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Full Length Research Paper

Active constituents of some *Satureja* L. species and their biological activities

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Methanol extracts from the aerial parts of three *Satureja* L. spp. belonging to the family Lamiaceae were studied. *S. cilicica* is endemic to Turkey; *S. icarica* and *S. coerulea* extend to West Anatolia; *S. icarica* extends to the Turkish-Greek border and *S. coerulea* extends to the Turkish-Bulgarian border. *S. icarica*, *S. coerulea*, and *S. cilicica* extracts had strong fungicidal effects at high concentrations and fungistatic effects at lower concentrations. Plant methanol extracts were investigated for their antibacterial, antifungal and antimycobacterial activity. To our knowledge, this is the first report of *S. cilicica* mycobactericidal activity against *M. tuberculosis.* Phenolic constituents were detected using high performance liquid chromatography (HPLC) analyses; the effective constituents of methanol extracts of these plants are presented, and their activities and their ethnobotanical uses are discussed.

Key words: Antibacterial, antifungal, antimycobacterial, Lamiaceae, *Satureja icarica, Satureja coerulea, Satureja cilicica.*

INTRODUCTION

Satureja L. is a genus of common aromatic plants belonging to the family Lamiaceae. *Satureja* is represented by 15 species in Turkey, which is an important biodiversity hotspot for the Lamiaceae family (Davis, 1982; Tumen et al., 2000). The endemism ratio of this genus is 33% in Turkey; *Satureja* species grow mainly in south and west Anatolia. *Satureja* species are economically and medicinally important because of their high content of essential oils. Previous studies on the essential oils of *Satureja* species found in Turkey have been reported (Tumen and Baser, 1996; Tumen et al., 1997, Tumen et al., 1998; Baser, et al., 2000).

Members of the *Satureja* genus are called *kekik* in Turkish, and some species are exported as the herb thyme (Satil et al., 2008). Traditional Turkish folk remedies use *S. cuneifolia* and *S. thymbra*, which are collected from the wild, and *S. hortensis*, which is known locally as “Cipriska” or “Koc Otu” (Sahin et al., 2003) and is cultivated for use as a diuretic and digestive aid in various regions of Turkey (Baytop, 1999; Sahin et al., 2003). The species *S. hortensis* is also native to southern Europe and has been naturalised to regions of North America. In Europe, summer savoury (*Satureja hortensis* L.) and winter savoury (*Satureja montana* L.) are the most important *Satureja* species for cultivation. *S. hortensis* has a sweeter and more delicate aroma and fragrance than *S. montana* (Skocibusic and Bezic, 2004; Bezic et al., 2005). Both summer and winter savoury are used to flavour food (Bowles, 2004). In previous studies, essential oils and extracts from *S. hortensis* demonstrated a variety of useful properties: antibacterial activity; antifungal activity, particularly against *Aspergillus flavus* under *in vitro* conditions (Dikbas et al., 2008; Gulluce et al., 2003; Sahin et al., 2003; Boyraz and Ozcan, 2006); antioxidant activity; antispasmodic activity; and anti-diarrheal as well as sedative properties (Deans and Svoboda, 1989; Gulluce et al., 2003; Hajhashemi et al., 2000; Madsen et al., 1996; Dorman and Hiltunen, 2004). Carvacrol and γ-terpinene were identified as the primary phenolic constituents of *S. hortensis* (Ryu et al., 2004). *S. montana* L., showed effective antibacterial activity against *E. coli*, methicillin-resistant *Staphylococcus aureus* and *Candida albicans* (*Skocibusic* et al., 2006). De Oliveira et al. (2011) reported that the antimicrobial effect of *S. montana* against *C. perfringens* type A in mortadella-type

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Table 1. Herbarium data of plants such as locality, altitude, collection time and herbarium number of species.

Genus species authority (Labiatae)	Locality	Altitude (m)	Collection time	Herbarium number
Satureja icarica P. H. Davis	Gökçeada, Çanakkale	250	09/22/2002	FS1024
Satureja coerulea Janka in Velen	Demirköy, Kırklareli	600	10/29/2001	FS1006
Satureja cilicica (endemic) P. H. Davis	Andıran, Geben kasabası, Kahraman Maraş	1400	08/27/2000	FS1180

sausages and Zavatti et al. (2011) showed that *S. montana* is a medicinal plant used to treat male sexual dysfunctions in rats.

Ozkan et al. (2007) determined antioxidant activities of *Satureja cilicica* essential oil in butter and *in vitro*. The essential oil of *S. cilicica* exhibited a strong antioxidant activity in butter. Carvacrol (59.2%) was the main component in the oils of *S. icarica*. The oil of *S. coerulea* contained caryophyllene (10.6%) and caryophyllene oxide (8.0%) as main constituents. The main component of *S. cilicica* are carvacrol, *p*-cymene, γ-terpinene (Kirimer et al. 1993). The main component is carvacrol (52.04-55.97%) in *S. icarica,* caryophyllene (10.3-12.2%) in *S. coerulea* (Tümen et al., 1998 a, c).

Satureja spp. are widely distributed across Turkey and neighbouring regions: *S. cilicica* is endemic to Turkey; *S. icarica* and *S. coerulea* extend to West Anatolia; *S. icarica* extends to the Turkish-Greek border; and *S. coerulea* extends to the Turkish-Bulgarian border (Davis, 1982). However, despite the ubiquity of these species, the antibacterial, antifungal and antimycobacterial activity of their methanol extracts has not been studied previously. The goal of this study was to identify the major phenolic constituents in methanolic extracts of *Satureja icarica* P. H. Davis, *Satureja coerulea* Janka in Velen and *Satureja cilicica* (endemic) P. H. Davis using HPLC, and to determine the antibacterial, antifungal and antimycobacterial activities of these constituents.

MATERIALS AND METHODS

Plant materials

Aerial parts (herbs in the flowering stage) of plants were collected from different parts of Turkey between 2000 and 2002. The plants were identified by Assoc. Prof. Dr. F. Satil at Balıkesir University, Turkey. Voucher specimens were deposited in the herbarium of Department of Biology, Balikesir University. Herbarium plant data, such as locality, altitude, and collection time and identification number of species are given in Table 1.

Preparation of plant extracts

The plants [*S. icarica* (23 g), *S. coerulea* (25 g), and *S. cilicica* (29 g), (endemic)], were air-dried at room temperature. Extracts were prepared using 1 L of methanol (98%) at room temperature over a period of ten days according to the method of Seshadri (1962). The methanol extracts were filtered through filter paper concentrated using a rotary evaporator and dried *in vacuo* at 40°C. The total yields from *S. icarica*, *S. coerulea* and *S. cilicica* were 1.10, 1.25 and 1.27 g, respectively. All stocks were stored at -20°C. To conduct antimicrobial activity tests, samples were dissolved in dimethyl sulphoxide (DMSO) and prepared at a concentration of 100 mg/ml. All samples were sterilised using syringe membrane filters.

High performance liquid chromatography (HPLC) conditions

HPLC was performed using a Shimadzu HPLC device according to published techniques for the preparation of phenolic constituents (Caponio et al., 1999). A DAD (diode array detector) detector (lmax = 278) and SIL-10ADvp auto sampler were used for reverse-phase gradient system chromatography. An SCL-10Avp system controller, a LC-10ADvp pump and a DGU- 14A degasser were used. A CTO-10Avp oven was used with an Agilent Zorbax Eclipse XDB-C18 (250 × 4.60 mm) 5 µm column. The A and B mobile phase components were 3% acetic acid and methanol, respectively, and the flow rate was 0.8 ml/min. The column temperature was 30°C and the injection volume was 20 µL. Gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, vitexin, rutin, naringin, hesperidin, apigenin-7-glucoside, rosmarinic acid, eriodictyol, quercetin, naringenin, luteolin, apigenin, and carvacrol were used as chromatography standards.

Preparation of microorganisms and inocula

A total of seven microorganisms were used for antimicrobial activity studies: *Staphylococcus aureus* (ATCC 6538P), *Klebsiella pneumoniae* (CCM 2318), *Escherichia coli* (ATCC 11230), *Pseudomonas aeruginosa* (ATCC 27853*), Proteus vulgaris* (ATCC 6897), *Bacillus cereus* (CCM 99) and *Candida albicans* (ATCC 10239). Methanol extracts from the three plants were examined against the following fungi: *Aspergillus niger* van Tiegh (TA 47-3), *Aspergillus flavus* Link (TA 41-17), *Aspergillus ochraceus* K. Wilh. (MUCL 39534), and *Fusarium proliferatum* (Matsushima) Nirenberg (TA 18-2). The fungi were subcultured on Czapek-Dox Agar (Oxoid CM 97), Malt Extract Agar (Oxoid CM 59), Sabouraud 2% Dextrose Agar (Merck) and Sabouraud 2% Dextrose Broth (Merck), respectively. *Mycobacterium tuberculosis* strain H37Ra from American Type Culture Collection (ATCC 25177) was used for the antimycobacterial bioassay.

Preparation of bacterial and fungal inocula

Day-old cultures of bacteria grown on nutrient agar (NA) plates were suspended in sterile saline solution until the turbidity was equal to a 0.5 McFarland standard of 10^6 colony forming units (CFU) per ml (Koneman et al., 1997). The plates were inoculated using the bacterial suspensions (15 µL per well) and were incubated overnight at 37°C. All tests were performed in triplicate.

For fungi, the isolates were subcultured on potato dextrose agar and incubated at 35°C for 7-14 days. Fungal suspensions were standardised at a spectrophotometric absorbance of 0.600 at 450 nm.

Antibacterial and antifungal activity tests

Stock solutions of all extracts were prepared in DMSO. The extracts were screened for antimicrobial activity using the agar diffusion technique. Sulphamethoxazole/trimethoprim (Oxoid) was used as a standard drug for bacteria (25 µg/disk) and amphotericin B (BioChemica) was used for fungi (30 µg/disk).

Petri dishes were inoculated with the bacterial/fungal suspensions (100 µL per dish). Filter paper disks (Whatman No. 1; 6 mm diameter) were soaked with 15 µL of each extract (100 and 50 mg/ml) and the disks were applied to the agar plate surfaces. The plates were incubated at 37°C overnight for bacteria and 27°C for two nights for fungi. Determination of the disc diffusion results for each extract was performed according to National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 2006a).

Inhibition zone diameters were measured three times at different angles, and the means and standard deviations were calculated. Fungal colony inhibition (I%) was calculated as a percentage according to the following equation, where Dc is the diameter of the control zone (mm), and Ds is the diameter of the sample zone (mm).

$$I\% = (Dc - Ds)/Dc*100$$

Minimal inhibitory concentrations (MICs) were determined using a modified microdilution method according to the National Committee of Clinical Laboratory Standard guidelines (NCCLS, 2006b) for bacteria and for fungi (NCCLS, 2008). Sterile 96-well microplates were used for the assay (0.2 ml volume, Fisher Scientific).

Samples were diluted to twice the desired initial test concentration using nutrient broth (NB) (Oxoid), and all microplate wells were filled with NB (100 µL). Each test sample (100 µL) was added to the first well, and serial two-fold dilutions were performed to obtain final concentrations within the range of 12.5–0.4 mg/ml. The lowest concentrations that were determined, after macroscopic evaluation, to inhibit the growth of the organisms tested were determined to be the MICs.

Preparation of Mycobacterium tuberculosis Inocula

Bacterial suspensions of *M. tuberculosis* were prepared either from Lowenstein–Jensen slants or from complete 7H9 broth cultures. To prepare an inoculum that was less than 15 days old from a culture grown on Lowenstein-Jensen medium, a suspension was prepared in Middlebrook 7H9 broth. The turbidity of the suspension was adjusted to a 1.0 McFarland standard. The suspension was vortexed for several minutes and was allowed to stand for 20 min for the initial settling of larger particles. The supernatant was transferred to an empty sterile tube and was allowed to stand for an additional 15 min. After being transferred to a new sterile tube, the suspension was adjusted to a 0.5 McFarland turbidity standard by visual comparison. One ml of the adjusted suspension was diluted in 4 ml of sterile saline solution.

To prepare *M. tuberculosis* inoculum using a BACTEC MGIT tube with positive growth, the positive tubes were used beginning from the day after the sample first became positive (day-1 positive), up to and including the fifth day (day-5 positive). The positive tubes that were older than five days were subcultured into fresh growth medium. Tubes that were day-1 and day-2 positive were used in the inoculation procedure for the susceptibility tests. The tubes that were between days 3 and 5 positive were diluted using 1 ml of the positive broth and 4 ml of sterile saline solution; the 5 ml diluted suspension samples were used for the inoculation procedures.

Antimycobacterial activity tests

Antimycobacterial bioassays were performed using the microplate Alamar blue assay (MABA) (Collins and Franzblau, 1997). The methanol extracts were sterilised by filtration using 13 mm diameter (0.22 µm pore size) filters (Millipore, Bedford, MA).

An oleic acid, albumin, dextrose and catalase (OADC) mixed supplement (0.5 ml) was added to Middlebrook 7H9 broth. The broth mixture was vortexed and 1.75 µL was added to the first microplate well. The remaining wells were filled with Middlebrook 7H9 Broth (100 µL). Extract (25 µL) was added to the first well, and the final extract concentration in the first well was 12.5 mg/ml. After mixing by pipetting several times, two-fold dilutions were performed from the first well to the next well (100 µL), excluding the positive and negative control wells. Final extract concentrations in the wells were between 12.5 mg/ml and 0.024 mg/ml. Streptomycin (STR), ethambutol (EMB), and isoniazid (INH) were used as standard drugs. Concentrations of STR, EMB and INH in the two-fold dilution series ranged from 41.5 to 0.040 µg/ml, 83 to 0.16 µg/ml, and 2.07 to 0.004 µg/ml, respectively.

Determination of minimal inhibitory concentrations (MICs) for mycobacterium tuberculosis

Microplates were inoculated with the bacterial suspension (20 µL per well except for the negative control wells) and incubated at 37 °C for 6 days. Alamar blue (15 µL, Trek Diagnostic system) was then added to the bacterial growth control wells (without extract) and the microplates were incubated at 37°C for an additional 24 h. If the dye turned from blue to pink (indicating positive bacterial growth); then Alamar blue solution was added to the other wells to determine the MIC values. All tests were performed in triplicate.

Determination of mycobactericidal activity

The plant extracts described above were used in mycobactericidal activity tests. Two-fold dilution series in triplicate sets of parallel microplate wells were used for each extract. To determine the minimum bactericide concentrations (MBCs), fresh Middlebrook 7H9 culture broth (185 µL) was transferred to each well, and 15 µL of an *mycobacterial* suspension, from MIC concentration and higher concentration wells obtained from the MIC test described above was added to each well, in order to determine the minimum bactericide concentration (MBC).

Two microplate wells were used as positive and negative controls, and each test was repeated in triplicate. For the negative controls, 200 ml of fresh broth (Middlebrook 7H9 culture medium and OADC) was used. For positive controls, including 185 µL and inoculums from former positive control wells (15 µL) was used. After 24 h of incubation and colour development using the Alamar blue solution, MBCs were recorded as the minimum extract concentration that did not cause any colour change in cultures when reincubated in fresh medium.

RESULTS

Standard compounds for HPLC chromatograms were gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, vitexin, rutin, naringin, hesperidin, apigenin- 7 -glucoside, rosmarinic acid, eriodictiol,

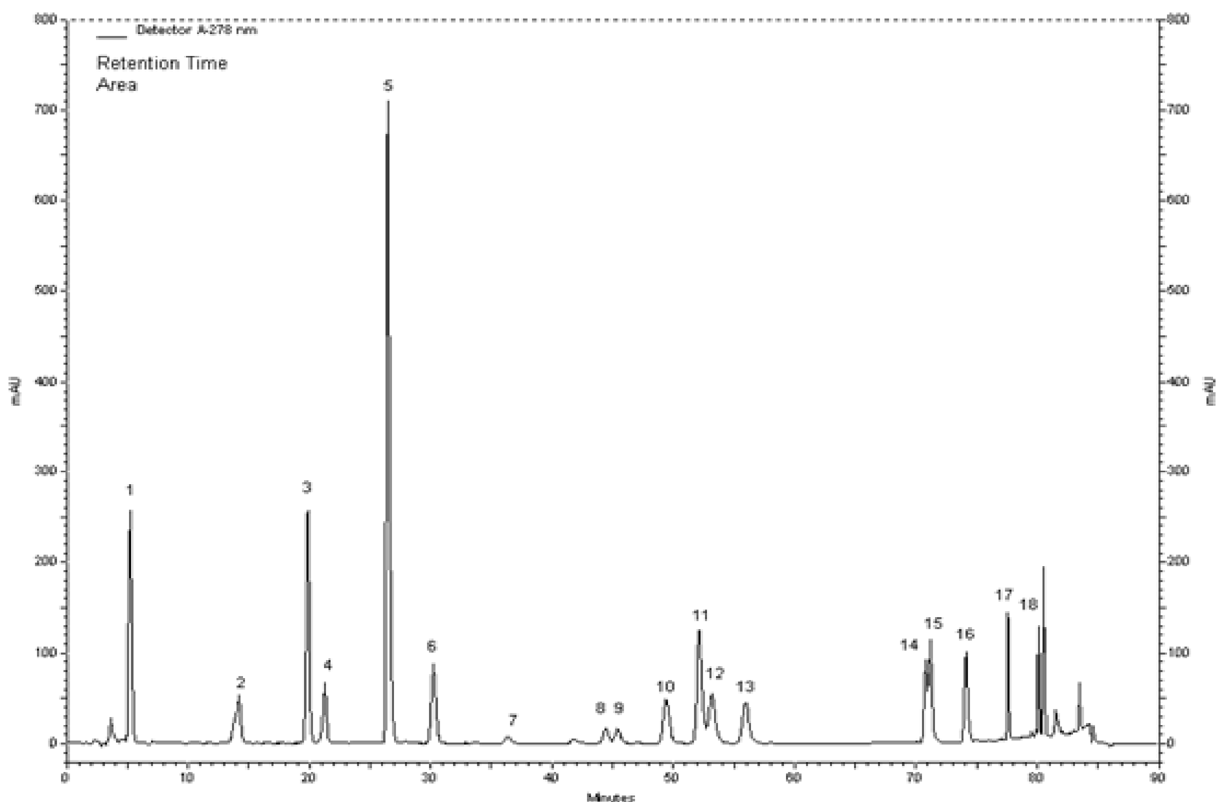

Figure 1. HPLC chromatogram for the standard compounds: (1) gallic acid, (2) catechin, (3) caffeic acid, (4) epicatechin, (5) p-coumaric acid, (6) ferulic acid, (7) vitexin, (8) rutin, (9) naringin, (10) hesperidin, (11) apigenin-7-glucoside, (12) rosmarinic acid, (13) eriodictyol, (14) quercetin, (15) naringenin, (16) luteolin, and (17) apigenin, (18) karvakrol.

quercetin, naringenin, luteolin, apigenin, and carvacrol. The major phenolic constituents determined by HPLC analyses of the methanol extracts were carvacrol, hesperidin and apigenin for *S. icarica*; rosmarinic acid, carvacrol and caffeic acid for *S. coerulea*; and rosmarinic acid, hesperidin and quercetin for *S. cilicica* (Figures 1, 2, 3 and 4; Table 2).

Inhibition of bacteria and fungi by methanol extracts of *Satureja* spices was tested by measuring the sizes of inhibition zones (mm). The microbial colony inhibition efficacy of the extracts was different in some cases, depending on which inhibition zone diameters were larger than the standard drug inhibition zones for bacteria and fungi (Table 3).

The extracts were tested for antibacterial activity against *P. vulgaris*, *K. pneumoniae*, *B. cereus, P. aeruginosa, E. coli,* and *S. aureus* and antiyeast activity against *C. albicans* (Table 4). *M. tuberculosis* was used for the antimycobacterial activity test (Table 5). Bacteria were tested for susceptibility to the reference drug Sulphamethoxazole/Trimethoprim (Oxoid). STR, EMB and INH (BD) were used as standard drugs for *M. tuberculosis*.

The *S. icarica* methanol extract was the most effective against *E. coli* (MIC of 0.8 mg/ml). Other bacteria (*B. cereus, P. vulgaris, P. aeruginosa, K. pneumoniae, S. aureus*) and yeast (*C. albicans*) showed inhibition between MICs of 1.6 to 6.3 mg/ml. The *S. icarica* extract exhibited bacteriostatic activity (MIC > 12.5 mg/ml) against other bacteria.

The *S. coerulea* methanol extract was the most effective against *B. cereus* (MIC of 6.3 mg/ml). The efficacy of inhibition against other bacteria (*P. vulgaris, P. aeruginosa, K. pneumonia, S. aureus, E. coli*) and yeast (*C. albicans*) was moderate (MIC of 12.5 mg/ml). *S. coerulea* extract showed bacteriostatic/fungistatic activity at the same concentration (MBC/MFC of 12.5 mg/ml) against *S. aureus, P. vulgaris* and *C. albicans.*

The methanol extract from *S. cilicica* was shown to be most effective against *P. vulgaris* and *S. aureus* (MIC of 1.6 mg/ml). MIC values for other bacteria varied between 3.1 and 6.3 mg/ml. *S. cilicica* extract showed bactericidal activity (MBC of 3.1 mg/ml) against *S. aureus* and bacteriostatic activity (MIC > 12.5 mg/ml) against other bacteria.

The filamentous fungi showed various sensitivities to the extracts that were tested. Strong activity was recorded for methanol extracts of *S. icarica, S. coerulea*, and *S. cilicica*, which completely inhibited fungi in a MIC range of 6.3 to 12.5 mg/ml. The fungus *F. proliferatum*

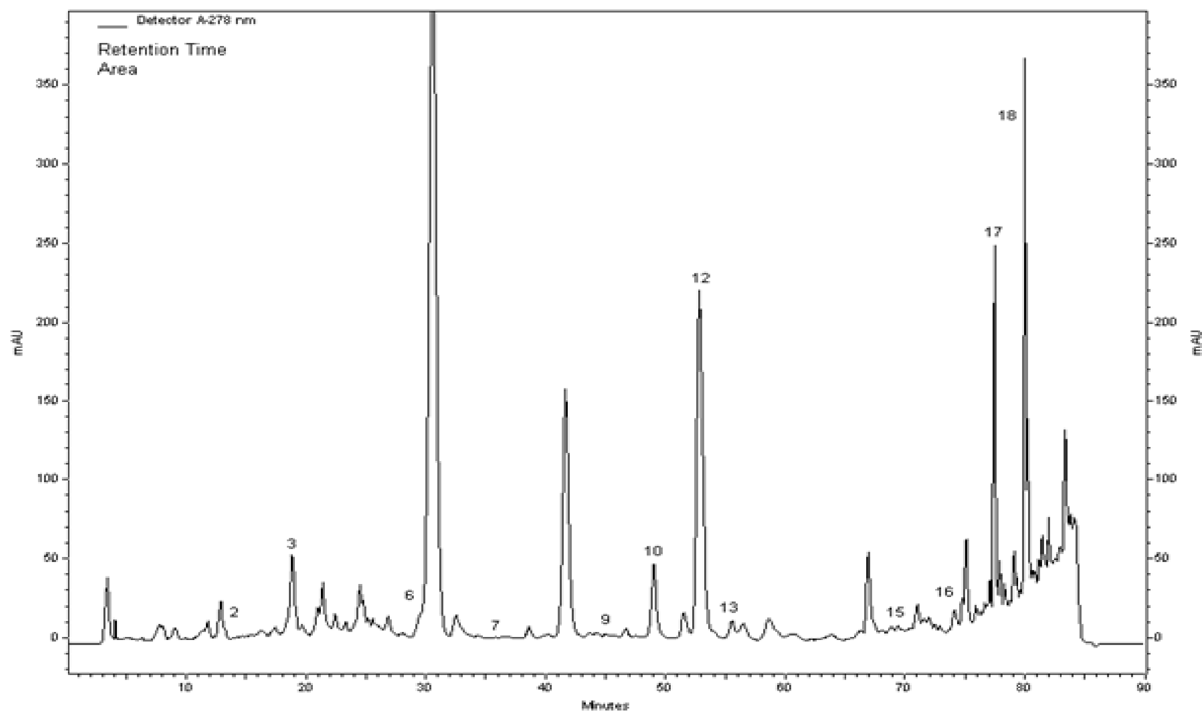

Figure 2. HPLC chromatogram of methanol extracts of *Satureja icerica*: (1) gallic acid, (2) catechin, (3) caffeic acid, (4) epicatechin, (5) p-coumaric acid, (6) ferulic acid, (7) vitexin, (8) rutin, (9) naringin, (10) hesperidin, (11) apigenin-7-glucoside, (12) rosmarinic acid, (13) eriodictyol, (14) quercetin, (15) naringenin, (16) luteolin, and (17) apigenin and (18) karvakrol.

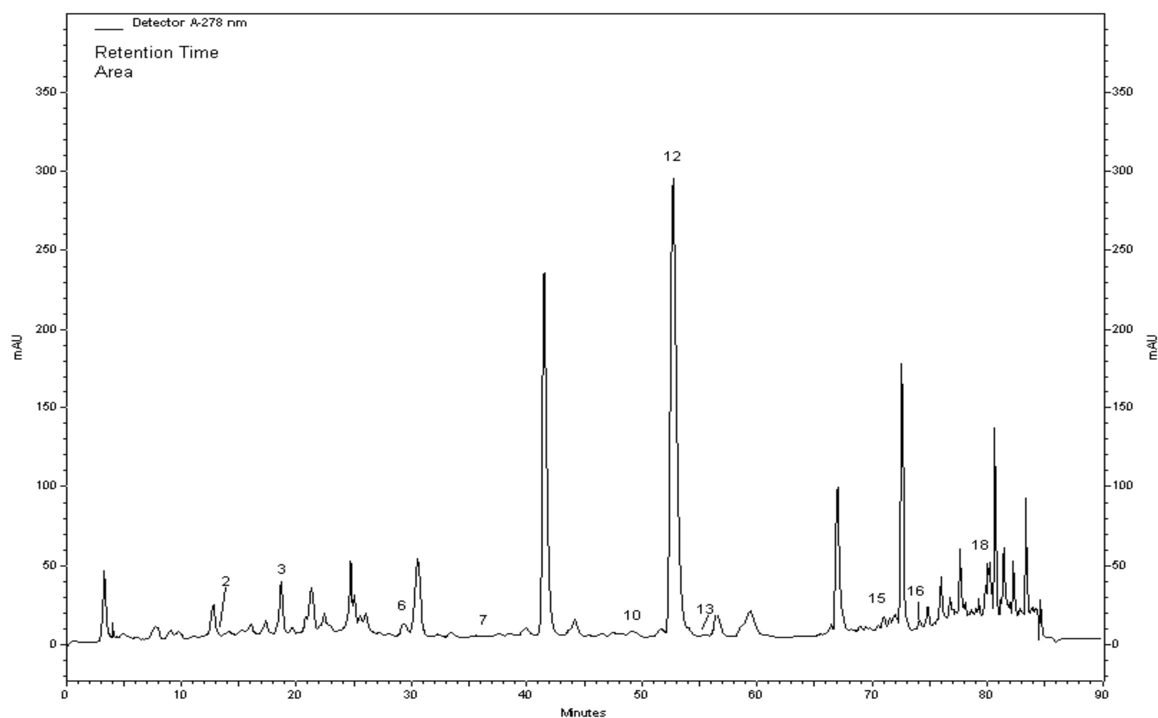

Figure 3. HPLC chromatogram of methanol extracts of *Satureja coerulea*: (1) gallic acid, (2) catechin, (3) caffeic acid, (4) epicatechin, (5) p-coumaric acid, (6) ferulic acid, (7) vitexin, (8) rutin, (9) naringin, (10) hesperidin, (11) apigenin-7-glucoside, (12) rosmarinic acid, (13) eriodictyol, (14) quercetin, (15) naringenin, (16) luteolin, and (17) apigenin and (18) karvakrol.

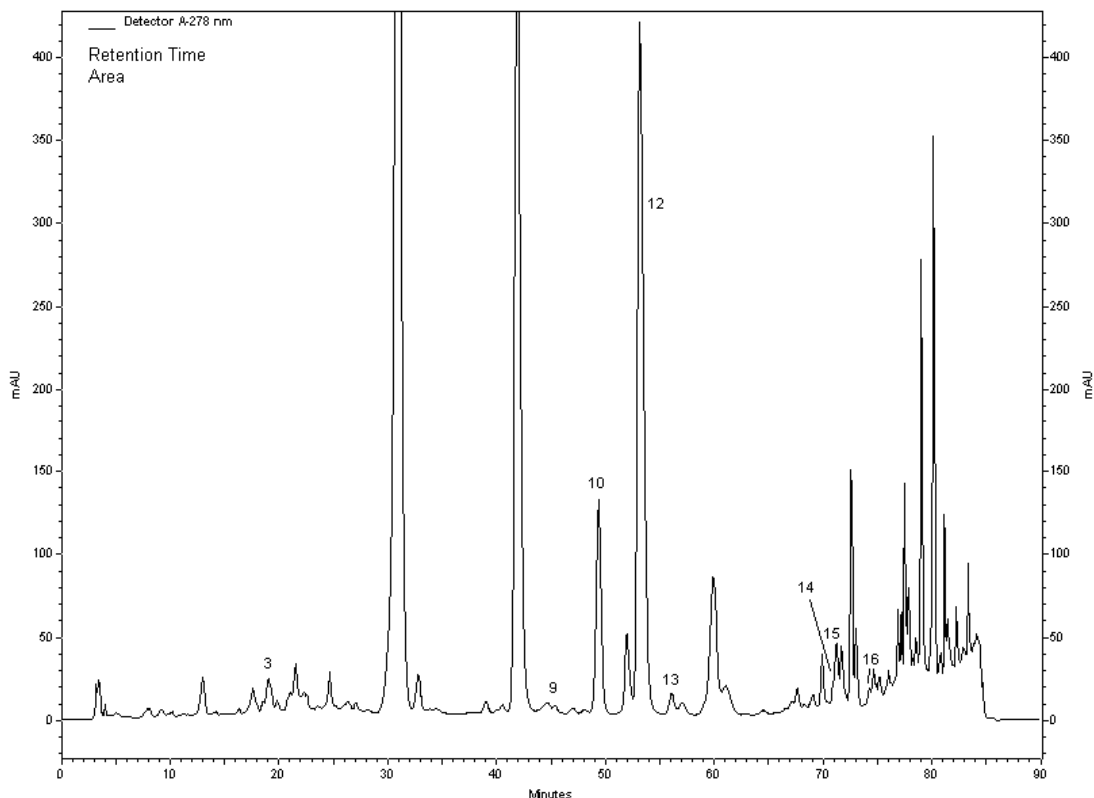

Figure 4. HPLC chromatogram of methanol extracts of *Satureja cilicica*: (1) gallic acid, (2) catechin, (3) caffeic acid, (4) epicatechin, (5) p-coumaric acid, (6) ferulic acid, (7) vitexin, (8) rutin, (9) naringin, (10) hesperidin, (11) apigenin-7-glucoside, (12) rosmarinic acid, (13) eriodictyol, (14) quercetin, (15) naringenin, (16) luteolin, and (17) apigenin and (18) karvakrol.

Table 2. Chemical concentrations in the methanol extracts of *Satureja* species.

Compound	*Satureja icarica*	*Satureja coerulea*	*Satureja cilicica*
gallic acid	n.a	n.a	n.a
catechin	322.7	298.3	n.a
caffeic acid	622.2	505.1	214.2
epicatechin	n.a	n.a	n.a
p-coumaric acid	n.a	n.a	n.a
ferulic acid	81.9	138.8	n.a
vitexin	95.2	134.0	n.a
rutin	n.a	n.a	n.a
naringin	127.8	n.a	239.1
hesperidin	2318.1	74.0	5663.2
apigenin-7-glucoside	n.a	n.a	n.a
rosmarinic acid	9716.0	13662	20358
eriodictyol	231.8	13.0	275.9
quercetin	n.a	n.a	179.3
naringenin	151.7	56.6	394.8
luteolin	165.3	117.0	241.5
apigenin	2256.0	n.a	973.5
carvacrol	13786.0	1219	n.a

*Results were given as P<0.01.

Table 3. Disk susceptibility testing for bacteria and fungi.

Microorganism	Statistics	Mean zone of inhibition (mm)								
		Satureja icarica			*Satureja coerulea*			*Satureja cilicica*		
		Concentration (mg/disc)								
		1.5	**0.75**	**Sd drug**	**1.5**	**0.75**	**Sd drug**	**1.5**	**0.75**	**Sd drug**
P. vulgaris	Mean± SD	15.5±0.57	8±0	36±3.46	6±0	6±0	38.75±2.21	12.25±1.5	9.5±1	10.87±4.5
	R%	56.94	77.77		84.51	84.51		-12.64	12.64	
K. pneumonia	Mean± SD	13±0.81	8.5±0.57	30±0	6±0	6±0	30±0	12.75±1.25	8±0	30±0
	R%	56.66	71.66		80	80		57.5	73.33	
B. cereus	Mean± SD	10±0	9.5±0.57	6±0	7.5±0.57	6±0	6±0	16.25±3.30	15±1.41	6±0
	R%	-66.66	-58.33		-25	0		-170.83	-150	
P. aeruginosa	Mean± SD	14.5±0.57	6±0	6±0	6±0	6±0	6±0	13±1.41	10.5±0.57	6±0
	R%	-141.66	0		0	0		-116.66	-75	
E. coli	Mean± SD	6±0	6±0	30±0	6±0	6±0	30±0	6±0	6±0	30±0
	R%	80	80		80	80		80	80	
S. aureus	Mean± SD	15.25±0.5	8±0	33±0	6±0	6±0	33±0	16.25±0.5	11.25±0.5	33±0
	R%	53.78	75.75		81.81	81.81		50.75	65.90	
C. albicans	Mean± SD	6±0	6±0	6±0	6±0	6±0	6±0	22±0	11±0	6±0
	R%	0	0		0	0		-266.66	-83.33	
A. ochraceus	Mean± SD	6±0	6±0	6±0	6±0	6±0	6±0	7.5±0.57	6±0	7±0.81
	R%	0	0	0	0	0	0	-7.14	14.28	
A. niger	Mean± SD	6±0	6±0	13.75±2.98	9±0.81	6±0	13±1.41	6±0	6±0	10.75±0.95
	R%	56.36	56.36		30.76	53.84615		44.18	44.18	
A. flavus	Mean± SD	37.5±6.45	32.5±2.08	6.75±0.5	6±0	6±0	6±0	6±0	6±0	7.25±0.95
	R%	-455.55	-381.48		0	0	0	20.83	20.83	
F. proliferatum	Mean± SD	6±0	6±0	8.5±2.38	6±0	6±0	6±0	12.75±2.21	8±0.81	7±0.81
	R%	29.41	29.41		0	0		-82.14	-14.28	

Table 4. Antibacterial and antifungal activities of methanol extracts from the plants. Results are shown as MICs and MBCs/MFCs.

Microorganism	*Satureja icerica*		*Satureja coerulea*		*Satureja cilicica*	
	A	B	A	B	A	B
P. vulgaris	1.6	>12.5	12.5	12.5	1.6	>12.5
K. pneumonia	3.1	>12.5	12.5	>12.5	6.3	>12.5
B. cereus	1.6	>12.5	6.3	>12.5	3.1	>12.5
P. aeruginosa	1.6	>12.5	12.5	>12.5	6.3	>12.5
E. coli	0.8	>12.5	12.5	>12.5	3.1	>12.5
S. aureus	6.3	>12.5	12.5	12.5	1.6	3.1
C. albicans	1.6	>12.5	12.5	12.5	6.3	>12.5
A. ochraceus	6.3	>12.5	6.3	12.5	12.5	>12.5
A. niger	6.3	>12.5	12.5	12.5	6.3	>12.5
A. flavus	12.5	>12.5	12.5	>12.5	6.3	>12.5
F. proliferatum	6.3	12.5	6.3	12.5	6.3	12.5

A: MIC (mg/ml); B: MBC (mg/ml).

Table 5. Antimycobacterial activity results for the methanol extracts against *M. tuberculosis* H37Ra (ATCC 25177) determined by MABA.

Plant	*S. icarica*		*S. coerulea*		*S. cilicica*		Standard drug					
							STR		EMB		INH	
M. tuberculosis	A	B	A	B	A	B	A	B	A	B	A	B
	n.a.	n.a.	n.a.	n.a.	0.8	0.8	0.16	0.16	1.2	1.29	0.01	0.016

A: MIC (mg/ml); B: MBC (mg/ml).

was the most sensitive to inhibition by the three plant extracts; the extracts also exhibited fungicidal activity against *F. proliferatum* (MFC of 12.5 mg/ml). *S. icarica* and *S. coerulea* extracts showed fungistatic activity against the other fungi that were tested (MICs of 6.3–12.5 mg/ml). *S. coerulea* extracts also showed fungicidal activity against *A. ochraceus* and *A. niger* (MIC of 12.5 mg/ml) and fungistatic activity against *A. flavus*.

According to the MABA test results for the activity of the three plant extracts against *M. tuberculosis*, *S. cilicica* extracts showed the highest activity (MIC and MBC of 0.8 mg/ml). The remaining extracts, *S. icarica* and *S. coerulea*, showed higher MBC values (1.6 mg/ml) than those of *S. cilicica*.

DISCUSSION

This study demonstrated that the addition of crude methanol extracts of *S. icarica, S. coerulea*, and *S. cilicica* to growth medium inhibited the growth of the mycotoxigenic filamentous fungi, *F. proliferatum*, *A. flavus*, *A. ochraceus* and *A. niger*. This property may provide a safe and effective method of protecting food from mycotoxigenic fungi. The results of this study showed that the activities of methanol extracts were concentration dependent: *S. icarica*, *S. coerulea*, and *S. cilicica* extracts had strong fungicidal effects at high concentrations and fungistatic effects at lower concentrations.

According to the results presented here, *S. icarica* had fungicidal activity against *F. proliferatum* and fungistatic activity against the other fungi and bacteria that were tested. *S. coerulea* also showed fungicidal activity against *A. ochraceus* and *A. niger* and showed bactericidal activity against *P. vulgaris, S. aureus* and *C. albicans.* Our previous research on the effects of plant-derived methanol extracts (including *T. spicata* and *O. minutiflorum*) against fungi (Askun et al., 2008) and bacteria (Askun et al., 2009) formed the basis for developing research on antimicrobial activity. *S. cilicica* extract showed bactericidal activity against *S. aureus* and fungicidal activity against *F. proliferatum*. *S. cilicica* extract also showed fungistatic and bacteriostatic activity against the other fungi and bacteria tested.

Methanol extracts of *Satureja* spp. were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* using the Alamar blue susceptibility test. The methanol extract of *S. cilicica* showed antimycobacterial activity at a concentration of 0.8 mg/ml. Although *S. icarica* extract showed the same MIC value (0.8 mg/ml) as *S. cilicica*, the MBC value of *S. icarica* was higher.

Similarly, the MIC value for *S. corulea* extract was 0.4 mg/ml, whereas the MBC was 1.6 mg/ml. However, all plant methanol extracts killed *M. tuberculosis*. MIC/MBC values for the standard drugs were 0.16/0.16 µg/ml, 1.29/1.29 µg/ml and 0.016/0.016 µg/ml for STR, EMB and INH, respectively. To our knowledge, this study is the first to report the mycobactericidal activity of *S. cilicica* against *M. tuberculosis.*

The use of MABA has several advantages when compared with other methods, such as the ability to use small quantities of extract in 200 µL wells (Molina-Salinas et al., 2006) and a shorter time requirement. The use of Middlebrook 7H9 Broth and OADC supplement in multi-well plates reduces the cultivation time from 3-4 weeks (using solid medium such as Lowenstein-Jensen) to 7–10 days (Sethi et al., 2004). A reduction in cultivation time is beneficial when conducting research, especially when working with rare species, as the impact on natural environments and the potential for over-collecting species is minimised.

Plant methanol extracts contain many chemicals such as alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids (Kumar et al., 2009). Methanol extracts may therefore yield a spectrum of antibacterial components that is different from those previously described. In a review of literature on the antimicrobial activity of different plant extracts, Parekh et al. (2005) noted that methanol extracts were more active than aqueous extracts.

The HPLC results demonstrated that none of the *Saturea* species extracts contained gallic acid, epicatechin, p-coumaric acid, rutin, or apigenin-7-glucoside. Compared to the other species, the major phenolic constituents in *S. icarica* were carvacrol, hesperidin and apigenin. While, the concentration of carvacrol in *S. icarica* was 11.3 times higher than that *S. coerulea*; the concentration of apigenin was 2.3 times higher than that *S. cilicica*. Although, caffeic acid and catechin were found in the extract of *S. icarica* but there was no quercetin present.

Although rosmarinic acid, carvacrol and caffeic acid were the major phenolic constituents of *S. coerulea*, the amounts of ferulic acid and vitexin were higher than in the other two species. In contrast, naringin, quercetin, and apigenin were not detected.

Rosmarinic acid, hesperidin and quercetin were the major phenolic constituents in the *S. cilicica* methanol extract. Although the amounts of naringin, hesperidin, rosmarinic acid, eriodictyol, naringenin, and luteolin were high relative to the other two species, the *S. cilicica* extract did not contain catechin, ferulic acid, vitexin, or carvacrol.

Extracts of natural products are a common starting point in the search for new antimycobacterial agents. As shown previously, rosmarinic acid might be responsible for the antimycobacterial activity observed in *S. cilicica* (Askun et al., 2009). The results of this study support the findings of our previous research. In addition, Mandalari et al. (2007) reported that paired combinations of eriodictyol, naringenin and hesperidin showed both synergistic and antagonistic interactions that were dependent on the test indicator organism and their cell wall structure. In addition to these interactions, the results presented here showed synergistic interactions, which supports the suggestion that rosmarinic acid and hesperidin may be the main bioactive antimicrobial constituents present in *S. cilicica* extract.

Rosmarinic acid is a natural phenolic compound with two phenolic rings (Petersen and Simmonds, 2003). Hesperidin is produced in high concentrations by members of the Rutaceae and Lamiaceae famillies, and it has been shown that dietary hesperidin deficiency has been linked to abnormal capillary leakiness (Martínez et al., 2011). Hesperidin is also well known for having antioxidant, analgesic and anticarcinogenic properties (Hirata et al., 2005) and sedative effects (Fernández et al., 2005). Naringin is present in grapefruit and citrus, and the antioxidant (Haenen et al., 1997), antimicrobial (Han and You, 1988) and anticancer (So et al., 1996) properties of naringin have been studied. Naringenin also occurs in citrus fruits. Renugadevi and Prabu (2009) showed that the nephroprotective potential of naringenin against Cd toxicity might be due to its antioxidant and metal chelating properties. In addition, naringenin has antimutagenic (Choi et al., 1994) and anticancer (So et al., 1997) properties. Rosmarinic acid and chlorogenic acid are caffeic acid esters that are common in Lamiaceae plants (Petersen et al., 2009). The biosynthesis of rosmarinic acid starts with the amino acids L-phenylalanine and L-tyrosine. Rosmarinic acid occurs in plants that are thought to have health benefits, and it has antiviral, antibacterial, anti-inflammatory and antioxidant properties. In plants, the role of rosmarinic acid is thought to be related to defence (Petersen et al., 2003). Carvacrol activity results from the interaction of the carvacrol hydroxyl group with the cytoplasmic membrane, which changes the membrane permeability with respect to protons and potassium ions (Ultee et al., 2002). Carvacrol has many biological properties, such as antibacterial (Lambert et al., 2001) and antifungal (Manohar et al., 2001) activity.

Arunasree (2010) identified the mechanism by which carvacrol induces cell death in human metastatic breast cancer cells and demonstrated that carvacrol induces apoptosis in breast cancer (MDA-MB 231) cells.

Furthermore, studies of bio-based food packaging have emphasised a need for the involving of natural active agents (for example, antioxidants or antimicrobial constituents). Recently, the antimicrobial packaging industry has shown a great interest in natural active agents for inhibiting or delaying the growth of pathogenic bacteria and fungi on foods (Padgett et al., 1998; Dorman and Deans, 2000).

To date, there have been relatively few published

accounts of the effects of plant extracts on *M.tuberculosis* (Adeniy et al., 2004; Rojas et al., 2006; Askun et al., 2009).

These results provide a basis for the selection of candidate plant species for further phytochemical and pharmacological investigation. From a practical perspective, *S. cilicica* extract may be a suitable candidate for the development of plant-based pharmaceutical products for use against tuberculosis.

After determining that some of the methanol extracts had high antibacterial, antifungal and antimycobacterial activities, further research should involve the isolation and purification of the effective constituents from the extracts.

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