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Original Article

Comparative Study on the Quantity and Chemical Composition of Essential Oil, Antioxidant Activity and Total Phenol Content of Some Iranian Native *Satureja* Species under the Same Conditions

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ABSTRACT

Satureja sp. belonging to the Lamiaceae family, has a wide variety and distribution in the world. In this research, essential oil content and components, phenol and antioxidant potential of five native *Satureja* species of Iran, including *Satureja bachtiarica* Bunge Bunge, *S. sahendica* Bornm., *Satureja spicigera* (K.Koch) Boiss., *S. macrantha* C.A.Mey. and *S. mutica* Fisch & C.A.Mey. were evaluated in the same conditions. The experiment was conducted based on randomized complete block design with three replications. The highest and the lowest essential oil content were obtained from *S. mutica* (2.05%) and *S. sahendica* (0.96%), respectively. Thymol was identified as the dominant component of essential oil in *S. spicigera* and *S. macrantha*, but in the other species, carvacrol was the dominant constituent in essential oil. In *S. sahendica*, monoterpene hydrocarbons, but in the other species, oxygenated monoterpenes were as the major constituents of the essential oil. Total phenols of the studied species were not significantly different, but the antioxidant potential of their extracts was significantly different. Cluster analysis based on phytochemical properties, divided the species to two groups: *S. spicigera* and *S. macrantha* in one group and the other species in a separate group. Results showed considerable phytochemical diversity among species and therefore the superior species will vary depending on the desired phytochemical properties.

INTRODUCTION

Different species belonging to the same plant genus have different abilities to produce active substances due to their different genetic potential [1]. In addition to genetics, the production and accumulation of active substances in plants are also affected by habitat conditions [2]. Therefore, to identify and determine the superior species in terms of quantity and quality of the active substance, different species of the same genus should be evaluated and compared under the same conditions, and then the superior species introduced for cultivation under those conditions.

Satureja consists of 15 species in Iran, of which 9 of them are endemic [3]. *Satureja*, due to its valuable compounds such as phenolic and terpenoid compounds, has several properties, including antioxidant, antifungal, antimicrobial, antispasmodic, antidiarrheal, anticancer, stomachic, blood purifier, anti-flatulence, antitussive, and expectorant [4,5]. The most important compounds in *Satureja* are thymol, carvacrol, γ-terpinene, and p-cymene [6]. Different species of *Satureja* have a wide distribution in Iran, which indicates their compatibility with most environmental conditions. Many studies have been done to determine the quantity and quality of *Satureja* essential oils, but few studies have been done to compare the quantity and quality of the essential oil of different species of *Satureja* under the same conditions. Noormand Moaied *et al*. [7] evaluated the phytochemical diversity of six *Satureja* species native to Iran (*S.*

sahendica, *S. spicigera*, *S. bachtiarica*, *S. macrantha*, *S. mutica*, and *S. atropatana*) under the same conditions and found that the highest essential oil and thymol content belonged to *S. spicigera* and the highest amount of carvacrol belonged to *S. bachtiarica*. In a study performed on two *Satureja* species, including *S. khuzistanica* Jamzad. and *S. rechingeri* Jamzad. in Khuzestan province, it was observed that *S. rechingeri* Jamzad. produced more essential oil compared to the *S. khuzistanica* Jamzad. [8]. Zarezadeh *et al*. [9], by evaluating the essential oil yield of *S. atropatana*, *S. bachtiarica*, *S. spicigera*, *S. isophylla*, *S. khuzestanica*, *S. macrantha*, *S. mutica*, *S. rechingeri*, *S. sahendica*, and *S. hortensis* in Yazd province conditions, found that the highest and lowest essential oil content belonged to *S. rechingeri* (5.9%) and *S. isophylla* (0.2%), respectively. In another study, which performed to identify the constituents of the essential oils of three *Satureja* species, including *S. macrantha*, *S. rechingeri*, and *S. spicigera* cultivated in the National Botanical Garden of Tehran, 16 compounds were identified in the essential oil of *S. macrantha* and thymol was dominant constituents (39.1%), in *S. rechingeri* essential oil, 15 compounds were identified and carvacrol was the major component (85.2%) and in *S. spicigera* essential oil, 16 compounds were detected, and thymol (46.7%) was the major constituent [10].

Due to the importance of comparing different species under the same conditions in order to identify the superior species in terms of quantity and quality of active ingredients under those conditions, in the present study, the amount of total phenol and antioxidant potential of the extract as well as essential oil content and components of five native *Satureja* species of Iran was evaluated in the of Kurdistan province conditions.

MATERIALS AND METHODS

Plant Materials

Areal part of five native *Satureja* species of Iran, including *S. bachtiarica* Bunge, *S. sahendica* Bornm, *S. spicigera* (K.Koch), *S. macrantha* C.A.Mey. and *S. mutica* Fisch & C.A.Mey. were harvested in full flowering stage from *Satureja* collection located in Kurdistan province (Sanandaj city: longitude 47°01'55" and latitude 35°16'34"). It should be noted that the plants were 6 years old. The experimental design was randomized complete block with three replications. The distance between planting rows and also between plants on each row was 1 meter. In each row, 25 plants belonging to the same species were planted. The plants were irrigated once a week for approximately four hours each time. The plants were harvested at the full flowering stage and then transferred to the laboratory for drying and essential oil extraction.

Essential Oil Extraction

Essential oil of plant samples isolated using water distillation method (Clevenger apparatus) for three hours [11] with three replications for each species. After extraction, the essential oils were dehydrated by anhydrous sodium sulfate, and their content (v/w%) was calculated and then poured into glass vials and kept at 4°C until analysis.

Essential Oil Analysis

GC/MS and GC/FID were used for quantitative and qualitative analysis of essential oil compounds. The specifications of the devices and their working conditions were as follows:

GC/MS

GC model Agilent 7990 B (USA) equipped with a 5988A mass spectrometer, HP-5MS column (30 m L, 0.25 mm i.d., 0.25 µm f.t., 5% phenyl methyl polysiloxane), He used as carrier gas with flow rate 1 mL/min and oven, injector and detector temperature were set at 240 °C, 230 °C and 240 °C, respectively. The essential oil was injected using split sampling technique by a ratio of 1:30; ionization voltage 70 eV, and the scanning range of the spectra was 40 to 400 m/z. The components of essential oil were identified by comparison of retention indices with commercial libraries [12].

GC/FID

GC model Agilent 7990 B (USA) equipped with FID detector, column VF-5MS (0.25mm i.d., 0.50 µm f.t., 30 m l., 5% phenyl methyl polysiloxane). The oven temperature program was the same to GC/MS. The volume of injected essential oil was 1 µL (diluted with n-hexane in a ratio of 1 to 100). The percentage of components was obtained by calculating the area under the peaks without correction factors.

Isolation of Extract

Extracts were isolated by soxhlet apparatus based on the continuous hot method for 4 hours. Methanol was used as the solvent. After extraction, a rotary apparatus was used to remove the solvent from the extract, and then in order to final drying, the extracts were placed in an oven at 50°C for 24 hours. The obtained dry extract was used to measure total phenol and antioxidant capacity.

Measurement of Total Phenol

The colorimetric method based on Folin-Ciocalteu instruction was used to measure total phenol content [13]. First, 1mL pure methanol was added to 1mg of dry extract. Then, 100 µL of the obtained mixture in the previous step, 100 µL Folin Ciocalteu reagent 10% and 200 µL Na2CO3 7.5%, were mixed. The resulting mixture was diluted to 2 mL with distilled water. The samples were kept at room temperature (25°C) for approximately 1h, and then their light absorption was measured by spectrophotometer (UV-2100) at 760 nm. Gallic acid was used as the standard, and finally, the amount of total phenol in the extracts was expressed based on mg of gallic acid per gram of dry extract.

Measurement of Antioxidant Capacity

The antioxidant capacity of the extracts was determined based on the DPPH radicals scavenging [14]. 1mL methanol was added to 1 mg dry extract. Then, 50 µL of the obtained solution was combined with 950 µL methanolic DPPH solution (0.2 mM) and incubated in the dark at 25°C for 15 min. After the mentioned time, the light absorption of the samples was measured by spectrophotometer (UV-2100) at 517 nm. A solution of methanol and DPPH was used as a control sample.

The percentage of antioxidant capacity (AOA%) was calculated based on the below formula (Formula 1):

Formula 1: AOA% = [(A blank – A sample)] /A blank ×100

In the above formula, AOA: antioxidant capacity, A blank: absorbance of the control sample, A sample: absorbance of the sample containing the plant extract

Finally, the amount of antioxidant capacity of the samples was calculated based on IC50 (concentration of the extract that can scavenge 50% of DPPH radicals).

Statistical Analysis

Analysis of data was performed by SPSS (21 ver) software. The means of data were compared based on Duncan's multiple range test at 5% probability level. Clustering of *Satureja* species was performed according to Ward's method and based on the square of the Euclidean distance.

RESULTS

Essential Oil Content and Components

The studied *Satureja* species had significant differences in essential oil content. The highest essential oil content (2.06%) belonged to *S. mutica*, which was not significantly different from *S. spicigera* (1.68%). The lowest essential oil content was obtained from *S. sahendica* (0.96%), which was not significantly different from *S. macrantha* (1.01%) and *S. bachtiarica* (1.25%) (Fig. 1).

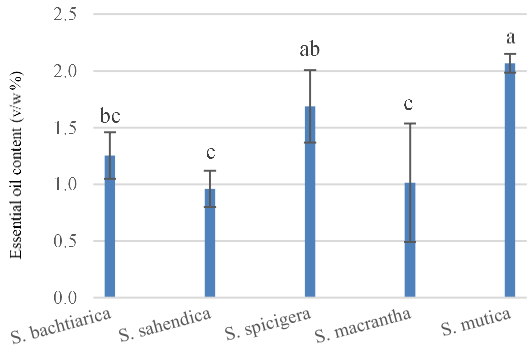

Fig. 1 Essential oil content of the studied *Satureja* species

The number, type, and amount of identified essential oil compounds in different species of *Satureja* species were different, so that in the essential oil of *S. bachtiarica*, 24 compounds were identified, which accounted for 90.42% of the essential oil. Carvacrol (48.56%), p-cymene (17.17%), γ-terpinene (7.54%), and thymol (5.92%) were dominant components in *S. bachtiarica* essential oil. In the essential oil of *S. sahendica*, 26 compounds were identified, which accounted for 95.52% of the essential oil. The major essential oil constituents of this species were carvacrol (20.92%), p-cymene (19.05%), limonene (17.78%), and thymol (17.5%), respectively. 24 compounds were identified in *S. spicigera* essential oil, which accounted for 95.75% of the essential oil. Thymol (44.69%), γ-terpinene (20.13%), p-cymene (12.24%), and carvacrol (7.78%) were the major

components in the essential oil of this species, respectively. The dominant components of *S. macrantha* essential oil were thymol (43.86%), p-cymene (13.58%), γ-terpinene (12.58%), and carvacrol (4.65%). In the essential oil of this species, 24 compounds were identified, which accounted for 92.76% of the essential oil. Carvacrol (46.89%), γ-terpinene (17.63%), p-cymene (11.12%), and thymol (10.39%) had the highest amount in the essential oil of *S. mutica*, respectively. In the essential oil of the mentioned species, 24 compounds were identified, which accounted for 95.33% of the essential oil. Some compounds were present only in the essential oil of one of the studied species. For example, cis-sabinene hydrate was found only in the essential oil of *S. macrantha* and trans- α- bergamotene only in the essential oil of *S. sahendica*. On the other hand, some compounds such as α-phellandrene, terpinolene, terpinen-4-ol, thymol acetate, spathulenol, and caryophyllene oxide were not present only in one species (Table 1).

In general, monoterpenes were the main classes of the identified compounds in essential oils in all the studied *Satureja* species. In *S. sahendica*, hydrocarbon monoterpenes, but in other species, oxygenated monoterpenes had the highest amount in the essential oil. The highest amount of hydrocarbon monoterpenes (50.39%) was observed in *S. sahendica* essential oil, and the lowest amount (28.76%) was observed in *S. bachtiarica* essential oil.

Table 1 Essential oil components of the studied *Satureja* species

Compound	RI[a]	RT[b]	*Satureja* species				
			S. bachtiarica	*S. sahendica*	*S. spicigera*	*S. macrantha*	*S. mutica*
n-Nonane	900	5.14	0.27±0.012	0.13±0.128	0.26±0.03	0.14±0.144	0.18 ± 0.06
α-Thujene	924	6.07	0.43±0.035	0.46±0.001	0.88±0.133	0.74±0.266	0.97±0.148
α-Pinene	932	6.31	0.69±0.015	0.61±0.11	0.57±0.098	1.74±1.05	0.65±0.005
Camphene	946	6.84	0.34±0.031	0.18±0.065	0.01±0.015	0.05±0.045	0.25±0.006
β-Pinene	974	7.92	0.22±0.008	0.22±0.022	0.1±0.098	1.59±1.372	0.22±0.028
β-Myrcene	988	8.56	0.98±0.045	1.02±0.264	1.43±0.068	0.83±0.828	1.43±0.193
n-Decane	1000	8.93	1.31±0.068	0.81±0.648	1.3±0.129	1.15±0.286	1.16±0.073
α-Phellandrene	1002	9.07	0.06±0.059	-	0.11±0.11	0.09±0.094	0.18±0.024
α-Terpinene	1014	9.61	0.9±0.11	1.06±0.415	2.18±0.059	1.35±0.96	1.83±0.167
p-Cymene	1020	10.02	17.17±1.23	19.05±19.05	12.24±2.03	13.58±1.89	11.12±2.13
Limonene	1025	10.15	0.29±0.056	17.78±17.33	0.35±0.058	0.46±0.048	0.33±0.04
γ-Terpinene	1059	11.56	7.54±1.36	9.92±3.29	20.13±0.42	12.58±7.45	17.63±1.21
cis-Sabinene hydrate	1065	11.87	-	-	-	0.07±0.07	-
Terpinolene	1086	12.88	0.15±0.012	0.08±0.021	0.05±0.049	-	0.05±0.047
Trans-Sabinene hydrate	1096	13.31	0.27±0.032	0.25±0.142	0.27±0.032	0.3±0.021	0.25±0.003
Linalool	1098	13.49	2.19±0.019	1.16±0.1	-	0.14±0.141	-
Borneol	1165	16.44	0.71±0.075	0.43±0.158	0.11±0.113	0.44±0.439	0.28±0.007
Menthol	1167	16.84	0.86±0.001	1.16±0.397	0.38±0.02	0.7±0.31	0.56±0.031
Terpinen-4-ol	1174	17.02	0.1±0.006	0.23±0.081	0.04±0.041	-	0.06±0.06
(E)-Anethole	1282	22.22	0.47±0.078	0.29±0.292	0.04±0.043	0.47±0.471	0.05±0.054
Thymol	1289	22.56	5.92±5.45	17.5±16.06	44.69±3.1	43.86±6.28	10.39±6.58
Carvacrol	1298	23.14	48.56±3.33	20.92±19.36	7.78±1.83	4.65±1.17	46.89±2.71
Thymol acetate	1349	25.17	0.07±0.069	0.05±0.049	0.07±0.072	0.18±0.184	-
Carvacrol acetate	1370	25.96	0.26±0.055	0.2±0.091	-	-	0.1±0.013
Trans-Caryophyllene	1417	27.83	0.67±0.54	1.19±0.147	2.48±0.233	2.6±1.377	0.17±0.027
Trans- α-Bergamotene	1432	28.84	-	0.04±0.044	-	-	-
Spathulenol	1577	34.19	-	0.1±0.096	0.12±0.124	2.85±2.571	0.25±0.019
Caryophyllene oxide	1582	34.39	-	0.68±0.05	0.16±0.159	2.19±1.96	0.33±0.047
Total	-	-	90.42±6.33	95.52±3.27	95.75±2.09	92.76±3.79	95.33±0.06

[a] Retention index [b] Retention time

The highest and lowest content of oxygenated monoterpenes were observed in essential oils of *S. bachtiarica* (59.41%) and *S. sahendica* (42.18%), respectively. *S. macrantha* essential oil was richer in sesquiterpenes than other species. The highest content of hydrocarbon sesquiterpenes (2.6%) was identified in *S. macrantha* essential oil, followed by *S. spicigera* (2.48%), *S. sahendica* (1.2%), *S. bachtiarica* (0.67%), and *S. mutica* (0.16%). The highest amount of oxygenated sesquiterpenes (5.04%) was observed in *S. macrantha* essential oil, followed by *S. sahendica* (0.78%), *S. mutica* (0.57%), *S. spicigera* (0.28%), and *S. bachtiarica* (0%). In addition to monoterpenes and sesquiterpenes, a number of other alcoholic compounds such as n-nonane and n-decane were also present in the essential oils of the studied *Satureja* species; the highest amount of these compounds (1.57%) was observed in *S. bachtiarica* essential oil (Fig. 2).

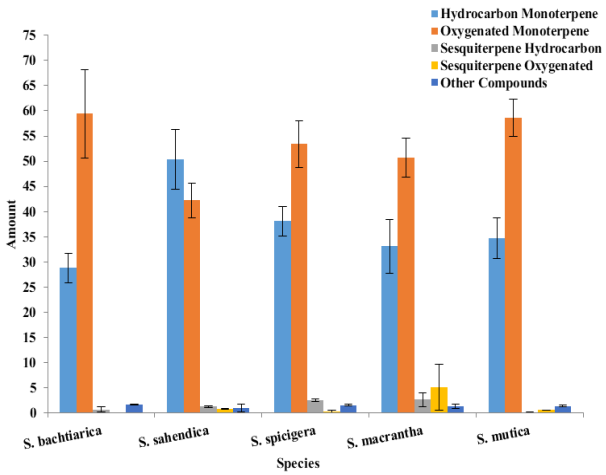

Fig. 2 The main groups of the essential oil in different *Satureja* species

Total Phenol and Antioxidant Capacity of Extract

The highest (176.3 mg GA/g DW extract) and the lowest (146.5 mg GA/g DW extract) total phenol content was observed in the extract of *S. mutica* and *S. bachtiarica*, respectively. However, it should be noted that there was no significant difference between the amounts of phenol in the extracts of the studied species (Fig. 3). The lower IC50 indicates the more antioxidant capacity of the extract. Accordingly, the extract of *S. mutica* had the lowest antioxidant capacity (IC50 = 0.055 mg/mL), although there was no significant difference with the antioxidant capacity of *S. spicigera* extract (IC50= 0.046 mg/mL). The highest antioxidant capacity (IC50 = 0.037 mg/mL) belonged to the *S. sahendica* extract; however, did not show a significant difference with *S. bachtiarica*, *S. macrantha*, and *S. spicigera* (Fig. 3).

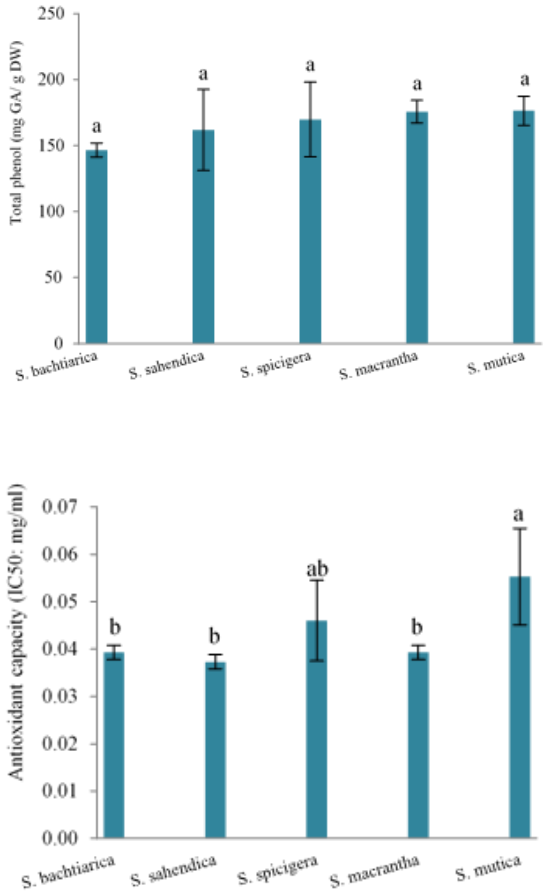

Fig. 3 Total phenol and antioxidant capacity of *Satureja* species extract

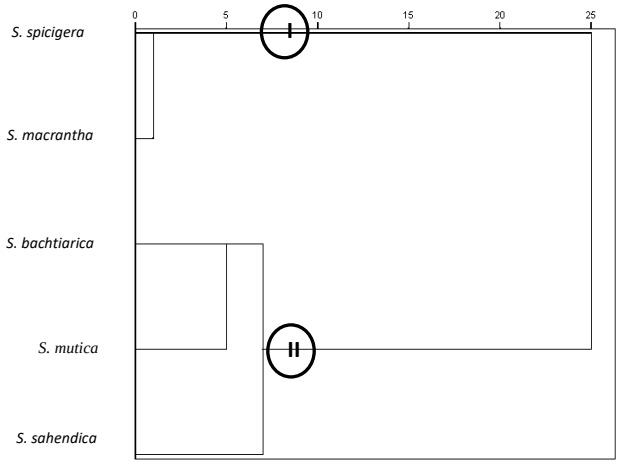

Fig. 4 Cluster analysis of *Satureja* species based on the essential oil content and components

Clustering of Species Based on the Phytochemical Properties

The clustering of the studied species based on the similarity in the measured phytochemical properties divided them into two separate clusters. *S. spicigera* and *S. macrantha* in one cluster and *S. bachtiarica*, *S. sahendica*, and *S. mutica* in the separate cluster (Fig. 4).

DISCUSSION

The production and accumulation of the active ingredients in medicinal plants depend on two main factors, including genetics and climatic conditions of the habitat [15]. The difference in the essential oil content of the studied *Satureja* species is due to their genetic potential, because they were grown under the same conditions, and the effect of environmental conditions on the content of their active ingredients was eliminated. The essential oil content of different *Satureja* species has been reported in many studies, but most of these studies have been done in different habitat conditions, and it is not possible to identify and report the superior species in terms of essential oil content. Therefore, to achieve a reliable result, all of them must be evaluated under the same conditions. The essential content of *S. macrantha* (0.2%) and *S. spicigera* (0.3%) [6], *S. macrantha* (1.8%) [16], *S. macrantha* (2.02%) [17], *S. macrantha* (1.48%) and *S. mutica* (2.31%) [18], *S. mutica* (1.9%) [19], *S. spicigera* (3.7%) [20], *S. spicigera* (3.82%) [21], *S. sahendica* (2.5%) [22], *S. sahendica* (2.8%) [23], *S. bachtiarica* (2.3%) [24], *S. bachtiarica* (2.34%) [11] and *S. bachtiarica* (2.7%) [25] have been reported. The observed differences in the amount of essential oil obtained from a similar species in different studies indicate that the essential oil content is highly influenced by habitat conditions, which can help us to determine the appropriate conditions for the cultivation of that species.

Different species of *Satureja*, in addition to differences in the essential oil content, also differ in the type and amount of essential oil components, which can affect the color, odor, taste, and medicinal properties of their essential oil [26]. Therefore, it is necessary to identify the essential oil components of the species and the amount of them in the essential oil for production, standardization, and export. As shown in the results, the number and amount of components identified in the essential oils of the studied species were different; however, in all species, monoterpenes formed the major part of the essential oil, and among the monoterpene compounds, thymol, carvacrol, p-cymene, and γ-terpinene were the dominant components in the essential oils of all species. Previous studies also confirm our results [6,11,15-23]. The biological properties of *Satureja* essential oil often depend on the presence of thymol, carvacrol, and γ-terpinene, so the presence of high amounts of these compounds in the essential oil can indicate better quality of *Satureja* essential oil [27-29]. Each of the essential oil components alone or in combination with other components cause different changes in the essential oil, so depending on the type of use of the essential oil, can be selected the plant species and suitable conditions for the essential oil production with desired quality.

Few studies have been done to determine the phenol content of different *Satureja* species. Phenol content of *S. bachtiarica* (38.4-44.5 mg GA/g DW extract) [11], *S. mutica* (26.8-36.8 mg GA/g DW extract) [19], and *S. sahendica* (24.8-25.6 mg GA/g DW extract) [22] have been reported. Despite the total phenol content, *Satureja* species were significantly different in terms of antioxidant capacity. The antioxidant capacity of the studied species according to IC50 varied from 0.037 to 0.055 mg/mL. In previous studies, the antioxidant capacity of *S. bachtiarica* and *S. sahendica* based on IC50 has been reported 37.24 and 0.008 mg/ml, respectively [11,22]. Although there is a positive correlation between total phenol content and antioxidant capacity [30], it certainly does not mean that whole antioxidant capacity is due to phenolic compounds, because the enzyme system, vitamins, organic acids, and the other compounds can be effective in the antioxidant capacity of the plant [31-33].

Placement of *S. spicigera* and *S. macrantha* species in the same cluster can be due to high thymol and lack of carvacrol acetate in their essential oil, because in other species, carvacrol had the highest amount in essential oil and also carvacrol acetate was identified as one of their essential oil components. Species clustering can be helpful in breeding projects, so that by crossing between species that are more distant from each other in terms of measured traits and are located in clusters with farther distances, new varieties can be achieved that can sometimes be very desirable.

Phytochemical evaluation of several species belonging to the same genus under the different conditions allows us to identify and introduce the superior species in terms of measured characteristics for domestication and cultivation. The results of this study indicated that in terms of essential oil content, *S. mutica* was superior compared to the other species. Nevertheless, in medicinal plants, in addition to the quantity of the active ingredient, its quality is also important, and which quality is superior depends on the type of use that is made from the plant or the active ingredient. For some industries, high levels of thymol may be desirable, but for other industries, high levels of carvacrol or antioxidant capacity or phenol content may be desirable. Therefore, the first should be determined the purpose of production and market of plant and then a superior species introduced. For example, if the amount of carvacrol in the essential oil is desirable, *S. bachtiarica* and *S. mutica* are superior, and if the high thymol content in the essential oil is important, *S. spicigera* and *S. macrantha* are superior, and if a high level of hydrocarbon monoterpenes is important, *S. sahendica* is preferred. Therefore, a particular species cannot be considered superior to other species in all of important characteristics.

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