Antimicrobial Activity, Essential Oil Composition and Micromorphology of Trichomes of *Satureja laxiflora* C. Koch from Iran

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The antimicrobial activity, essential oil composition and micromorphology of trichomes of *Satureja laxiflora* C. Koch, a native plant from Iran, were studied. The essential oil was obtained from the aerial parts at the flowering stage by hydrodistillation, and analyzed by GC and GC/MS. Thirty-three compounds representing 99.1% of the total oil were characterized. The major compounds were thymol (63.9%) and γ-terpinene (11.9%) followed by carvacrol (4.8%), *p*-cymene (3.9%), geraniol (3.2%) and geranyl acetate (3.1%). Furthermore, the essential oil and its three main components were tested against two bacteria and three fungi. The result of the bioassays has been shown that the oil possesses potent antimicrobial property. Chemical studies confirmed that a major portion of this antimicrobial activity is due to thymol present in the oil. Micromorphological analysis by SEM of both vegetative and reproductive organs revealed the presence of abundant sessile capitate and sparse short-stalked glandular trichomes along with retrorse eglandular hairs, giving useful diagnostic characters for identification of this medicinal plant.

Key words: Satureja laxiflora, Micromorphology, Antimicrobial Activity of Oil

Introduction

The genus *Satureja* L. (Lamiaceae) comprises more than 200 species of aromatic herbs and shrubs, widely distributed in the Mediterranean region. This genus in flora of Iran is represented by 12 species distributed commonly in rocky mountains (Rechinger, 1982; Jamzad, 1992, 1994, 1996). *S. laxiflora* C. Koch with the common Persian name Marzeh is an annual aromatic plant native to Iran (Mozaffarian, 1996). Its strong aromatic odour is due to the presence of volatile oils especially thymol. The essential oils composition and antimicrobial activities of some *Satureja* species have been studied (Capone *et al.*, 1989; Deans and Svoboda, 1989; Tumen *et al.*, 1998; Sefidkon and Jamzad, 2000; Sefidkon and Ahmadi, 2002; Ciani *et al.*, 2000; Ghannadi, 2002; Sajjadi and Baluchi, 2002; Tzakou and Skaltsa, 2003; Goren *et al.*, 2004). Aerial parts of *S. laxiflora* and other species of *Satureja* are used widely as a flavouring agent for much kind of food products and also as a traditional herbal medicine for the treatment of gastrointestinal disorders. The preparation made with the essential oil of *S. khuzistanica* Jamzad is known as "dentol drop" and used in herbal medicine of Iran for healing of the toothache. As part of our study on the characterization of Iranian aromatic medicinal plants, here essential oil composition, antimicrobial activity of the oil and its three major constituents and micromorphology of trichomes of *S. laxiflora* are reported.

Material and Methods

Plant material

The aerial parts of *Satureja laxiflora* were collected in July 2003, from the Arasbaran protected area, at an altitude of 1900 m, Ardabil, Iran. A voucher specimen (No. 200360) was deposited in the Medicinal Plants Research Institute Herbarium (MPRIH), Shahid Beheshti University, Tehran, Iran.

Isolation procedure

Air-dried aerial parts (25 g) were subjected to hydrodistillation for 2 h, using a Clevenger-type

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apparatus. The distillate was dried over anhydrous sodium sulphate and stored in a sealed vial at low temperature until analysis.

Gas chromatography (GC)

GC analysis of the essential oil was performed using a Varian CP-3800 instrument equipped with a DB-1 capillary fused silica column (25 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 4 °C/min, then held for 10 min. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively.

Gas chromatography/mass spectrometry (GC/MS)

GC/MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min; transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; split ratio was 1/50. The quadrupole mass spectrometer was scanned over the 45–465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of components

The constituents of the oil were identified by calculation of retention indices under temperature programmed conditions for *n*-alkanes (C_6–C_{24}), and the essential oil on the same chromatographic conditions (60 °C–250 °C at 5 °C/min) and comparison of their retention indices and mass spectra with authentic compounds or with data published in the literature (Adams, 2001; Shibamoto, 1987).

Antimicrobial activity

Antimicrobial activities of the oil and its three major compounds were evaluated by a disc diffusion method using Mueller-Hinton agar for bacteria and by the Agar-well diffusion method using Sabouraud Dextrose Agar for fungi at different oil volumes of 0.3, 0.6, 1.2 and 2.4 μl with determination of inhibition zones. The 5 microbial strains used as test organisms were as follows: *Candida albicans* ATCC 5027, *Aspergillus niger* ATCC 16404, *Saccharomyces cerevisiae* ATCC 9763, *Klebsiella pneumoniae* ATCC 3583 and *Enterococcus faecalis* ATCC 15753.

Standard reference antibiotics were used in order to control the sensitivity of the tested bacteria (ampicillin) and fungi (nystatine). The incubation conditions used were 24 h at 37 °C for bacteria and 48–72 h at 24 °C for fungi. All the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

Scanning electron microscopy (SEM)

For observation by scanning electron microscopy fragments of leaves, stem and flower were mounted on stubs and sputter-coated with gold then examined with a JEOL JXA-840 scanning electron microscope at 10 kV. Detailed technical data has been described by Cavaleiro *et al.* (2002).

Results and Discussion

Essential oil analysis

Essential oil of *S. laxiflora* was obtained by hydrodistillation using a Clevenger-type apparatus and analyzed with GC and GC/MS. The yield of the oil was 2.0% and the oil was yellow in colour. Thirty-three compounds were identified, representing 99.1% of the total oil. The qualitative and quantitative compositions are presented in Table I, where compounds are listed in order of their elution on the DB-1 column. The oil was characterized by a high concentration of oxygenated monoterpenes (76.3%) of which thymol (63.9%) being the major compound followed by carvacrol (4.8%) and geraniol (3.2%). Monoterpene hydrocarbons constituted 18.1% of the oil and γ-terpinene (11.9%) and *p*-cymene (3.9%) were the main compounds of this fraction. Sesquiterpenoids comprised 4.7% of the total oil. A literature survey on the oils of *Satureja* species from Iran revealed that three chemotypes (thymol, carvacrol and *p*-cymene) can be recognized. The oil of *S. laxiflora* that contained thymol as well as its biogenetic precursors, γ-terpinene and *p*-cymene, can be included in the thymol chemotype.

Antimicrobial activity

Antimicrobial activity by disc diffusion method showed that the oil of *S. laxiflora* was active against all tested microorganisms. Table II shows the *in vitro* antimicrobial property of the essential

Table I. Constituents of the essential oil of *S. laxiflora*.

Compounds	RI[a]	%	Identification method[b]
Tricyclene	0926	0.2	RI, MS
α-Pinene	0934	0.1	RI, MS, CoI
Camphene	0948	tr	RI, MS
Sabinene	0967	tr	RI, MS
β-Pinene	0974	0.1	RI, MS, CoI
Myrcene	0980	0.6	RI, MS
α-Phellandrene	0999	0.1	RI, MS
3-Carene	1007	tr	RI, MS
α-Terpinene	1013	0.9	RI, MS
p-Cymene	1015	3.9	RI, MS, CoI
Limonene	1024	0.1	RI, MS
(*Z*)-β-Ocimene	1035	0.1	RI, MS
γ-Terpinene	1053	11.9	RI, MS, CoI
trans-Sabinene hydrate	1059	0.5	RI, MS
Terpinolene	1081	0.1	RI, MS
Linalool	1083	tr	RI, MS, CoI
Z-Tageton	1131	0.1	RI, MS
Borneol	1155	0.2	RI, MS
4-Terpineol	1166	0.4	RI, MS
α-Terpineol	1176	0.1	RI, MS
Geraniol	1236	3.2	RI, MS
Thymol	1271	63.9	RI, MS, CoI
Carvacrol	1277	4.8	RI, MS
δ-Elemene	1336	0.1	RI, MS
Geranyl acetate	1355	3.1	RI, MS
β-Caryophyllene	1424	1.8	RI, MS
(*Z*)-β-Farnesene	1443	0.2	RI, MS
α-Humulene	1456	0.1	RI, MS
Germacrene-D	1480	0.2	RI, MS
Germacrene-B	1495	1.4	RI, MS
β-Bisabolene	1499	0.2	RI, MS
Spathulenol	1570	0.6	RI, MS
β-Eudesmol	1628	0.1	RI, MS
Monoterpene hydrocarbons		18.1	
Oxygenated monoterpenes		76.3	
Sesquiterpene hydrocarbons		4	
Oxygenated sesquiterpenes		0.7	
Total identified		99.1	

[a] RI, retention index relative to C_6–C_{24} *n*-alkanes on the apolar DB-1.
[b] MS, mass spectrum; Co I, co-injection with an authentic sample.
tr, trace (< 0.1%).

Table II. Antimicrobial activity of the essential oil of *S. laxiflora* and its three major compounds.

Microorganism	Inhibition zone [mm][a]								
	Oil volume [μl]				Major constituents			Standard antibiotics	
	0.3	0.6	1.2	2.4	Thymol (10 μl)	*p*-Cymene (10 μl)	γ-Terpinene (10 μl)	Ampicillin[b]	Nystatine[c]
Enterococcus faecalis	10	17	22	28	24	8	–	12	nt
Klebsiella pneumoniae	8	10	14	17	20	–	–	–	nt
Candida albicans	–	9	12	16	36	9	–	nt	18
Saccharomyces cerevisiae	–	9	14	18	40	–	–	nt	18
Aspergillus niger	–	8	11	15	38	–	–	nt	16

[a] Includes diameter of disc (6 mm).
[b] Tested at 10 μg/disc.
[c] Tested at 30 μg/disc.
(–), Inactive; (7–14), moderately active; (> 14), highly active; nt, not tested.

oil of *S. laxiflora* and its three main compounds and the inhibition zones formed by standard reference antibiotic discs. The oil at all volumes showed potent inhibitory activity against the tested microorganisms. The Gram-(+) bacterium *Enterococcus faecalis* was found to be more sensitive to the oil than the Gram-(–) bacterium *Klebsiella pneumoniae*. The oil at 0.3 μl was inactive against three tested fungi, but at 0.6 and 1.2 μl showed moderate activity. The growths of tested fungi at 2.4 μl were highly inhibited, then it was considered that these organisms were sensitive to the oil. The antimicrobial property of the oil may be associated with the contribution of the monoterpene thymol (63.9%), which has been examined previously and was found to have a significant antimicrobial activity (Salgueiro *et al.*, 2003). From this study it is clear that the antifungal activity of *S. laxiflora* oil at high volume (2.4 μl) mainly is similar to that of the standard antibiotic, nystatine. In comparison, the higher volume of the oil indicated a potent inhibitory property against the tested bacteria than the positive control, ampicillin. The essential oil composition and the observed antimicrobial properties show that the oil has a good potential for use in aromatherapy and pharmacy, and support the popular uses of this plant in the folk medicine of Iran.

Micromorphology

The vegetative (leaf and stem) and reproductive (flower) organs of *S. laxiflora* are covered by an indumentum containing both eglandular and glandular trichomes (Fig. 1). The eglandular ones are simple, needle-shaped, uniseriate, uni-multicellular (up to 4-celled), retrorse and have a cuticle with papillae (Fig. 1a, d). On the outer surface of calyx eglandular hairs are erect to patent (Fig. 1b). On the outer surface of petal the eglandular hairs are of two types: 1) 2-celled that the terminal cell is flattened and 2) 3-celled that the middle cell is flattened (Fig. 1c). In this study we gave most emphasis to the type and distribution of glandular trichomes secreting essential oils. The glandular hairs are more abundant on the abaxial leaf surface and calyx, whilst these trichomes on the adaxial leaf surface, stem and corolla are sparsely distributed. The glandular ones are of two types: 1) sessile capitate trichomes consisting of a multicellular head covered by a thick cuticle (Fig. 1e), and 2) short-stalked capitates occuring only on the outer surface of calyx (Fig. 1f). The micromorphological analysis of the trichomes gave useful diagnostic characters for identification of this medicinal plant.

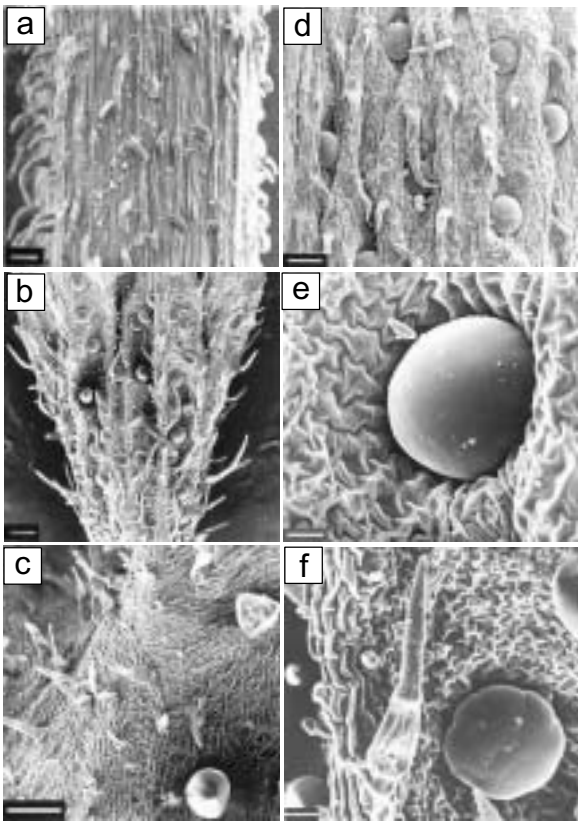

Fig. 1. Micrographs (SEM) of leaf, stem and flower of *Satureja laxiflora*. Scale bars in a–d = 100 μm, e, f = 25 μm. a) Retrorse eglandular hairs on the stem; b) outer surface of calyx covered with glandular and eglandular trichomes; c) eglandular and glandular hairs on the outer surface of petal; d) trichomes on the abaxial leaf surface covered with glandular and retrorse eglandular; e) capitate sessile glandular trichome on the abaxial leaf surface; f) two types of glandular hairs and a eglandular hair on the calyx.

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