanol and hexane served as negative controls.

3. Results and Discussion

3.1. Fatty acids, vitamins and sterol contents in the seeds of S. hortensis and M. spicata subsp. spicata

Essential fatty acids, are called polyunsaturated fatty acids, can not be produced by body and they must be taken from dietary sources (Singh, 2005). The intake of polyunsaturated fatty acids have been shown to reduce the risk of coronary artery, other cardiovascular and some chronic diseases (Campos et al., 2008; Rajaram, 2014). The *Lamiaceae* is characterized by high percentage of unsaturated fatty acids (Azcan et al., 2004). Similarly, present study demonstrated that *S. hortensis* and *M. spicata* subsp. *spicata* from *Lamiaceae* have high polyunsaturated fatty acids.

The fatty acid compositions of *S. hortensis* and *M. spicata* subsp. *spicata* are given Table 1. It has been showed that palmitic acid (C16:0; 3.61 ± 0.32%), stearic acid (C18:0; 1.52 ± 0.22%), oleic acid (C18:1 n9; 7.13 ± 0.49%), linoleic acid (C18:2 n6; 15.19 ± 0.51%), α-linolenic acid (C18:3 n3; 66.24 ± 1.42%), γ-linolenic acid (C18:3 n6; 1.04 ± 0.14%) and stearidonic acid (C18:4; 4.63 ± 0.39%) are the major fatty acids of *S. hortensis*.

Table 1. Fatty acid compositions in seeds of *S. hortensis* L. and *M. spicata* L. subsp. *spicata*.

Fatty acids	*S. hortensis*	*M. spicata*
C16:0	3.61 ± 0.32	5.11 ± 0.41
C18:0	1.52 ± 0.22	1.92 ± 0.21
C20:0	0.12 ± 0.07	0.21 ± 0.07
ΣSFA	**5.25 ± 0.2**	**7.24 ± 0.23**
C16:1 n9	0.39 ± 0.01	0.52 ± 0.14
C18:1 n9	7.13 ± 0.49	8.19 ± 0.42
C20:1 n9	0.11 ± 0.02	0.21 ± 0.06
C24:1	0.12 ± 0.04	-
ΣMUFA	**7.75 ± 0.14**	**8.92 ± 0.2**
C18:2 n6	15.19 ± 0.51	31.14 ± 0.98
C18:3 n3	66.24 ± 1.42	48.17 ± 1.01
C18:3 n6	1.04 ± 0.14	2.07 ± 0.22
C18:4	4.63 ± 0.39	3.02 ± 0.18
ΣPUFA	**87.1 ± 0.61**	**84.4 ± 0.59**

Gören et al. (2003) determined that seeds of *Satureja thymbra* riched in oleic acid (43.9%), linolenic acid (30.2%), stearic acid (14.1%) and palmitic acid (11.4%) contents. They also demonstrated that *Satureja cuneifolia* has palmitic acid (34.6%) and oleic acid (10.1%) contents (Gören et al., 2003). On the other hand, Tepe and Cilkiz (2016) indicated that *Satureja* has palmitic acid, oleic acid, linoleic acid and linolenic acids as fatty acids. Also, present study showed that the main fatty acids of *M. spicata* are palmitic acid (C16:0; 5.11 ± 0.41%), stearic acid (C18:0; 1.92 ± 0.21%), oleic acid (C18:1 n9; 8.19 ± 0.42%), linoleic acid (C18:2 n6; 31.14 ± 0.98%), α-linolenic acid (C18:3 n3; 48.17 ± 1.01%), γ-linolenic acid (C18:3 n6; 2.07 ± 0.22%) and stearidonic acid (C18:4; 3.02 ± 0.18%). Rao and Lakshminarayana (1988) determined that the major fatty acids of *Mentha arvensis* are linolenic acid (C18:3), palmitic acid (C16:0) and linoleic acid (C18:2). Also, the studies showed that *Mentha* species (including *M. spicata*) contained high α-linolenic acid (Pereira et al., 2001). On the contrary, the results of El-Sayeda et al. (2014) conflict with present study. They indicated that *Mentha* has palmitic acid (C16:0; 45.27%), stearic acid (C18:0; 5.67%), oleic acid (C18:1 n9; 5.3%), linoleic acid (C18:2 n6; 7.86%) and linolenic acid (C18:3 n6;19.26%) contents (El-Sayeda et al., 2014). Similarly, Tulukcu (2011) determined that *Mentha* has high saturated fatty acids such as palmitic acid (C16:0) is 24.5% and stearic acid (C18:0) is 20.01%. And also, Tulukcu (2011) found that oleic acid (C18:1) and linoleic acid (C18:2) are lowest (4.80%, 7.30%, respectively) while linolenic acid (C18:3) content is high (29.5%). Furthermore, Conforti et al. (2011) found that linoleic acid and linolenic acid contents of *Mentha spicata* are trace amounts.

Phytosterols are known as total and LDL cholesterol reducing effect in patients with metabolic syndrome and diabetes (Fassbender et al., 2008; Sialvera et al., 2012; Demonty et al., 2013). Also, they might also protect against certain types of cancer such as colon, breast and prostate (Tasan et al., 2006). Sitosterol, campesterol and stigmasterol are the most common plant sterols in nature (Jong et al., 2003). It has been demonstrated that ergosterol (263.1 ± 2.14 µg/g), stigmasterol (39.07 ± 0.91 µg/g) and beta-sitosterol (14.64 ± 0.49 µg/g) have been found in *M. spicata* subsp. *spicata*, while ergosterol (69.41 ± 1.75 µg/g) and beta-sitosterol (19.81 ± 1.14 µg/g) have been found in *S. hortensis* in the present study (Table 2). It has been found that the sterol contents of *M. spicata* subsp. *spicata* are higher than those of *S. hortensis* in the present study. The study done by El-Sayeda et al. (2014) found that *Mentha* has β-sitosterol (5.6%) and stigmasterol (1.9%) contents whilst Conforti et al. (2008) found that γ-sitosterol is the predominant sterol in the *Mentha* investigated.

The fat soluble-vitamins (ADEK) have various biological actions related to protection the human health and their absence cause to important problems such as cancer, diabetes, osteoroporosis and immune system defects (Blanco et al., 2000; Mazzini et al., 2006; Albahrani and Greaves, 2016). Vitamin E are serious compounds in scavenging membrane phospholipids against free radicals attacts (Chai et al., 2012; Rizvi et al., 2014; Borel and Desmarchelier, 2016). Also, vitamin D are act as absorption of calcium and phosphate from intestinal and in the storage of these minerals in the bone (Capote et al., 2007; Chai et al., 2012). In addition to the protective role of vitamin A in the eye it has also effective in the carbohydrate, lipids and protein metabolism whilst vitamin K has role in the blood coagulation (Chen et al., 2011; Albahrani and Greaves, 2016). Some vitamins such as vitamin A and D play role as hormones and operate their role at intracellular receptor sites (Ravinaskar et al., 2015). The present study showed that *S. hortensis* contains α-tocopherol (35.18 ± 0.92 µg/g), retinol (6.33 ± 0.51 µg/g), K2 (1.61 ± 0.21 µg/g) D3 (86.8 7 ± 1.51 µg/g), and D2 vitamins (0.25 ± 0.07 µg/g) whilst *M. spicata* subsp. *spicata* contains r-tocopherol (31.51 ± 1.01 µg/g), D3 (162.23 ± 2.41 µg/g), α-tocopherol (0.84 ± 0.17 µg/g) and retinol-acetate (0.21 ± 0.07 µg/g) as lipide-soluble vitamins (Table 2). Chanwitheesuk et al. (2005) found that two *Mentha* species contained 0.0054-0.0294 mg% vitamin E content.

3.2. Flavonoid contents and radical scavenging capacities in the seeds of S. hortensis and M. spicata subsp. spicata

Phenolics, are one of main group herbal compounds, have potent to high antioxidant capacity against free radical damage (Benabdallah et al., 2016). It has been indicated that species from *Lamiaceae* have strong antioxidant capacity mostly due to phenolic compounds (Hossain et al., 2010). The variety of phenolics reduce cancer growth by capturing cancer cells in the certain phases of the cell cycle, heart disease and diabetes (Berdowska et al., 2013;

Table 2. Lipide-soluble vitamin and sterol contents in seeds of *S. hortensis* L. and *M. spicata* L. subsp. *spicata*.

	Lipide-soluble vitamins (µg/g)								Sterols (µg/g)		
Taxa	K2	K1	R-Tocopherol	D2	D3	A-tocopherol	Retinol	Retinol acetate	Ergosterol	Stigmasterol	Beta-sitosterol
S. hortensis	1.61 ± 0.21	-	-	0.25 ± 0.07	86.87 ± 1.51	35.18 ± 0.92	6.33 ± 0.51	-	69.41 ± 1.75	-	19.81 ± 1.14
M. spicata	-	-	31.51 ± 1.01	-	162.23 ± 2.41	0.84 ± 0.17	-	0.21 ± 0.07	263.1 ± 2.14	39.07 ± 0.91	14.64 ± 0.49

Shahidi and Ambigaipalan, 2015). Flavonoids are most abundant compounds of phenolics in the plants and contained 6000 chemicals (Gomaa et al., 2015). Total nine flavonoids (rutin, myricetin, morin, quercetin, kaempferol, catechin, naringin, naringenin, resveratrol) are studied in this study (Table 3). It has been found that *S. hortensis* contained naringenin (612.57 ± 2.57 µg/g), morin (86.97 ± 1.12 µg/g), quercetin (22.87 ± 0.75 µg/g), and kaempferol (20.11 ± 0.94 µg/g). However, myricetin, catechin and naringin aren't identified in the seeds of *S. hortensis.* Literatures showed that *Satureja* has natural phenolic compounds (Zheng and Wang, 2001; Zeljkovic et al., 2015). It has been determined that *Satureja* has luteolin and naringenin contents studies done by different researchers (Skoula et al., 2005; Kosar et al., 2005). Oke et al. (2009) suggested that the amounts of total phenols found in the *Satureja* methanolic extract are very high. Also, Tepe and Cilkiz (2016) reviewed that *Satureja* has catechin, naringin, naringenin, kaepmherol, apigenin, luteolin, rutin and myricetin whilst Boroja et al. (2018) demonstrated that *Satureja hortensis* contains apigenin, kaempherol, luteolin, naringin, naringenin and quercetin. On the other hand, naringenin (135.91 ± 1.91 µg/g), naringin (61.23 ± 2.15 µg/g), quercetin (47.51 ± 1.17 µg/g) have been identified as the major flavonoids in the seeds of *M. spicata* subsp. *spicata*. But myricetin and catechin constituents are not detected in the seeds of *M. spicata* subsp. *spicata.* Bimakr et al. (2011) found that *Mentha* contains catechin, epicatechin, rutin, myricetin, luteolin, apigenin and naringenin. Also, Farzaei et al. (2017) showed that *Mentha* has luteolin and apigenin contents. However, Tang et al. (2016) determined apigenin and naringenin as minor compounds. Furthermore, different studies suggested that *Mentha* has very high total phenolic content and the ability to scavenge the free radical DPPH (Capecka et al., 2005; Stringaro et al., 2018).

The results related to the radical-scavenging potential of *S. hortensis* and *M. spicata* subsp. *spicata* are summarized in Table 3. It has been found that 25 and 50 µL methanolic extracts of *S. hortensis* (89.62 ± 1.17%, 85.24 ± 1.24%, respectively) exhibited higher radical scavenging activity than those of *M. spicata* subsp. *spicata* (66.85 ± 1.01%, 89.91 ± 2.12%, respectively); this might be due to the high flavonoid concentration of *S. hortensis.* Several studies indicated that methanol extracts of *Satureja* species exhibited high antioxidant activity (Eminagaoglu et al., 2007; Oke et al., 2009; Alonso-Carrillo et al., 2017). Dorman and Hiltunen (2004) suggested that the crude and ethyle acetate extracts of *Satureja* are capable of scavenging reactive free radical species. It has been suggested that the extracts of *S. hortensis* may be able to defend sensitive constituents such as amino acids, DNA, lipoproteins, polyunsaturated fatty acids, sugars and proteins from oxidative stress (Dorman and Hiltunen, 2004; Zahedifar and Najafian, 2015). Besides, different studies demonstrated that *Mentha* species represent strong antioxidant activity and high phenolic constituent (Tawaha et al., 2007; Benabdallah et al., 2016; Tang et al., 2016). Unver et al. (2009) and Conforti et al. (2008) indicated that *Mentha* has high free radical scavenging capacity. Also, Sytar et al. (2018) found that *Mentha spicata* subsp. *spicata* has phenolic content and high antioxidant capacity. Furthermore, Motamed and Naghibi (2010) indicated that *Mentha* (93.68%) and *Satureja* species (93.39%) have the highest DPPH radical scavenging activity. These results suggested that methanol extracts of *S. hortensis* and *M. spicata* subsp. *spicata* have significant free radical scavenging activity.

3.3. Antimicrobial activities of lipid soluble vitamins, flavonoids and fatty acids in the seeds of S. hortensis and M. spicata subsp. spicata

The antimicrobial capacities of seed extracts have many practices comprising pharmaceuticals, food protection, natural therapies and alternative medicine (Reynolds, 1996; Lis-Balchin and Deans, 1997; Kelen and Tepe, 2008). The antimicrobial activities of vitamins, flavonoids and fatty acids of the studied species, negative control group and standart antibiotics have been showed in Table 4. It has been found that the extracts of vitamins and flavonoids in seeds have antibacterial and antifungal activity against the microorganisms tested but it seems that the antimicrobial activities of fatty acids extracts of seeds are lower than flavonoids and vitamins extracts (Table 4).

Table 4 shows that the vitamin extracts of *S. hortensis* have the maximum antimicrobial activity against all of the tested microorganisms: *E. coli* (12.1 ± 0.1 mm), *K. pneumoniae* (23.2 ± 0.3 mm), *S. aureus* (30.7 ± 0.1 mm*), B. megaterium* (19.1 ± 0.2 mm), *C. albicans* (22.4 ± 0.3 mm) *C. glabrata* (17.5 ± 0.2 mm), *Epidermophyton* sp. (23.4 ± 0.3 mm), *Trichophyton* sp. (21.1 ± 0.3 mm). Similarly, the flavonoid extracts of *S. hortensis* showed the maximum antimicrobial activity against the tested microorganisms, listed from high to low as: *E. coli* (35.1 ± 0.1 mm), *S. aureus* (34.4 ± 0.3 mm), *K. pneumoniae* (25.1 ± 0.2 mm), *C. albicans* (23.1 ± 0.2 mm), *B. megaterium* (21.4 ± 0.4 mm), *Epidermophyton* sp. (21.3 ± 0.4 mm), *Trichophyton* sp. (16.2 ± 0.2 mm) and *C. glabrata* (15.3 ± 0.3 mm). The fatty acids in seeds of *S. hortensis* did not show any antifungal activity against *C. albicans, C. glabrata*, *Epidermophyton* sp. and *Trichophyton* sp. However, the fatty acids in seeds

Table 3. Flavonoid and radical scavenging capacities in seeds of *S. hortensis* L. and *M. spicata* L. subsp. *spicata*.

	Flavonoids (µg/g)							inhibiton %	
	Myricetin	**Morin**	**Quercetin**	**Kaempferol**	**Catechin**	**Naringin**	**Naringenin**	**25 µL**	**50 µL**
S. hortensis	-	86.97 ± 1.12	22.87 ± 0.75	20.11 ± 0.94	-	-	612.57 ± 2.57	89.63 ± 1.17	85.24 ± 1.24
M. spicata	-	0.13 ± 0.06	47.51 ± 1.17	0.36 ± 0.04	-	61.23 ± 2.15	135.91 ± 1.91	66.85 ± 1.01	89.91 ± 2.12

Table 4. Antimicrobial activities of seed extracts containing vitamins, flavanoids and fatty acids.

Microorganisms	Inhibition zone (mm)								
	Vitamins		Flavonoids		Fatty acids		Control		
	S.h.	M. s.	S.h.	M. s.	S.h.	M. s.	Methanol	Hexan	Standart
E. coli	12.1 ± 0.1	15.1 ± 0.2	35.1 ± 0.1	23.1 ± 0.2	11.2 ± 0.1	-	-	15.3 ± 0.4	10.1 ± 0.2**
K.pneumoniae	23.2 ± 0.3	25.7 ± 0.3	25.1 ± 0.1	32.4 ± 0.4	8.2 ± 0.4	13.2 ± 0.1	-	14.2 ± 0.4	9.4 ± 0.1**
S. aureus	30.7 ± 0.1	21.2 ± 0.5	34.4 ± 0.3	13.3 ± 0.4	13.1 ± 0.3	-	-	13.2 ± 0.2	13.2 ± 0.2**
B. megaterium	19.1 ± 0.2	25.6 ± 0.4	21.4 ± 0.4	35.7 ± 0.5	8.3 ± 0.2	11.4 ± 0.1	-	12.1 ± 0.1	9.3 ± 0.2**
C. albicans	22.4 ± 0.3	24.1 ± 0.3	23.1 ± 0.2	17.6 ± 0.6	-	-	-	17.1 ± 0.1	18.1 ± 0.3*
C. glabrata	17.5 ± 0.2	18.4 ± 0.3	15.3 ± 0.3	11.1 ± 0.3	-	-	-	11.4 ± 0.7	12.3 ± 0.1*
Trichophyton sp.	23.4 ± 0.3	24.3 ± 0.2	16.2 ± 0.2	-	-	-	-	17.3 ± 0.6	NT
Epidermophyton sp.	21.1 ± 0.3	18.1 ± 0.1	21.3 ± 0.4	11.5 ± 0.2	-	-	-	9.1 ± 0.1	NT

S.h. = *S. hortensis* L., M.s. = *M. spicata* L. subsp. *spicata*. Standart: *Nystatin (30 µg/disc), **Streptomysin sulphate (10 µg/disc); Control: methanol and hexan (10 µL); NT = not tested.

of *Satureja hortensis* showed antibacterial activity against *E. coli* (11.2 ± 0.1 mm), *K. pneumoniae* (8.2 ± 0.4mm), *S. aureus* (13.1 ± 0.3 mm), and *B. megaterium* (8.3 ± 0.2 mm) (Table 4). Several studies revealed that *Satureja* species showed major activity against the gram positive and gram microorganisms, fungi and yeast (Dikbas et al., 2009; Choulitoudi et al., 2016; Tepe and Cilkiz, 2016; Valdivieso-Ugarte et al., 2019; Vitanza et al., 2019). On the other hand, the results obtained from study of Sahin et al. (2003) showed that hexane extract of *S. hortensis* don't have antifungal, but they observed antibacterial activity against four strains of three *Bacillus* species.

Furthermore, the vitamin extracts in the seeds of *M. spicata* subsp. *spicata* have strongly antimicrobial effect over some tested microrganisms; *K. pneumoniae* (25 mm), *B. megaterium* (25 mm), *C. albicans* (24 mm), *Epidermophyton* sp. (24 mm), *S. aureus* (21 mm), *C. glabrata* (18 mm), *Trichophyton* sp. (18 mm) and *E. coli* (15 mm) (Table 4). Also, the extracts of flavonoids in the seeds of *M. spicata* have antimicrobial activity on *E. coli, K. pneumoniae*, *S. aureus*, *B. megaterium*, *C. albicans, C. glabrata* and *Trichophyton* sp. (13 mm, 23 mm,13 mm, 35 mm, 17 mm, 11 mm and 19 mm zone of inhibition respectivelly) while it has not antimicrobial activity on *Epidermophyton* sp. However; the fatty acids in the seeds of *M. spicata* subsp. *spicata* exhibited antimicrobial effect over *K. pneumoniae* (13 mm) and *B. megaterium* (11 mm), where as those have not antimicrobial effect on the other tested microrganisms*: E. coli*, *S. aureus*, *C. albicans, C. glabrata, Epidermophyton* sp. and *Trichophyton* sp. It has been reported that chemical compositions of *Mentha* species have antifungal properties against human pathogens (*Malassezia furfur, Trichophyton rubrum*, and *Trichosporon beigelii*) and inhibited efficiently antimicrobials (Yadegarinia et al., 2006; Mahboubi and Haghi, 2008; Scherer et al., 2013; Biswas et al., 2014; Singh et al., 2015; Alexa et al., 2018). On the contrary, Gulluce et al. (2007) reported the methanol extract from aerial parts of *Mentha* showed no antimicrobial activities.

4. Conclusions

The present study found that α-linolenic acid (C18:3 n3) is the major polyunsaturated fatty acid of *S. hortensis* (87.1 ± 0.61%) and *M. spicata* subsp. *spicata* (84.4 ± 0.59%). It has been showed that the saturated fatty acids of *S. hortensis* and *M. spicata* subsp. *spicata* low. Also, ergosterol has been found to be a major sterol in the studied taxa. However, it has been found that *S. hortensis* and *M. spicata* subsp. *spicata* have low lipide-soluble vitamin content. On the other hand, naringenin and quercetin have been identified as the predominant flavonoids in *S. hortensis* and *M. spicata* subsp. *spicata.* Furthermore, present results suggested that methanol extracts of *S. hortensis* and *M. spicata* subsp. *spicata* display significant free radical scavenging activity. In addition, the present results indicated that *S. hortensis* and *M. spicata* subsp. *spicata* showed major activity against gram-positive and gram-negative microorganisms, fungi and yeast.

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