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GC-MS Analysis and Antibacterial Activity of Cultivated *Satureja cuneifolia* Ten. Essential Oil

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The composition of the essential oil of *Satureja cuneifolia* Ten. cultivated in Konya, Turkey, was investigated by capillary GC-MS. The compounds were characterized by comparison with library searches. Six main compounds were identified. Carvacrol was the dominant component, comprising 59.28% of the essential oil. The oil also contained 15.72% thymol, 9.69% p-cymene, 4.16% γ-terpinene, 1.70% linalool and 1.25% borneol.

The antibacterial activity of the essential oil of *S. cuneifolia* and its components was determined by a semiquantative disc-diffusion method, and the minimum inhibitory concentration (MIC) was determined based on a micro-well dilution method against strains of *Pseudomonas aeruginosa, Bacillus cereus, Sarcina lutea, Escherichia coli*, and *Staphylococcus aureus*.

Key Words: *Satureja cuneifolia*, Lamiaceae, GC, Essential oil, Antibacterial activity.

Introduction

Satureja cuneifolia Ten. is known as "Dağ Kekiği" in Turkey and commonly used as a spice in Turkish cuisine[1]. It is a well-known aromatic plant used to produce essential oil and aromatic water in the mountain regions of the Aegean and Mediterranean parts of Turkey[2]. The genus *Satureja* belongs to the family *Lamiaceae,* sub-family *Nepetoideae,* and the tribe *Mentheae*. The genus embraces over 30 species whose center of distribution is located in the eastern part of the Mediterranean. These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky habitats[3]. The genus *Satureja* is represented in Turkey by 15 species, 5 of which are endemic[4]. Most aromatic plants belonging to the family *Lamiaceae,* such as *Satureja, Sideritis, Salvia, Origanum* and *Thymus*, are used as herbal teas in Turkey[5].

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The chemical composition of the essential oils has been noted to depend on climatic, seasonal, geographic and soil conditions; harvest period; and distillation technique. The antibacterial activity of the essential oils is dependent on the composition and concentration of the essential oil and the type and concentration of the target microorganism[6].

The aim of the present study was to investigate the antibacterial activity of the essential oil of *Satureja cuneifolia* Ten. by a semiquantitative disc-diffusion method and its chemical composition by GC and GC/MS.

Experimental

Materials

Samples of wild savory (*Satureja cuneifolia* Ten.) were collected during the flowering period in the Konya region at 1200 m altitude. The plant samples were identified by Prof. Dr. H. Duman. The specimens were left to grow in a field of 3% organic content under controlled conditions by irrigating once a week, fertilizing once with 0.4 t per hectare of organic fertilizer (sheep feces incubated for 1 year). During the growth period (April-October) of the plant, the average values for temperature, humidity and rain in the region were as follows: 16 °C, 52%, and 112 mm, respectively. The voucher specimens are kept at the Herbarium of Ankara University, Faculty of Pharmacy, Ankara, Turkey (AEF 22848-22849).

Essential oil distillation

Aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil (yield 1.70%).

Chemicals

Thymol (Sigma; T-0501), carvacrol (Aldrich; 28,219-7), and p-cymen (Roth; 5282) were used as reference compounds for identification and MIC determinations.

Chromatographic conditions

Gas chromatographic analysis was carried out on a Varian-Chrompack CP-3800 coupled to FID and Varian-Chrompack Saturn 2000 MS under electron impact ionization (70 eV). The MS scan range was 30-200 atomic mass units (AMU). The chromatographic column for the analysis was a fused silica WCOT-Fused Silica capillary column (30 m x 0.25 mm i.d.; CP-Sil 5CP, 025 μm). The carrier gas used was helium at a flow rate of 0.7 mL/min. Samples were analyzed with the column held initially at 100 °C for 3 min and then increased to 150 °C with a 8 °C/min heating ramp and then kept at 150 °C for 3 min. Finally, temperature was increased to 250 °C with a 15 °C/min heating ramp and kept at 250 °C for 3 min. The injection was performed in split mode (50:1) at 250 °C. The identification of the oil components was based on the Wiley and Nist mass spectral library.

Reference bacteria

Standard strains of *Sarcina lutea ATCC 9341, Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 29998 were used. The strains were

obtained from Selçuk University, Faculty of Veterinary Medicine.

Determination of antibacterial activity by semiquantative disc diffusion

A semiquantative disc-diffusion method was used to determine the antibacterial activity[7]. The essential oil from *S. cuneifolia* Ten. and the pure components were prepared with 70% ethanol to the test concentrations (0.1%, 0.3%, 0.5%, 1%, 2%, 5%, and 10%) and adsorbed onto the discs (50 μL) and the same volume (50 μL) of 70% ethanol was used as a control. Gentamycin was employed as a positive control. The minimum inhibitory concentration (MIC) of *S. cuneifolia* Ten. and its components carvacrol, thymol and p-cymene was determined for the individual strains of *P. aeruginosa, B. cereus, S. lutea, E. coli*, and *S. aureus* by disc diffusion on Mueller-Hinton Agar (MH, Oxoid). Overnight cultures of these strains were prepared in Tripticase Soy Broth (TSB; Oxoid) and adjusted to the McFarland No. 0.5 barium sulfate tube and used to inoculate Mueller-Hinton Agar (MHA) plates. Then 0.1 mL of inoculum was immediately and evenly distributed with a Drigalski spatula. Discs impregnated with different diluted amounts of the essential oil were placed on the surface of these plates and incubated at 37 °C for 18 h. The solvent control (ethanol) did not show any antibacterial activity. The radius of the zone of growth inhibition around the discs was measured with a ruler. The results were quoted after subtracting the radius of the disc. All antibacterial tests were performed in triplicate.

Preparation of inoculum

All the microorganisms were cultured overnight at 37 °C in trypticase soy broth (Oxoid) and used as inoculum. The turbidity of the suspensions was adjusted to the McFarland 0.5 turbidity standard.

Micro-well Dilution Assay

The inocula of the bacterial strains were prepared from 18 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils were diluted in 70% ethanol to the highest concentration (500 μg/mL) to be tested. Then serial 2-fold dilutions were made in a concentration range from 7.8 to 500 μg/mL in nutrient broth. MIC values of the essential oils and pure compounds against the strains were determined twice based on a micro-well dilution method with some minor modifications as previously described[8]. In brief, the wells of 96-well plates were dispensed with 95 μL of nutrient broth and 5 μL of the inocula. A 100 μL aliquot from the stock solutions of the essential oil initially prepared at the concentration of 500 μg/mL was added to the first wells. Then 100 μL from their serial dilutions was transferred into 6 consecutive wells. The last well containing nutrient broth without compound and the same amount of inocula with previous wells on each strip was used as a negative control. The final volume in each well was 200 μL. Gentamycin (Sigma, G-1397) at the concentration range of 500-7.8 μg/mL was used as a positive control. The plate was incubated at 37 °C for 24 h. Microbial growth was determined by plating 5 μL samples from the last and first 3 consecutive wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Results and Discussion

The antibacterial activity of the essential oil from the aerial parts of *S. cuneifolia* Ten. was examined against microorganisms in the present study. The results (Table 1) showed that activity against several Gram positive (*S. aureus, B. cereus, S. lutea*) and Gram negative (*P. aeruginosa, E. coli*) bacteria was observed with essential oil from the aerial parts of *S. cuneifolia*.

The semiquantative disc-diffusion method is easy to perform, and is useful to detect the activity of antimicrobial agents on large numbers of cultures. It also gives an opportunity to evaluate the antibacterial activity to some extent without performing a MIC assay. The in vitro antibacterial activities of the essential oil from *Satureja* species have recently been evaluated against a panel of some foodborne bacteria[9]. The results from our study were comparable to those reported by Chorianopoulos et al. However, we also tested a strain of *Pseudomanas aeruginosa*, which is known to possess a degree of intrinsic resistance to some antibacterial agents. Essential oil of *S. cuneifolia* has antibacterial activity to *Ps. aeruginosa* by a MIC of 31.2 μg/mL. This suggests that the essential oil of *S. cuneifolia* represents a useful source of natural mixtures of antibacterial compounds that may exhibit potential for use in medicine and veterinary medicine.

The essential oil composition of different *Satureja* species growing in Turkey showed variation. The main components of *S. boissieri* were reported to be carvacrol (40.8%) and γ-terpinene (26.4%)[5].

The essential oil components are given in Table 2. The compounds were characterized by mass spectral interpretation and comparison with library searches. The 6 main compounds were p-cymene, γ-terpinene, linalool, borneol, carvacrol, and thymol. Carvacrol was the dominant component, making up 59.28% of the essential oil. The oil also contained 15.72% thymol, 9.69% p-cymene, 4.16% γ-terpinene, 1.70% linalool and 1.25% borneol.

Detailed studies of the essential oils of *S. cuneifolia* originating from Turkey have been carried out. The majority of essential oil samples were rich in carvacrol (26% -72%), while thymol (22% -58%) was the main constituent in other oil samples [2].

Analysis of the essential oil components of some *Satureja* species has previously been performed[4,10−12]. Azaz et al. have reported that carvacrol was the main component in the oils of *S. icarica, S. boissieri, S. pilosa, S. macrantha, S. cuneifolia,* and *S. thymbra* (59.2%, 44.8%, 42.1%, 64.4%, 48.7%, and 39.0% respectively)[4,13]. Our study revealed that the ratio of carvacrol in *S. cuneifolia* was similar to that previously reported (59.28%). However, carvacrol is not necessarily the major component of other species like *S. hortensis* as it has been reported that thymol (43.4%) was the main component and higher than carvacrol (40.6%). P-cymene was reported as the main constituent of *S. aintabensis*[13]. Germacrene D has also been detected as the main compound of *S. coerulea* oil[10]. However, in another paper by the same authors, beta-caryophyllene (10.6%) and caryophyllene oxide (8.0%) were determined to be the main components in the oil of *S. coerulea*[4].

Our results are in accordance with the reports on carvacrol and thymol content in the essential oil of *S. cuneifolia*. However, total amounts of oxygen-containing phenolic monoterpenes carvacrol and thymol were higher than previous results[2,6]. The essential oils of Croatian origin *S. cuneifolia* contained low percentages of thymol and carvacrol but they were relatively rich in γ-terpinene and p-cymene[14].

The antibacterial activity of *S. parnassica* subsp. *parnassica* essential oil has been attributed to a considerable degree to the existence of linalool and carvacrol[15]. Previous studies showed that the antibacterial

Table 1. Antibacterial activity of *Saturea cuneifolia* Ten. essential oil against the bacteria strains tested.

Strains		MIC*	Concentration w/v (%)**							
			0.3	0.5	1	2	5	10	G	G_{MIC}
P. aeruginosa ATCC 27853	EO	31.2	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	2.4 ± 0.2	3.8 ± 0.4	11 ± 0.8	16 ± 0.8	125
	Car	62.5	0.9 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.9 ± 0.2	2.1 ± 0.3	5.4 ± 1.2		
	Thy	125	0	0	0	0	0.7 ± 0.2	3.9 ± 1.5		
	P-cy	125	0	0	0	0	0.5 ± 0.2	1.2 ± 0.3		
B. cereus ATCC 11778	EO	125	1.5 ± 0.2	1.8 ± 0.2	1.9 ± 0.1	2.9 ± 0.3	4.4 ± 0.7	12 ± 1.7	18 ± 0.6	7.8
	Car	125	0.6 ± 0.1	1.1 ± 0.3	1.1 ± 0.2	1.9 ± 0.2	2.1 ± 0.3	5.4 ± 1.2		
	Thy	250	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.4	1.3 ± 0.5	3.9 ± 0.7		
	P-cy	250	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.6 ± 0.4		
E. coli ATCC 29998	EO	62.5	0	0	1.2 ± 0.1	1.9 ± 0.9	2.4 ± 1.3	6.3 ± 2.7	16 ± 0.6	15.6
	Car	62.5	0	0	0.3 ± 0.1	0.9 ± 0.8	2.5 ± 0.8	6.0 ± 2.9		
	Thy	250	0	0	0	04 ± 0.2	0.9 ± 0.2	1.5 ± 0.5		
	P-cy	500	0	0	0.2 ± 0.1	0.3 ± 0.3	1.1 ± 0.7	1.3 ± 1.1		
S. aureus ATCC 6538	EO	15.6	0	0	0	2.9 ± 1.1	2.8 ± 1.7	7.8 ± 3.2	21 ± 0.6	31.2
	Car	62.5	0	0	0	0.9 ± 0.1	1.2 ± 1.1	3.2 ± 2.8		
	Thy	125	0	0	0	14 ± 1.2	1.8 ± 1.3	4.8 ± 3.1		
	P-cy	500	0	0	0	0	0.4 ± 0.2	0.8 ± 0.1		
S. lutea ATCC 9341	EO	7.8	0.2 ± 0.1	0.3 ± 0.2	1.2 ± 0.1	2.2 ± 0.2	4.8 ± 0.4	7.3 ± 2.8	21 ± 0.2	3.9
	Car	15.6	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	1.7 ± 0.1	2.9 ± 0.7	4.3 ± 1.2		
	Thy	15.6	0.2 ± 0.1	0.2 ± 0.3	0.4 ± 0.2	0.2 ± 02	1.8 ± 0.5	3.3 ± 1.0		
	P-cy	31.2	0	0.1 ± 0.1	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4	1.7 ± 1.9		

EO: Essential oil of *Satureja cuneifolia*; **Car:** Carvacrol, **Thy:** Thymol, **P-cy:** P-cymen,
G: Gentamycin
**: Inhibition zone in diameter (mm) around the discs.
*: Minimal Inhibitory concentrations (as μg/mL).

properties of the essential oils rich in phenolic compounds, such as carvacrol, are widely reported to possess high levels of antimicrobial activity[6,16−19].

Table 2. The main components of essential oil of *Satureja cuneifolia* Ten.

Compounds	Concentration %
p-Cymene	9.69 ± 0.49
$\gamma-$ Terpinene	4.16 ± 0.22
Linalool	1.70 ± 0.14
Borneol	1.25 ± 0.28
Carvacrol	59.28 ± 1.88
Thymol	15.72 ± 0.50

Essential oils' components are used as flavoring agents in the food industry; they nowadays represent a highly interesting source of natural antimicrobials for food preservation due to their antimicrobial and antioxidative activity. Carvacrol is a phenolic compound present in the essential oil fraction of both oregano and thyme and is Generally Recognized as Safe (GRAS) for flavoring. It has been shown to exhibit antibacterial and antifungal activities and its primary site of toxicity is the membrane and leakage of vital intracellular constituents[20].

It is observed that essential oil of cultivated *S. cuneifolia* is rich in phenolic compounds possessing antimicrobial activity. It can be used as a natural preservative ingredient in the food and pharmaceutical industries.

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