Antimicrobial Activity of Some *Satureja* Essential Oils

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The genus *Satureja* is represented by fifteen species of which five are endemic and *Satureja pilosa* and *S. icarica* have recently been found as new records for Turkey. Aerial parts of the *Satureja pilosa, S. icarica, S. boissieri* and *S. coerulea* collected from different localities in Turkey were subjected to hydrodistillation to yield essential oils which were subsequently analysed by GC and GC/MS. The main constituents of the oils were identified, and both antibacterial and antifungal bioassays were applied. Carvacrol (59.2%, 44.8%, 42.1%) was the main component in the oils of *S. icarica*, *S. boissieri* and *S. pilosa*, respectively. The oil of *S. coerulea* contained β-caryophyllene (10.6%) and caryophyllene oxide (8.0%) as main constituents.

Introduction

The genus *Satureja* (Lamiaceae) is represented in Turkey by fifteen species of which five are endemic (Davis, 1982; Tümen *et al.*, 1998a).

Several *Satureja* species are locally known as "keklik otu", "kılıç kekik", "firubu", "çatlı" or "kekik" in the regions where they grow and used as culinary or medicinal herbs in various regions of Turkey. Dried herbal parts constitute an important commodity for export. The uses of Satureja species have been reported in our previous works (Başer, 1995; Başer *et al.*, 2001; Tümen *et al.*, 1992; Tümen *et al.*, 1993; Tümen and Başer, 1996; Tümen *et al.*, 1997; Tümen *et al.*, 1998a,b,c).

There is a large demand for fungicides for use in agriculture, food protection and medicine. Antifungal chemotherapy relies heavily on fungicides and many efforts have been made to standardize test procedures in order to increase reproducibility (Cormican and Pfaller, 1996). As a result, the National Committee for Clinical Laboratory Standards (NCCLS) proposed in 1997, an antifungal susceptibility test for yeast, with guidelines for macrodilution and microdilution methods. A modification of the said method (M38) for filamentous fungi appears promising and its standardization is reported to be in progress (Espinel-Ingroff, 1998). However, since filamentous fungi do not grow as single cells, standardization appears to be more challenging in the case of unicellular yeast and bacteria (Hadecek and Greger, 2000).

Here, we report on the gas chromatographic (GC) and gas chromatography/mass spectrometric (GC/MS) analyses of the major constituents of the essential oils of four *Satureja* species: *S. coerulea*, *S. icarica*, *S. pilosa* and *S. boissieri* and their antibacterial and antifungal properties against common pathogenic and saprophytic fungi.

Experimental

Plant material and isolation of the oils

Information on the plant material used in this study is given in Table I. Air dried aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus. Percentage yields of oils calculated on moisture free basis are also indicated in Table I. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE).

Gas chromatography (GC)

GC analysis using a Shimadzu GC-17A system. An CP-Sil 5CB column (25 m × 0.25 mm inner

diameter and 0.4 μm film thickness) was used with nitrogen as carrier gas (1 ml/min). The oven temperature was kept at 60° C and programmed to 260° C for at a rate of 5° C/min, and then kept constant at 260° C for 40 min. Split flow was adjusted at 50 ml/min. Injector temperature was 250° C. The percentages were obtanied from electronic integration measurements using flame ionization detection (FID, 250° C).

Gas chromatography / Mass spectrometry (GC/MS)

A Shimadzu GCMS-QP5050A system, with CP-Sil 5CB column (25 m × 0.25 μm film thickness) was used with helium as carrier gas. GC oven temperature was kept at 60° C and programmed to 260° C for at a rate of 5° C/min, and then kept constant at 260° C for 40 min. Split flow was adjusted at 50 ml/min. The injector temperature was at 250° C. MS were taken at 70 eV. Mass range was between *m/z* 30 to 425. Library search was carried out using the in-house "TBAM Library of Essential Oil Constituents". Relative percentage amounts of the separated compounds were calculated from FID chromatograms. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). The components identified in the oils are listed in Table I.

Antimicrobial bioassay

Microdilution broth susceptibility assay was used (Koneman *et al.*, 1997). Stock solutions of essential oils were prepared in dimethylsulfoxide (DMSO). Serial dilutions of essential oils were prepared in sterile distilled water in 96-well microtiter plates. Freshly grown bacterial suspensions in double strength Mueller Hinton Broth (Merck) and yeast suspension of *Candida albicans* in yeast medium were standardised to 108 CFU/ml (McFarland No: 0.5). Sterile distilled water served as growth control. 100 μl of each microbial suspension were then added to each well. The last row containing only the serial dilutions of antibacterial agent without microorganism was used as negative control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Human pathogens used for this assay were obtained both from the Microbiology Department, Faculty of Sciences in Anadolu University and Microbiology Department of Medical Faculty of Osmangazi University, Eskişehir (Table II).

Fungal spore inhibition assay

In order to obtain conidia, the fungi were cultured on Czapex Dox Agar medium (Merck) in 9 cm Petri dishes at 25 °C, for 7–10 days. Harvesting was carried out by suspending the conidia in a 1% (w/v) sodium chloride solution containing 5% (w/v) DMSO. The spore suspension was then filtered and transferred in to tubes and stored at –20 °C, accordingly to Hadacek and Greger (2000). The 1 ml spore suspension was taken thereof, diluted in a loop drop until one spore could be captured (Hasenekoğlu *et al.*, 1990).

One loop drop from the spore suspension was applied onto the centre of the Petri dish containing Czapex Dox Agar (Merck), Malt Extract Agar (Mast Diagnostics) and Potato Dextrose Agar (Acumedia) medium (Merck). 0.2 ml of each essential oil was applied onto sterile paper disks (9 mm diameter) and placed in the Petri dishes and incubated at 25 °C for 72 h. Spore germination during the incubation period was followed using a microscope (Olympus BX50) in 8 h intervals. The fungi *Aspergillus niger* (BUB Czp.30), *Penicillium sublateritium* (BUB Czp. 69), *P. canescens* (BUB Czp. 38) and *P. steckii* (BUB Czp. 28) used for this assay were isolated from various soil samples and deposited in Balikesir University, Faculty of Science and Letters, Department of Biology (BUB), Balikesir, Turkey.

Results and Discussion

Water distilled essential oils from aerial parts of *S. coerulea*, *S. icarica*, *S. pilosa* and *S. boissieri* collected from four different localities in Turkey have been analysed by means of GC and GC/MS. The resulting main components of the oils are shown in Table I along with other collections and yield information.

The analyses showed that carvacrol (42.1%–59.2%) was the main component in the oils of *S. icarica*, *S. pilosa* and *S. boissieri.* Other major components were identified as *p*-cymene (8.1%–35.5%) and borneol (4.5%–6.3%), besides other monoterpenes. In contrast, *S. coerulea* contained mainly sesquiterpenes such as ï-caryophyllene (10.6%), germacrene D (4.7%), and caryophyllene

Table I. Information on collection of *Satureja* sp. and essential oil compositions.

Satureja sp	Collection site and date	ESSE[a]	Oil Yields (%)	RRI[b]	Main components	(%)[c]
S. boissieri Hausskn. ex Boiss.	C7: Adıyaman 30.09.2001	13985	1.1	1273	carvacrol	44.8
				1007	*p*-cymene	35.5
				1048	γ-terpinene	6.5
				1276	thymol	2.3
S. coerulea Janka in Velen.	A1: Kırklareli: Dereköy 29.10.2001	13983	0.6	1422	β-caryophyllene	10.6
				1565	caryophyllene oxide	8.0
				1151	borneol	6.3
				1471	germacrene D	4.7
				1022	limonene	4.3
				1374	β-bourbonene	3.6
				1563	spathulenol	3.0
S. icarica P. H. Davis	A1: Ḍanakkale: Gökçeada 23.09.2001	13984	0.8	1273	carvacrol	59.2
				1007	*p*-cymene	15.7
				1151	borneol	4.5
				1048	γ-terpinene	4.4
				1218	methyl carvacrol	2.0
S. pilosa Velen.	B1: Balıkesir: Edremit 22.09.2001	13986	0.4	1273	carvacrol	42.1
				1007	*p*-cymene	8.1
				1238	geraniol	7.6
				1151	borneol	4.7
				1490	β-bisabolene	4.3
				1218	methyl carvacrol	3.1

[a] ESSE: Acronym of the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey.
[b] RRI: Relative retention indices calculated against *n*-alkanes on non-polar column (CP Sil5CB).
[c] (%): Relative percentage from FID.

oxide (8.0%) as main components (See also Table I).

In an earlier work, the essential oil of *S. icarica* was reported to contain carvacrol (52.0%–56.0%), borneol (5.2%–5.8%), γ-terpinene (5.8%–6.9%), *p*-cymene (13.1%–17.0%) as main constituents. The essential oil of *S. coerulea* was reported to contain β-caryophyllene (10.3%–12.2%), caryophyllene oxide (3.9%–5.7%), borneol (4.4%–8.2%), 1,8-cineole (0.1%–1.5%), limonene (0.3%–5.1%) and germacrene D (12.8%–20.6%), and the essential oil of *S. pilosa* was reported to contain carvacrol (5.1%–53.5%), *p*-cymene (4.7%–17.4%), geraniol (1.2%–4.5%), and borneol (1.0%–8.8%) being the main constituents as investigated by Tümen *et al.* (1998a and 1998c). However, to the best of our knowledge, the essential oil composition of *S. boissieri* has not previously been investigated.

In our previous work (Başer *et al.*, 2001), antibacterial activity of the essential oils of *S. wiedemanniana* obtained from various samples was shown. Carvacrol and thymol were shown to inhibit pathogenic microorganisms. Furthermore, antimicrobial activities of different *Satureja* species were shown in other previous studies (Müller-Riebau *et al.*, 1995; Akgül and Kıvanç, 1988; Kıvanç and Akgül, 1989).

In this present study, using the microdilution broth assay (Koneman *et al.*, 1997), the essential oil of *S. pilosa* showed a minimal inhibitory concentration value of 31.25 μg/ml against the pathogenic yeast *Candida albicans*. The other oils tested were also found as active against *C. albicans* in various inhibitory concentration ranges (see Table II). *Pseudomonas aeruginosa* was best inhibited by the oil of *S. icarica* and the other oils tested also showed inhibitory activities. The pathogen *Enterobacter aerogenes* was inhibited by both *S. pilosa and S. icarica* essential oils with a MIC value of 62.5 μg/ml, stronger than the standard Chloramphenicol. *Salmonella typhimurium* was inhibited by all oils except for *S. coerulea* as good as the standard antimicrobial agent. As a general result, all the bacteria assayed showed inhibition when tested against the *Satureja* oils (see Table II).

Table II. Antimicrobial activity (MIC) of *Satureja* essential oils.

Microorganisms	Source	A	B	C	D	St
Escherichia coli	ATCC 25292	125	125	125	62.5	62.5
Staphylococcus aureus	ATCC 6538	62.5	125	125	62.5	7.81
Pseudomonas aeruginosa	ATCC 27853	125	125	125	62.5	250
Enterobacter aerogenes	NRRL 3567	125	125	62.5	62.5	125
Proteus vulgaris	NRRL 123	125	125	62.5	62.5	31.25
Salmonella typhimurium	NRRL 4420	62.5	125	62.5	62.5	62.5
Candida albicans	OGU	62.5	62.5	31.25	62.5	125*
Aspergillus niger	BUB Czp. 30	–	–	–	–	+*
Penicillium sublateritium	BUB Czp. 69	–	–	–	–	+*
Penicillium canescens	BUB Czp. 38	+	+	+	+	+*
Penicillium steckii	BUB Czp. 28	–	–	–	–	+*

A: *S. boissieri*
B: *S. coerulea*
C: *S. pilosa*
D: *S. icarica*

St: Chloramphenicol
* Ketoconazole
(–): Spore germination
(+): Spore inhibition

When the fungal spore inhibition assay was applied to the oils, observation during the three-day incubation period showed that *Penicillium canescens* spores were strongly inhibited, while germination of *Aspergillus niger, Penicillium steckii* and *P. sublateritium* were not inhibited by the tested samples.

In conclusion, the essential oil compositions when compared to previous studies (Tümen *et al.*, 1998a,b) have been confirmed. In addition, it is evident that these antimicrobial activities do not result only from monoterpenes such as carvacrol and thymol as reported in the previous work (Başer *et al.*, 2001) but also *S. coerulea* essential oil rich in sesquiterpenes, which also displayed activity. It may be worthwhile to investigate the individual components in antibacterial and antifungal assays.

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