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COMPARISON OF SOME *SATURJA* SPECIES BY PHYLOGENETIC AND CHEMOTAXONOMIC ANALYSIS

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ABSTRACT

In this study, the phylogenic relationship between five species of *Satureja* was investigated by RAPD-PCR. Genetic distance was calculated in order to construct phylogenic dendrograms of closely related samples. Results indicate that *S atropatana* has the closest relationship with two samples of *S. mutica* and also *S. spicigera*. Phylogenetic distances show that *S. macrantha* not only is far from *S. mutica* and *S.spicigera* but also, has different patterns of RAPD-PCR compare to others. The taxonomical classification, supported by GC and GC/ MS analysis of the leaf components, show that thymol is the major compound in the oils of *S mutica, S. atropatana* and *S. spicigera.* The oils of these three species are enriched of monoterpenes (more than 70%) compare to sesquiterpenes and a high amount of oxygenated compounds. The main component in the volatile oil of *S. macrantha* is a sesquiterpene, spathulenol.

Key-words: *Satureja atropatana, S. macrantha, S. mutica, S. spicigera*, RAPD, Chemotaxonomy

INTRODUCTION

Satureja genus belongs to Labiatae family and comprises 13 species in Iran. Some of them like *S.atropatana* and *S. khuzistanica* excursively grow in Iran and others also grow in Iraq, Turkemania, and Anatoly (Mozaffarian, 1996). *Satureja parvifalia, S.odora* and *S. macrantha* contain potent cytotoxic and antitumor agents (Mongelli *et al.*, 1996; Gohari *et al.*, 2006a). Some species, like *S.obovata*, contain flavonoids such as luteolin, naringin. Antifungal effect of the essential oil of *S.montana* is reported (Yamazaki *et al.*, 1998; Perrucci *et al.*, 1994). Also antidiarrhea, antispasmodic, antioxidative and pesticide activity of *S.hortansis* has been reported (Hajhashemi *et al.*, 2000; Madsen *et al.*, 1998). The essential oil of several species of the genus *Satureja* have been examined, exp. *S. cuneifolia, S. montana, S. pilosa, S.parvifolia* and include thymol, carvacrol, *p*-cymene, *γ*-terpinene (Tumen *et al.*, 1998a; Kustrak *et al.*, 1996; Tumen *et al.*, 1998b; Muschietti *et al.*, 1996). Previous studies have shown that not only the major compound in many species of *Satureja* is thymol but also monoterpenes presented in a higher proportion than sesquiterpenes. Among the *Satureja* species, *S. coerulea* showed sesquiterpenes as the dominant fraction (Duke *et al.*, 2001).

Literature reviews show that there is no report to study genetic relationship between *Satureja* species. Here, the phylogenic relationship between five species of *Satureja* has been investigated by RAPD-PCR for the first time and the taxonomical classification supported using GC and GC/ MS analysis of the volatile oils.

MATERIALS AND METHODS

Plant material

Plant materials (leaves), used in this study, are shown in table.1 along with their origin. Voucher specimens of these plants are all deposited at the Herbarium of the Institute of Forests and Rangelands Researches. Plant specimens were identified by Dr. Vali-allah Mozaffarian from the same institute.

Total DNA Extraction

Dried leaves of species were used for total DNA extraction. Plant samples grind to fine powder and followed by DNA extraction was carried out by GMO DNA extraction Kit (Bioneer, Cat No.K3031) according to the manufacturer's instruction.

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RAPD Analysis

In order to use single primers for DNA amplification, twenty 10-mer oligonucleotides (Alpha DNA, Canada) were applied for PCR (table 2).

Amplification mixtures contained 2.5µl buffer, 0.5 µl dNTP mixture (10mM of each dATP, dTTP, dCTP and dGTP), 0.25 µl Supertaq DNA polymerase (AB gene), 40ng template DNA and ddH_2O up to 25 µl.

The PCR program was: 94°C (20") 36°C + 0.1°C /sec (4'), 72°C (3') for 30 cycles in a Primus thermal cycler. PCR products were analyzed on 1% agarose gel and the number and RF of bands were determined.

To trace the matrix and calculate the genetic distance between *Satureia* species, single matching coefficient (Ssm) was calculated for each pair of samples, based on the presence or absence of the unique and shared fragments. UPGMA cluster was analyzed by using this matrix (Fig1) (Sneath *et al.*, 1973).

Detection of the main volatile components

Leaves of the plants (30 g) were dried at room temperature, cut into small pieces and hydro-distilled using a Clevenger-type apparatus for 5 hr. The oils were dried over anhydrous sodium sulfate and stored at 4-6°C.

FID-GC was carried out using a Varian GC 3600 chromatograph with DB-5 (methyl phenyl siloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness) or DB-1 (60 m × 0.25 mm i.d., 0.25 µm film thickness); carrier gas, He; split ratio, 1:15, and flame ionization detector. Temperature programming was performed from 60º C (2 min) to 240°C at 5º C/min, injector temperature 250°C and detector temperature 260º C.

GC-MS was performed on a cross-linked 5% methyl phenyl siloxane (HP-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness) or DB-1 (see GC): carrier gas, He; split ratio, 1:15; quadruple mass spectrometer (Hewlett-Packard 5973) operating at 70 eV ionization energy. The retention indices for all the components were calculated by using retention times of n-alkanes (C8-C25) that were injected after the essential oil at the same temperature and conditions. The components were identified by comparison of retention indices (RI, DB-5 or DB-1) with those reported in the literatures and also by comparison of their mass spectra with the published mass spectra or Wiley library. The percentage of each component was calculated on the basis of the peak area.

RESULTS AND DISCUSSION

Simple matching coefficient (Ssm) and genetic distance (d), derived from RAPD banding patterns using 20 primers, are shown in Table 3. The genetic distance between the two samples of *S.mutica* (from Golestan) and *S.spicigera* was considered to be short (0.780) and their RAPD banding patterns were quite similar to each other also there is a close relationship between two samples of *S. mutica* (harvested from Gilan) and *S. spicigera* (0.817). The cladogram constructed on the bases of the genetic distances derived from RAPD analysis is shown in Fig 1. Clustering analysis was based on UPGMA (unweighed pair- group method with arithmetical averages).

In this cladogram *S atropatana* has the closest relationship with two samples of *S. mutica* and also *S. spicigera*. Phylogenetic distances show that *S. macrantha* not only is far from *S. mutica* and *S.spicigera* but also, has different patterns of RAPD-PCR compare to others. Comparison of the main components in the volatile oils of *Satureja* species, derived from GC/ MS analysis, summarizes in Table 4. Results indicate that thymol is the major compound in the oils of *S mutica* (56.9%-62.6%) and *S. atropatana* (62.1%). Also, the essential oil of *S. spicigera* includes the high amount of thymol. The oils of these three species are enriched of monoterpenes (more than 70%) compare to sesquiterpenes. These oils have a high amount of oxygenated compounds. *Satureja macrantha* show some differences for the structures and amounts of the main compounds in the oil which contains higher amount of sesquiterpenes. Although, thymol (4.2%) can be detected in the volatile oil of *S. macrantha*, the main component is a sesquiterpene, spathulenol. Literature review shows that monoterpenes presented in a higher proportion than sesquiterpenes in the oils of other *Satureja* plants. Among *Satureja* plants only *S. coerulea* indicated sesquiterpenes as a dominant fraction (Gohari *et al.*, 2006b; Muschietti *et al.*, 1996; Tumen *et al.*, 1996; Senatore *et al.*, 1998).

In conclusion, molecular biological assay show *S. mutica* and *S. Spicigera* are very similar to each other phylogenetically and *S. macrantha* has a far genetic distance from other species of *Satureja*, mentioned in this paper. Accordingly, the chemotaxonomic investigation on the volatile components of those plants supports the proposed hypothesis that *S. macrantha* has not close relationship to other Iranian species of *Satureja*.

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Table 1. Different species of *Satureja*, utilized in RAPD-PCR, harvested in various parts of Iran.

Species of *Satureja*	**Origin**
S.mutica Fisch et al Mey	Gilan and Golestan
S.atropatana Burge	Tabriz
S.macrantha C.A.Mey	Eurumia
S.spicigera (C.koch)Bioss	Heyran

Table 2. Oligonucleotids used as 10-mer primers in PCR.

code	**5' to 3'**	**code**	**5' to 3'**
Z1	GGTCGGAGAA	Z2	TCGGACGTGA
Z3	AGACGTCCAC	Z4	GGAAGTCGCC
Z5	AGTCGTCCCC	Z6	CTGCATCGTG
Z7	GAAACACCCC	Z8	TGTAGCTGGG
Z9	ACGCGCATGT	Z10	GACGCCACAC
Z11	ACCAGGTTGG	Z12	AATGGCGCAG
Z13	CACTCTCCTC	Z14	GAATCGGCCA
Z15	CTGACCAGCC	Z16	GGGAGACATC
Z17	ACAACGCGAG	Z18	CCGCCTAGTC
Z19	GGAGGAGAGG	Z20	TCATCCGAGG

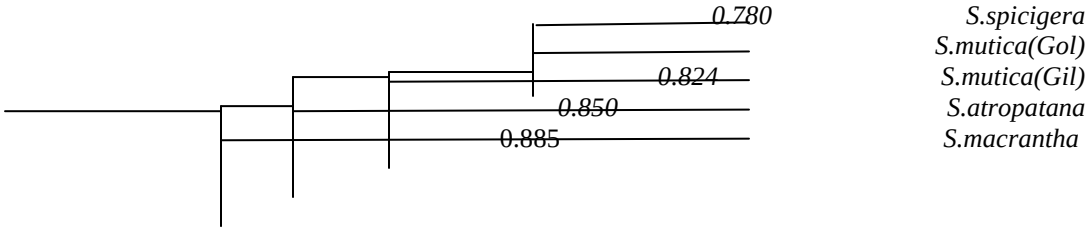
Table 3. simple matching coefficient (Ssm, above the diagonal) and genetic distances (d, below the diagonal) between pairs of *Satureja* plants based on RAPD-PCR.

	S.macrantha	*S.spicigrern*	*S.mutica* (Golestan)	*S.atropatana*	*S.mutica* (Gilan)
S.macrantha	-	0.272	0.2828	0.032	0.264
S.spicigera	0.853	-	0.391	0.277	0.332
S. mutica (Golestan)	0.847	0.780	-	0.290	0.307
S.atropatana	0.984	0.850	0.847	-	0.259
S.mutica (Gilan)	0.858	0.817	0.872	0.862	-

Table 4. Comparative percentage of the main components in the essential oils of *Satureja* species.

Compounds	*S. atropatana*	*S. mutica* (Golestan)	*S. mutica* (Gilan)	*S. macrantha*	*S. spicigera*
Main components	*thymol*	*thymol*	*thymol*	*Spathulenol*	*thymol*
thymol	*62.1%*	*56.9%*	*62.6%*	*4.2%*	*37.3%*
Monoterpene pecentage	*75.9%*	*70.3%*	*83.7%*	*27.2%*	*89.9%*
Sesquiterpene percentage	*10.4%*	*9.7%*	*9.1%*	*29.8%*	*7.3%*
CxHy	*14.8%*	*25.1%*	*21.2%*	*25.4%*	*40.6%*
CxHyOz	*74.8%*	*71.8%*	*77.3%*	*59.2%*	*56.6%*
Total identification	*89.6%*	*96.9%*	*98.5%*	*84.6%*	*97.2%*

Fig 1. Cladogram of genetic relationships between five samples of *Satureja*.



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