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The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Satureja rechingeri* Jamzad

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Abstract

The aerial parts of *Satureja rechingeri* were collected in two stages of plant growth (at the beginning of and full flowering stage) from Ilam province in the west of Iran. The essential oils were isolated by steam, hydro- and water-steam-distillation from the aerial parts at complete flowering stage. In addition, the essential oil of plant material at the beginning of flowering was obtained by the hydro-distillation. The oils were analyzed by capillary GC and GC–MS. The highest oil yield was obtained by hydro-distillation method and the lowest by steam-distillation. The highest oil yield was obtained at the beginning of flowering (4.72% w/w). The oil yields at full flowering stage were 2.46–4.24% (the highest for hydro-distillation and the lowest for steam-distillation).

Fifty-three compounds were identified in the oil of *S. rechingeri* at the beginning of flowering, with carvacrol (56.1%), *p*-cymene (14.0%) and α-thujone (4.7%) as the main components. Twenty-three constituents were characterized in the oils at the full flowering stage. The main components in all of the oils were carvacrol (84.0–89.3%).

So, *S. rechingeri* can be introduced as a rich carvacrol source in the complete flowering period.

Keywords: *Satureja rechingeri*; Essential oil; Distillation methods; Carvacrol

1. Introduction

Satureja is a member of the *Lamiaceae*: Nepetoideae, mainly distributed in the Mediterranean region. In Iran, 14 species are present in northern, northwestern and western parts. *Satureja rechingeri* was described as a new species from Iran, and its relationship to *S. khuzistanica*, *S. edmondi* and *S. macrantha* has previously been reported (Jamzad, 1996). *S. rechingeri* was characterized by its yellow flowers, dense white villous hairs and a dense covering of punctate glands on both leaf surfaces (Jamzad, 1996; Sefidkon & Ahmadi, 2000; Sefidkon & Jamzad, 2000, 2004; Sefidkon, Jamzad, & Mirza, 2004; Sefidkon & Jamzad, 2005). There are two important famous species of *Satureja* used as culinary herbs: *Satureja hortensis* L. and *Satureja montana* L.

The main constituents of the essential oil of *S. hortensis* are the phenols, carvacrol and thymol, as well as *p*-cymene, beta-caryophyllene, linalool and other terpenoids. Essential oil of *S. montana* includes the phenols, carvacrol and thymol, as well as *p*-cymene, linalool, terpineol, borneol and various esters of organic acids.

The green leaves and herbaceous parts of stems from both species are used fresh and dried as flavouring agents in seasonings, stews, meat dishes, poultry, sausages, and vegetables. *S. hortensis* has a sweeter and more delicate aroma and fragrance than does of *S. montana*, and is therefore the more popular of the two species. Both the essential oils, obtained by steam-distillation and the oleoresin, are used in the food industry. In addition, the essential oils

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of both species have been used in the perfume industry, either alone or blended with other essential oils.

As a medicinal plant, *S. hortensis* has been traditionally used as a stimulant, stomachic, carminative, expectorant, antidiarrheic, and aphrodisiac. The essential oil has demonstrated antimicrobial and antidiarrheic activity because of the phenols in the oil.

Due to these various usages of *Satureja* species or their oils, we were interested to study the essential oil content and composition of *Satureja* species in Iran.

The essential oil compositions of nine *satureja* species of Iran: *S. khuzistanica* (Sefidkon & Ahmadi, 2000) Jamzad, *S. bachtiarica* (Sefidkon & Jamzad, 2000) Bunge, *S. spicigera* (Sefidkon & Jamzad, 2004) (C. Koch) Boiss., *S. sahandica* (Sefidkon et al., 2004) Bornm. and *S. mutica* (Sefidkon & Jamzad, 2005) Fisch. & C. A. Mey, *S. macrantha* (Sefidkon & Jamzad, 2005) C. A. Mey., *S. intermedia* (Sefidkon & Jamzad, 2005) C. A. Mey., *S. edmondi* (Sefidkon & Jamzad, in press) Briquet and *S. isophylla* (Sefidkon & Jamzad, in press) Rech. f. were reported previously.

The major components of *S. khuzistanica* were *p*-cymene (39.6%) and carvacrol (29.6%), while those of *S. bachtiarica* were thymol (44.5%) and γ-terpinene (23.9%). The main constituents of the essential oil of *S. spicigera* were thymol (35.1%), *p*-cymene (22.1%) and γ-terpinene (13.7%). The main constituents of the essential oils of eight populations of *S. sahandica* were thymol (19.6–41.7%), *p*-cymene (32.5–54.9%) and γ-terpinene (1.0–12.8%). The main compounds of *S. mutica* were carvacrol (30.9%), thymol (26.5%), γ-terpinene (14.9%) and *p*-cymene (10.3%), those of *S. macrantha* were *p*-cymene (25.8%), limonene (16.3%) and thymol (8.1%) and those of *S. intermedia* were thymol (32.3%), γ-terpinene (29.3%) and *p*-cymene (14.7%).

The major components of *S. edmondi* oil were *p*-cymene (61.1%), γ-terpinene (9.6%) and thymol (5.0%), while those of *S. isophylla* were α-eudesmol (11.3%), β-eudesmol (9.6%), camphor (7.1%) and β-caryophyllene (6.1%).

Literature review showed variation between the chemical compositions of different *Satureja* species oils. For example, the main components of *S. boissieri* (Kurcuoglu, Tumen, & Baser, 2001) oil from Turkey were reported to be carvacrol (40.8%) and γ-terpinene (26.4%). The main constituents of *S. brownei* (Rojas & Usubillaga, 2000) oil from Venezuela were found to be pulegone (64.3%) and menthone (20.2%). The main compound of *S. parvifolia* (Viturro et al., 2000) oil from Argentina was piperitone oxide and those of *S. Boliviana* (Kurcuoglu et al., 2001) oil were γ-terpinene, β-caryophyllene and germacrene D. Menthone and isomenthone were the main components of the oils of *S. boliviana* and *S. brevicalix* from Peru (Senatore, Urrunaga Soria, Urrunaga Soria, & Della Porta, 1998). The essential oil of *S. punctata* was dominated by citral A, citral B and farnesene (Teklu, Alemayehu, & Abegaz, 1998). In the essential oil of *S. cuneifolia*, thymol and carvacrol were the main components (Tumen, Baser, Demirci, & Ermin, 1998).

Germacrene D has also been detected as the main compound of *S. coerulea* (Tumen, Kirimer, Ermin, & Baser, 1998) oil from Turkey. The main components of *S. hortensis* (Baher, Mirza, Ghorbanli, & Rezaii, 2002) cultivated in Iran were carvacrol and γ-terpinene.

From our literature searches, the oil of *S. rechingeri* has not been the subject of previous studies. In this paper, in addition to identification of volatile constituents from the aerial parts of *S. rechingeri* at the beginning of flowering, the effects of different distillation methods on quantity and quality of the oil were investigated at the full flowering stage.

There are three main distillation methods for obtaining essential oil, namely, (i) water-distillation (or hydro-distillation), (ii) steam-distillation and (iii) water- and steam-distillation.

In the manufacture of essential oils using the method of water-distillation, the botanical material is completely immersed in water and the whole is brought to the boil. This method protects the oils so extracted to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. When the condensed material cools down, the water and essential oil is separated and the oil decanted, to be used as essential oil.

When steam-distillation is used in the manufacture and extraction of essential oils, the botanical material is placed in a still and steam is forced over the material. The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are held in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. The temperature of the steam needs to be carefully controlled, just enough to force the plant material to release the essential oil, yet not too hot as to burn the plant material or the essential oil. The steam which then contains the essential oil, is passed through a cooling system (to condense the steam), which forms a liquid from which the essential oil and water is then separated.

The water- and steam-distillation method is basically a marriage between normal water-distillation and that of steam-distillation. The botanical material is immersed in water in a still, which has a heat source, and live steam is fed into the water and plant material mixture.

The effect of different distillation methods on oil content and composition of aromatic plants have been reported previously. The rose-scented geranium (*Pelargonium* sp.), was processed by various distillation methods, which revealed that water-distillation of the herb gave a higher oil yield (0.16–0.22%) than did water-steam-distillation (0.09–0.12%) or steam-distillation methods (0.06–0.18%). The distillation methods also had effects on the percentages of oil components (Kiran, Babu, & Kaul, 2005).

The effects of distillation methods and stage of plant growth on the essential oil content and composition of *Thymus kotschyanus* were also been studied. The highest

oil yield was obtained by the hydro-distillation method and the lowest by steam-distillation. The oil yield, related to distillation method and stage of plant growth, was 0.28–1.80% w/w (the highest for complete flowering stage by hydro-distillation method) (Sefidkon, Dabiri, & Rahimi-Bidgoly, 1999).

2. Materials and methods

2.1. Plant material

The aerial parts of *S. rechingeri* were collected at the beginning of and full flowering stage, from Ilam, banehroshan, altitude 600 m in August (beginning of flowering) and November (full flowering) 2004. A voucher specimen has been deposited in the national herbarium of Iran (TARI).

2.2. Isolation procedure

Dried aerial parts (50–70 g, three times) of *S. rechingeri*, at the beginning of flowering period, were subjected to the hydro-distillation of 3 h, using an all glass Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983) to produce oil.

For investigation of the oil composition at full flowering stage and the effect of distillation method on oil content and composition of *S. rechingeri*, the flowering shoots were subjected to hydro-distillation, water- and steam-distillation and direct steam-distillation. The oil yields are presented in Table 1. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (2 °C) before analysis.

2.3. Gas chromatography

GC analyses were performed using a Shimadzu GC-9 A gas chromatograph equipped with a DB-5 fused silica column (J & W Scientific Corporation) (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50 °C for 5 min and then programmed to 240 °C at a rate of 3 °C/min. Detector (FID) temperature was 265 °C and injector temperature was 250 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The percentages of compounds were calculated by the area normalization method, without considering response factors.

Table 1
Oil yields of *Satureja rechingeri* by different methods of distillation

No.	Stage of plant growth	Distillation method	Oil yield (%)
1	Beginning of flowering	Hydro-distillation	4.72
2	Full flowering	Hydro-distillation	4.24
3	Full flowering	Water- and steam-distillation	3.61
4	Full flowering	Steam-distillation	2.46

2.4. Gas chromatography–mass spectroscopy

GC–MS analyses were carried out in a Varian 3400 GC–MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 50–240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas, helium, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40–300 amu.

2.5. Identification of components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Adams, 1995, Shibamoto, 1987, Davies, 1990). Mass spectra from the literature were also compared (Adams, 1995, Stenhagen, Abrahamson, & McLaffery, 1974). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

3. Results and discussion

The oils isolated by different methods of distillation from the aerial parts of *S. rechingeri* were found to be yellow liquids in yields shown in Table 1.

The aerial parts of *S. rechingeri* produced higher oil yield at the beginning of flowering. In addition, it seems that the distillation method had a significant effect on the content of *S. rechingeri* essential oil. The highest oil yield was obtained by hydro-distillation and the lowest by steam-distillation. This may be due to this fact that, in the steam-distillation method, the state of the plant material, e.g. type of plant material, mode of comminution, mode of charging and grade of insulation are much more important than in other distillation methods. These results are in agreement with previous work on the effect of distillation methods on oil content and composition of other essential oil-bearing plants (Kiran et al., 2005, Sefidkon et al., 1999).

Fifty-three components were identified in the essential oil of *S. rechingeri* at the beginning of the flowering stage, representing 99.9% of the oil. The main components were carvacrol (56.1%), *p*-cymene (14.0%) and α-thujone (4.7%). Chemical composition of the oil can be seen in Table 2. The components are listed in order of their elution on the DB-5 column.

At full flowering stage, the oil obtained by hydro-distillation, consisted of 20 compounds, representing 99.3% of the oil. The main components were carvacrol (86.6%) and *p*-cymene (2.4%). Seventeen components were identified in the oil obtained by water- and steam-distillation representing 98.8% of the oil. The major constituents were carvacrol (89.3%) and *p*-cymene (1.9%). Twenty-two compounds were characterized in the oil obtained by direct steam-distillation, representing, 98.7% of the oil. The main

Table 2
Oil composition of *Satureja rechingeri* at the beginning of flowering

No.	Compound	RI*	Percentage (%)	Methods of Identification
1	α-Thujene	926	0.9	RI, MS
2	α-Pinene	934	0.5	RI, MS, CoI
3	Camphene	950	0.2	RI, MS, CoI
4	Thuja-2,4(10)-diene	953	0.1	RI, MS
5	Sabinene	974	0.1	RI, MS
6	β-Pinene	976	0.2	RI, MS, CoI
7	Myrcene	988	1.3	RI, MS, CoI
8	3,6-Heptadiene-2-ol	995	0.2	RI, MS
9	α-Phellandrene	1001	0.2	RI, MS
10	δ-3-Carene	1008	0.1	RI, MS
11	α-Terpinene	1014	0.7	RI, MS, CoI
12	*P*-Cymene	1023	14.0	RI, MS, CoI
13	Limonene	1028	9.6	RI, MS, CoI
14	1,8-Cineol	1030	0.3	RI, MS, CoI
15	(*E*)-β-Ocimene	1047	0.1	RI, MS
16	γ-Terpinene	1058	2.1	RI, MS, CoI
17	*cis*-Sabinene hydrate	1066	0.4	RI, MS
18	Artemisia Alcohol	1081	0.2	RI, MS
19	Terpinolene	1086	0.1	RI, MS, CoI
20	Linalool	1097	0.8	RI, MS
21	α-Thujone	1102	4.7	RI, MS, CoI
22	β-Thujone	1114	1.5	RI, MS, CoI
23	*cis*-*p*-Menth-2-en-1-ol	1119	0.1	RI, MS
24	*trans* Pinocarveol	1138	0.2	RI, MS
25	*cis*-Verbenol	1140	0.1	RI, MS
26	Camphor + *trans* Verbenol	1142	1.0	RI, MS
27	Pinocarvone	1161	t	RI, MS
28	Borneol	1164	0.3	RI, MS, CoI
29	Santolinyl acetate	1171	0.7	RI, MS
30	Terpin-4-ol	1175	0.5	RI, MS
31	*p*-Cymen-8-ol	1182	0.1	RI, MS
32	α-Terpineol	1188	0.1	RI, MS
33	*cis*-Dihydrocarvone	1193	0.1	RI, MS
34	Iso-Dihydrocarveol	1213	t	RI, MS
35	Methyl Carvacrol	1243	0.2	RI, MS
36	*cis*-Chrysanthenyl acetate	1263	0.2	RI, MS
37	Perillaldehyde	1274	0.1	RI, MS
38	Thymol	1290	0.3	RI, MS
39	Carvacrol	1300	56.1	RI, MS, CoI
40	Eugenol	1356	0.1	RI, MS
41	Carvacrol acetate	1372	0.4	RI, MS
42	α-Copaene	1377	t	RI, MS
43	β-Caryophyllene	1420	0.3	RI, MS, CoI
44	α-Humulene	1455	0.1	RI, MS
45	β-Bisabolene	1511	0.4	RI, MS
46	δ-Cadinene	1526	t	RI, MS
47	(*Z*)-β-Sesquisabinene hydrate	1543	t	RI, MS
48	Elemol	1547	0.1	RI, MS
49	Spathulenol	1579	t	RI, MS
50	Caryophyllene oxide	1584	0.1	RI, MS
51	Guaiol	1596	0.1	RI, MS
52	epi-α-Bisabolol	1687	t	RI, MS
53	Total	–	>99.9	–

RI, retention indices in elution order from DB-5 column.
MS, mass spectrometry; CoI, co-injection; *t* = less than 0.05%.

components were carvacrol (84.0%) and limonene (2.6%) (see. Table 3).

The results showed that, although the main compound at the oils of *S. rechingeri* at full flowering stage (by three distillation methods) was carvacrol, the relative percentage of this valuable compound in the oil obtained by water- and steam-distillation was higher.

Table 3
Percentage composition of the *Satureja rechingeri* oils obtained by different distillation methods at full flowering stage

No.	Compound	Hydro-distillation (%)	Water- and steam-distillation (%)	Steam-distillation (%)
1	α-Thujene	0.9	–	–
2	α-Pinene	0.4	0.6	0.8
3	Camphene	–	–	0.1
4	β-Pinene	0.1	0.1	0.2
5	Myrcene	1.1	0.9	t
6	α-Phellandrene	0.2	0.1	1.3
7	δ-3-Carene	0.1	0.1	0.3
8	α-Terpinene	0.5	0.4	0.1
9	*p*-Cymene	2.4	1.9	0.6
10	Limonene	0.2	0.2	2.6
11	1,8-Cineol	–	–	0.3
12	(*E*)-β-Ocimene	t	1.5	0.1
13	γ-Terpinene	2.2	t	2.3
14	*n*-Octanol	0.7	–	1.0
15	β-Thujone	0.1	0.1	2.2
16	Borneol	0.7	t	0.1
17	Terpin-4-ol	–	t	0.5
18	Neral	0.2	0.2	0.1
19	Bornyl acetate	0.1	–	0.2
20	Carvacrol	86.6	89.3	84.0
21	β-Caryophyllene	0.2	0.2	0.3
22	β-Bisabolene	0.5	0.7	1.3
23	(*Z*)-β-Sesquisabinene hydrate	0.1	–	0.3
	Total	99.3	98.8	98.7

t = less than 0.05%.

In addition, at full flowering stage the percentage of *p*-cymene was decreased and, instead, carvacrol was increased significantly. In essential oil-bearing plants, *p*-cymene was supposed to be a precursor of carvacrol. It seems, in *S. rechingeri* that the carvacrol formation was greater with progress of flowering. Some other minor components of the oil, at the beginning of flowering, were also not found in the oil at full flowering.

Comparison of oil composition of *S. rechingeri* with other *Satureja* species showed the highest percentage of carvacrol in this oil. Carvacrol was also reported as the major compound of *S. boissieri* (Kurcuoglu et al., 2001) (40.8%), *S. mutica* (Sefidkon & Jamzad, 2005) (30.9%), *S. khuzistanica* (Sefidkon & Ahmadi, 2000) (29.6%) and *S. hortensis* oils, but its percentage was lower than that of *S. rechingeri*. It seems that the oil of this Iranian *Satureja* species could have important medicinal properties because of its high percentage of carvacrol. Of course, the individual experiments are needed for the testing of medicinal properties.

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