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Chemical Compositions and Bacteriological Activity of Essential Oil of *Satureja sahandica Bornm.*

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The volatile constituents of the essential oil of *Satureja sahandica Bornm.* growing wild in Kurdistan, Iran were investigated by GC and GC/MS techniques. Twenty-one compounds, representing 90.5 % of the total oil were identified. The main components were: *p*-cymene (47.81 %), thymol (33.57 %), γ-terpinene (3.41 %), caryophyllene oxide (3.18 %), *p*-cymene-8-ol (1.61 %), linalool (1.51 %), α-pinene (1.33 %), (+)-spathulenol (1.24 %) and borneol (0.98 %). The bacteriological activity of this herb was investigated by the use of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. *Pseudomonas aeroginosa* was chosen as a relevant bacterium.

Key Words: *Satureja sahandica Bornm.*, Gas chromatography, Mass spectroscopy, Essential oil compounds, *Pseudomonas aeroginosa*.

INTRODUCTION

Satureja genus has 14 species in Flura Iranica and 11 species in Iran[1-3]. The local name of *Satureja sahandica Bornm*. is *Jater-e* (JΛter-e). It is utilized as the medicinal herb for the various purposes in local and traditional medicine in Kurdistan, Iran. One of its utility in traditional medicine is removing the food poisoning with positive and quick effect. Additionally, using the herb makes the mouth smells good, also it removes the toothache.

In notice to the medicinal effects of this herb the bacteriological study was investigated by the use of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. In this study,

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Pseudomonas aeroginosa was chosen as a relevant bacterium in according with quality control strains (QCS). These strains (13 strains) were selected from Persian Type Culture Collection (PTCC 1074) and clinical isolated (12 strains) which were identified by standard methods as the *P. aeroginosa*[4,5].

EXPERIMENTAL

The *Satureja sahandica Bornm.* materials of this study were collected from west of Iran (Kurdistan, 15 Km after Bijar to Tekab, protected area, altitude 1900 m above sea level) in August 2004. A voucher specimen has been deposited in herbarium of Research Center of Agriculture & Natural Resources, Sanandaj-Kurdistan, Iran.

GC/Mass method: Dried aerial parts of *Satureja sahandica Bornm.* were subjected to hydrodistillation for 5 h using Clevenger-type apparatus to produce a yellow oil in 0.28 % (w/w) yield. The oil of the aerial parts of *Satureja sahandica Bornm.* was examined by GC/MS (GC:HP 6890, MS:HP 5973), column (HP5-MS, 30 m × 0.25 mm fused silica capillary column, film thickness 0.32 μm) by temperature program 60 °C (3 min)-210 °C (2 min) at the rate of 6 °C/min (injection temperature 250 °C, carrier gas: helium (with purity 99.999 %), detector temperature 150 °C, ionization energy in mass was 70 eV, mass range 10-300 amu and scan time was 1 s.

Minimum inhibitory concentration (MIC) method[4]: (1) Dried *Satureja sahandica* was soaked in acetone for 48 h and then filtered to separate extraction from the plant. The extraction was concentrated by leaving it in the air. The concentrated extraction was diluted in sterile distilled water (60 g/100 mL). 1 mL Mueller-Hinton broth (Merck Co.) was poured in 13 tubes and was added 1 mL from 60 % dilution of extraction to the first tube, then 1 mL from that transferred to the second tube and it continued unlit twelfth tube (range of dilution was from 30 to 0.015 %) and 1 mL from tube 12 was discarded. The thirteenth tube had no extracted, it was control tube (only Mueller-Hinton broth without extraction). (2) The strains were cultured on blood agar to refresh. After 24 h incubation 37 °C, four or five colonies were suspended in 5 mL of normal saline (0.9 % NaCl solution) and were diluted to obtain turbidity equal of MacFarland standard of 0.5 (1.5×10^8 CFU/mL) and then diluted until 5×10^6 (CFU/mL). In this turbidity[4]; sensitivity and specificity of present test were 99 %. To each tubes 5×10^6 CFU/mL were added the bacteria (*P. aeroginosa*) and were incubated for 24 h in 37 ºC. After incubating period we controlled tubes for growth of the bacteria, but as the first three tubes were colourful and it was not possible to assay them for turbidity, so we obliged to culture them directly.

Minimum bactericidal concentration (MBC) method: For performing MBC, the first four tubes were cultured. In this procedure, one loop from

each tube was taken and spread completely on the surface of the blood agar (Merck Co.) plate. Results compared with rejection value[4], in this study the rejection value was 227 CFU/mL.

RESULTS AND DISCUSSION

The components were analyzed by GC and GC/MS and identified by comparing their MS spectra with authentic compounds (Table-1). The identifications were confirmed by comparison of their retention indices either with those of authentic compounds or with data in the literature[6-8]. In the aerial parts of *Satureja sahandica Bornm.,* the major identified components were: *p*-cymene (47.81 %), thymol (33.57 %), γ-terpinene (3.41 %), caryophyllene oxide (3.18 %), *p*-cymene-8-ol (1.61 %), linalool (1.51 %), α-pinene (1.33 %), (+)-spathulenol (1.24 %) and borneol (0.98 %).

TABLE-1
ESSENTIAL OIL CONSTITUENTS OF
Satureja sahandica Bornm.

No.	Name of compounds	Scan	K.I.*	RT**	%
1	α-Thujene	160	924.08	5.45	0.22
2	α-Pinene	180	931.02	5.62	1.33
3	Camphene	222	945.71	5.98	0.44
4	β-Pinene	303	973.88	6.67	0.17
5	Myrcene	343	988.16	7.02	0.54
6	α-Terpinene	423	1014.79	7.69	0.62
7	*para*-Cymene	464	1028.79	8.05	47.81
8	γ-Terpinene	551	1057.59	8.79	3.14
9	1-Methyl-4-(1-methylethyl)benzene	643	1088.33	9.58	0.78
10	Linalool	677	1099.61	9.87	1.51
11	Borneol	876	1166.80	11.57	0.98
12	Terpinene-4-ol	907	1177.07	11.83	0.80
13	*para*-Cymene-8-ol	937	1187.35	12.09	1.61
14	Thymol	1252	1306.08	14.78	33.57
15	β-Caryophyllene	1557	1421.23	17.39	0.57
16	Methyl-4-hydroxymethyl benzoate	1605	1440.57	17.80	0.26
17	(+)-Spathulenol	1943	1584.37	20.68	1.24
18	Caryophyllene oxide	1955	1590.10	20.79	3.18
19	Unknown	2076	1643.75	21.82	0.41
20	d-Nerolidol	2121	1663.54	22.20	0.45
21	Unknown	2152	1677.60	22.47	0.38

*Kovats index; **Retention time.

A literature survey revealed that some of the *Satureja* genus were investigated earlier[9-11]. The essential oils of the wild and cultivated *Satureja khuzistanica Jamzad* (Lamiaceae) from Iran were isolated by hydrodistillation and its chemical compositions was examined by GC and GC/MS[9]. The carvacrol percentages in the wild and cultivated plant were 93.9 and

80.6 %, respectively. The thymol in the wild form of this plant was 0.6 % and the percentage of *p*-cymene in wild and cultivated forms were 0.8 and 4.8 %, respectively.

The compositions of the essential oil obtained from *Satureja hortensis* L. seeds from Iran contained carvacrol (59.7 %) and *p*-cymene (9.3 %) as the main compounds. The thymol is present in trace amount among the compounds. Some of the biologica activities from the *Satureja* genus from Iran such as antimycotic activity of the essential oil of *Satureja mutica* Fisch were also reported[9]. The chemical compositions of the essential oil of *Satureja boissieri Hausskn. ex Boiss* was investigated by GC/MS[10]. In this genus, the main constituents were carvacrol (70.1 %), *p*-cymene (6.3 %) and γ-terpinene (6.8 %) (Table-2).

Satureja sahandica Bornm. contrary to the other genus of *Satureja* from Iran[9-11], *p*-cymene and thymol are the main constituents among those compounds (Table-2). However, in present investigated plant, carvacrol is absent. Perhaps, the high densities of the main compounds give some biological activities to the essential oil or to this herb.

TABLE-2
COMPARISON OF THE MAIN COMPONENTS IN SOME OF THE *Satureja* GENUS OF IRAN WITH *Satureja sahandica Bornm.* OF IRAN

No.	Name of *Satureja* genus	AA	BB	CC	DD	EE	FF
1	*Satureja khuzestanica Jamzad* (Wild)	93.9	0.8	0.6	Trace*	Trace*	–
2	*Satureja khuzestanica Jamzad* (Cultivated)	80.6	4.8	Trace*	1.5	2.1	–
3	*Satureja hortensis L.* (Seeds)	59.7	9.3	Trace*	Trace*	12.8	Trace*
4	*Satureja boissieri hausskn.* (ex Boiss.)	70.1	6.3	Trace*	Trace*	6.8	4.0
5	*Satureja sahandica Bornm.* (This study)	–	47.81	33.57	0.54	3.41	0.57

AA = Carvacrol %; BB = *p*-Cymene %; CC = Thymol %; DD = Myrcene %; EE = γ-Terpinene %; FF = β-Caryophyllene %
*The percentages of compounds under 0.5% were introduced by "Trace" word.

Bacterialogical activities: In this study, the first three tubes with the concentrations of 30, 15 and 7.5 (w/v) shown no growth and 69.2, 23.1 and 7.7 % of strains didn't grow in 3.25, 1.62 and 0.8 (w/v) concentrations, respectively. Moreover, in 3.25 % concentration, 99.9 % of standard strain (*P. aeroginosa* PTCC 1074) was killed. Therefore, as a total MBC, we considered 7.5 % (w/v) concentration because of 99.9 % of *P. aeroginosa* strains were killed at this concentration.

Conclusion

Satureja sahandica Bornm. is one of the *Satureja* genus of Iran and utilized as the medicinal herb for the various purposes in local and traditional medicine in Kurdistan, Iran. Twenty-one components in the essential oil of *Satureja sahandica Bornm.* were identified by GC and GC/MS techniques. In this herb, contrary to the other genus of *Satureja* from Iran, *p*-cymene (47.81 %) and thymol (33.57 %) have the most percentages among compounds of the essential oil. The MIC and MBC tests with different concentrations were investigated on *Pseudomonas aeroginosa* which showed the antibacterial activity of *Satureja sahandica Bornm* on *P. aeroginosa*.

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