



## Original Article

# Phytochemical Composition and the Evaluation of Antioxidant Activity of Methanolic Extract and Essential Oil of *Satureja Rechingeri* Extract and Essential Oil

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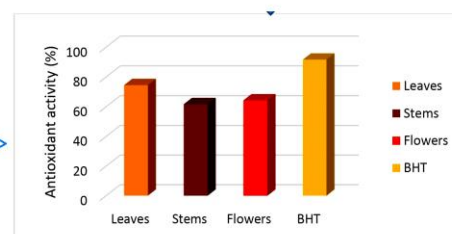
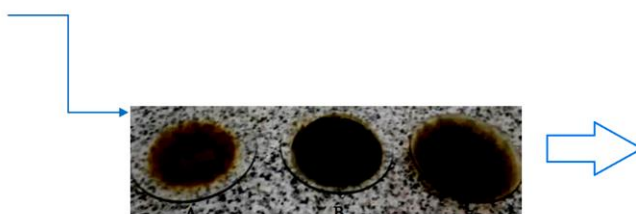
Essential oils

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### ABSTRACT

Aromatic herbs have special applications in pharmacy and cosmetics due to their secondary and medicinal compounds. For this reason, aromatic plants are especially suitable for multifunctional sustainable crop models. The main purpose of this study was to evaluate of chemical compounds of essential oils in different organs of *Satureja rechingeri* belongs to Asteraceae family. The plant was collected and then dried in the shade for a month at room temperature. Essential oils were extracted by hydro distillation and were analyzed by GC-MS apparatus. The results of this study showed that the different organs can be effective in main compounds of essential oils. Antioxidant efficacy of extract and essential oil of *S. rechingeri* was determined by DPPH method. The most important compound identified in of *S. rechingeri* essential oil was carvacrol, which was found to be 83.90%, 81.11% and 95.70% in the leaves, flowers and stems of this plant, respectively. The results of the present study showed the antioxidant activity of methanolic extract of the plant. This is probably due to the presence of flavonoid and polyphenolic compounds. *Satureja* essential oil showed moderate antioxidant activity of *S. rechingeri* extract had stronger antioxidant effects compared with its essential oil. Therefore, *S. rechingeri* extract can be useful in food hygiene and medicine.

### GRAPHICAL ABSTRACT



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## Introduction

The genus *Satureja* contains medicinal plants that belong to the family Lamiaceae. Medicinal plants of this genus contain about 0.5% of essential oil [1-2]. Studies show that there are differences in essential oils between species and subspecies of this plant genus [3]. During recent years, antiviral [4], anti-nociceptive and anti-inflammatory [5], anti-bacterial and anti-fungal [6-8], anti-spasmodic and remedy of diarrhea [9] and vasodilatory [10] effects have been reported for species of *Satureja*.

Medicinal plants of the genus *Satureja* usually grow in the northern and western regions of Iran. Two important species of *Satureja* are used as culinary plants. Important ingredients of *Satureja* essential oil include carvacrol and thymol, p-cymene,  $\beta$ -caryophyllene, linalool and other terpenoids [12].

*Satureja rechingeri* is one of the exclusive species of savory in Iran that grows in dry, sunny and calcareous rocky soils of southwestern Iran in Lorestan, Ilam and Khuzestan provinces. Due to the limited distribution of this plant species "Rechingeri savory" in natural habitats and the expansion of demand for plant raw materials, it is necessary to create high quality and homogeneous cultivars for cultivation in the agricultural system and supply plant raw materials required by the pharmaceutical industry. This study aimed to investigate phytochemical compounds of methanolic extract and essential oil of safflower and to investigate and determine its antioxidant effect.

## Material and methods

### Plant collection

Green plant material was collected from around Khorramabad area, Lorestan, Iran, and identified at the herbarium of the Lorestan University (Figure 1) (Voucher specimen (57587)). Fresh green plant parts were dried in the shade. The dried plant was pulverized by a milling machine.

### Isolation of essential oil

The essential oil of the plant was extracted by the Clevenger apparatus using the Hydrodistillation method for 6 hours. The extracted essential oil was stored in the dark at 4 ° C.



**Figure1:** *Satureja rechingeri* plant

### Preparation of extracts

10 grams of dried powder from plant was extracted by refluxing in 50 ml of %95 methanol three times for 72 hours, filtered off, and concentrated using a rotary evaporator under reduced pressure at 40°C. The crude extract was placed in a dark bottle and stored at -20°C prior to further use.

### GC-MS analysis of essential oil

Gas chromatography was performed with a Shimadzu model GC-17A (Kyoto, Japan) instrument equipped with a Shimadzu Quadropole-MS (QMS) model QP5050 detector. The present phytochemical study was performed based on the methods of Adams (2007), Baher et al. (2002), Jennings and Shibamoto (1998) and Adams (2001) [13-16]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Helium, 99.999%, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). The alkane mixture consisting of the C8-C26 alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used.

### Determination of total phenols and flavonoids in extracts

Total phenolic content was developed by Chang et al. (2001) based on the Folin-Ciocalteu method [17].

### DPPH radical scavenging assay

The radical scavenging activity of the extracts was performed with a few changes in the method of Abe et al. (1998) and Bozin et al. (2006) [19, 20].

## Result and Discussion

### GC-MS analysis of essential oil

#### Identification of essential oil compounds of *Satureja rechingeri* leaves

Hydro distillation of *S. rechingeri* leaves produced greenish yellow essential oil. 25 compounds were identified in the essential oil of *S. rechingeri*, which accounted for 100% of the total essential oil. Chemical composition of the essential oil was determined by gas chromatography and mass spectrometry (Table 1). Among these compounds, the main

compounds were carvacrol (83.90%), gamma-terpinene (4.57%) and paracymene (2.92%). The results of this table also show that mono terpenes occupy more than 99% of the volume of *S. rechingeri* essential oil, and among these, cavacrol as a phenolic mono-ring monoterpene with nearly 84% is the most significant composition of this essential oil. With the exception of mono terpenes, sesquiterpenes such as farnesene as a linear sesquiterpene and bisabolone as a single-ring sesquiterpene form a small fraction (less than one percent) of *S. rechingeri* essential oil.

**Table 1:** Ingredients of *S. rechingeri* leaves essential oil

NO	Retention time(min)	Retention Index	Katz Index	Chemical composition	Area Part%
1	12.43	928.9699571	931	Alpha.-Thujene	0.904933
2	12.766	936.1802575	939	Alpha.-Pinene	0.438516
3	13.579	953.6266094	953	Camphene	0.043592
4	14.901	981.9957082	980	Beta.-Pinene	0.122292
5	15.445	993.6695279	991	Myrcene	1.357049
6	16.238	1011.343964	1005	Alpha.-Phellandrene	0.223432
7	16.701	1021.890661	1018	Alpha. Terpinene	1.136154
8	17.158	1032.300683	1026	P-Cymene	2.922726
9	17.265	1034.738041	1031	Limonene	0.134734
10	17.362	1036.947608	1031	Beta.-Phellandrene	0.132919
11	18.01	1051.708428	1050	Trans.-Beta.-Ocimene	0.060284
12	18.597	1065.079727	1062	Gamma.-Terpinen	4.575447
13	19.265	1080.296128	1074	Cis-Sabinene Hydrate	0.35999
14	19.716	1090.569476	1088	Terpinolene	0.062903
15	20.47	1108.333333	1098	Linalool	0.527414
16	23.919	1192.867647	1190	4-Terpineol	0.334034
17	24.906	1218.315789	1206	Alpha. Terpeneol	0.154182
18	26.013	1247.447368	1244	Carvacrol Methyl Ether	0.236436
19	27.157	1277.552632	1285	Isopiperitenone	0.084059
20	28.419	1311.521127	1297	Thymol	0.17428
21	29.034	1328.84507	1312	Carvacrol	83.90177
22	30.754	1377.295775	1376	Carvacryl Acetate	1.022339
23	32.555	1429.790419	1427	Caryophyllene	0.264087
24	35.115	1506.803797	1508	Farnesene	0.359413
25	35.329	1513.575949	1509	Beta. Bisabolene	0.401137

As for essential oil compounds of flower *S. rechingeri*, according to Table 2, 25 compounds

were identified in the essential oil of flower *S. rechingeri*, which constituted 100% of the total

essential oil. Among these compounds, the main results of this table show that most of the compounds were carvacrol (83.11%), gamma-terpinene (4.66%) and paracymene (3.1%). The essential oil of this plant is composed of carvacrol.

**Table 2:** Ingredients of *S. rechingeri* flower essential oil

NO	Retention time(min)	Retention Index	Katz Index	Chemical composition	Area Part%
1	12.4	929.0772532	931	Alpha.-Thujene	0.94
2	12.8	936.2660944	939	Alpha.-Pinene	0.5
3	13.6	953.8626609	953	Camphene	0.1
4	14.9	982.1888412	980	Beta.-Pinene	0.1
5	15.5	993.8841202	991	Beta.-Myrcene	1.42
6	16.2	1011.389522	1005	Alpha.-Phellandrene	0.31
7	16.7	1022.209567	1018	Alpha.-Terpinene	1.24
8	17.2	1032.574032	1026	Para - Cymene	3.1
9	17.3	1034.965831	1031	L-Limonene	0.16
10	17.4	1037.357631	1031	Beta.-Phellandrene	0.12
11	18	1051.936219	1050	Beta.-Trans-Ocimene	0.05
12	18.6	1065.375854	1062	Gamma.-Terpinene	4.66
13	19.3	1080.637813	1085	Cis-Sabinene Hydrate	0.5
14	19.7	1090.774487	1088	Terpinolen	0.06
15	20.5	1108.70098	1098	Linalool	0.56
16	23.9	1193.014706	1190	Terpinene-4-Ol	0.53
17	24.9	1218.157895	1206	Alpha.-Terpineol	0.12
18	26	1247.763158	1244	2-Isopropyl-1-Methoxy-4-Methylbenzene	0.25
19	28.4	1311.408451	1311	Thymol	0.15
20	29	1328.450704	1317	Carvacrol	83.11
21	30.5	1371.408451	1374	Eugenol	0.08
22	30.8	1377.605634	1376	Carvacryl Acetate	0.99
23	32.6	1430.239521	1427	Caryophyllene	0.26
24	35.1	1507.120253	1509	Alpha.-Farnesene	0.23
25	35.3	1513.924051	1510	Beta.-Bisabolene	0.37
					1.00

As for the constituents of the essential oil of the stem of *S. rechingeri*, according to Table 3, 18 compounds were identified in the essential oil of safflower stalk, which constituted 100% of the total essential oil. Among these compounds, the main compounds were carvacrol (95.70%),

gamma-terpinene (0.66%) and paracymene (0.74%). The results of Table 3 show that most of the essential oil of this plant is composed of carvacrol and the percentage of carvacrol essential oil of stem is higher than that of leaves and flowers.

**Table 3:** Compounds of the stem *S. rechingeri* essential oil

NO	Retention time(min)	Retention Index	Katz Index	Chemical composition	Area Part%
1	12.415	928.6480687	931	Alpha.-Thujene	0.153742
2	12.769	932.3234624	939	Alpha.-Pinene	0.053772
3	15.435	993.0523918	991	Myrcene	0.205394
4	16.69	1021.640091	1018	Alpha. Terpinene	0.168802
5	17.136	1031.799544	1026	P-Cymene	0.742896
6	18.561	1064.259681	1062	Gamma.-Terpinen	0.668627
7	19.284	1080.728929	1074	Cis-Sabinene Hydrate	0.09125
8	20.467	1108.259804	1098	Linalool	0.217794
9	23.912	1192.696078	1190	4-Terpineol	0.269079
10	24.899	1218.131579	1206	Alpha. Terpeneol	0.112325
11	26.013	1247.447368	1244	Carvacrol Methyl Ether	0.270842
12	28.422	1311.605634	1297	Thymol	0.209918
13	29.009	1328.140845	1312	Carvacrol	95.70719
14	30.544	1371.380282	1357	Eugenol	0.104387
15	30.741	1376.929577	1376	Carvacryl Acetate	0.199333
16	32.553	1429.730539	1427	Caryophyllene	0.216571
17	35.108	1506.582278	1508	Farnesene	0.165499
18	35.329	1513.575949	1509	Beta. Bisabolene	0.357623
					0. 100

According to the results of Tables 1, 2, 3, the most important constituents of essential oils of different organs were carvacrol, gamma terpinene and parasyment [21]. The essential oil yield of *S. rechingeri* aerial parts collected from the habitat at the beginning of flowering by water distillation was reported to be 4.72%. At the full flowering stage, the essential oil yield of the same sample in different distillation methods was reported between 2.46% and 4.24%. The highest essential oil yield was obtained by water distillation and the lowest yield was obtained by steam distillation [22].

At the beginning of flowering, 43 compounds in the essential oil were identified as carvacrol (56.1%) and para-cymene (14%) as major components. Also, in the full flowering stage, 23 compounds were identified in the essential oil, of which carvacrol (84.3% -89.3%) was the most important essential oil compound. Research has

shown that the essential oil of this species has a strong antimicrobial effect [23, 24].

#### *Total phenols and flavonoids*

Phenolic compounds have been reported to exhibit various biological activities like antioxidant, antimicrobial, and etc. Total phenolic compounds in extracts were determined by Folin-Ciocalteu method and expressed as Gallic acid equivalents (GAEs). Many studies have revealed that the phenolic contents in the plants are associated with their antioxidant activities probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [25].

Folin-Ciocalteu was used as a reagent to measure phenolic compounds. In this method, gallic acid was used as a standard and methanolic extract of different organs was prepared at concentrations of 2000 µg/ml. It was then prepared and diluted in 100 µl of extracts with 1500 µl of Folin-

Ciocaltue 0.1% and with 1000 µl Mix distilled water; after 1 minute, 1500 µl of 20% sodium carbonate solution was added, the samples were transferred to a dark place at room temperature for two hours and then absorbed at 760 nm. The microplate reader was read.

To measure flavonoid compounds, methanolic solutions with a concentration of 2000 µg / ml were prepared, then 500 µl of the extract was mixed with 1500 µl of methanol, 100 µl of 10% aluminum chloride, 100 µl of 1 M potassium acetate and 2800 µl of distilled water. The samples were kept at room temperature for 40 minutes and their absorbance was read at 420 nm using a microplate reader. Quercetin was used as the standard.

The results of comparing the mean values of phenolic compounds showed that the leaves of S.

rechingeri with 223.71 µg of gallic acid per mg of extract had the highest phenol content and the stem with 158.95 µg of gallic acid per mg of extract containing the lowest number of phenolic compounds. Therefore, the phenolic content of different organs of savory sage is in the form of stem <flower <leaves.

The results of comparing the mean values of flavonoid compounds showed that the flower of Satureja rechingeri with 76.08 µg quercetin per mg of extract had the highest phenol content and the stem with 30.48 µg quercetin per mg of extract contained the lowest number of compounds, e.g. phenolic. Therefore, the phenolic content of different organs of S. rechingeri is in the form of stem <leaves <flower.

**Table 4:** Antioxidant activity, phenol and flavonoid contents of essential oil and methanolic extracts in different organs of *S. rechingeri*

Essential oil or methanolic extract	Antioxidant activity		Phenol (µg Gallic acid /mg extract)	Flavonoid (µg quercetin /mg extract)
	DPPH (IC <sub>50</sub> (µg/ml))	β-carotene linoleic acid		
Leaf methanolic extract	74.58	41.10	223.71	58.83
Stem methanolic extract	61.75	64.86	158.95	30.48
Flower methanolic extract	64.31	83.74	204.66	76.08
Leaf essential oil	77.44	87.12	-	-
Flower essential oil	90.25	69.56	-	-
Stem essential oil	78.63	75.48	-	-
BHT	91.64	83.74	-	-

#### *Evaluation of antioxidant activity of the studied samples by beta-carotene linoleic acid*

Unsaturated fatty acids such as linoleic acid are very sensitive to the oxidation process; therefore, inhibition of oxidation of this substance is used as a valuable method in determining antioxidant activity. In this method, antioxidant activity is measured by inhibiting the oxidation of linoleic acid and preventing the formation of volatile compounds and conjugated hydroperoxides. First, stock solution was prepared from beta-carotene linoleic acid solution, by dissolving 0.5 mg of beta-carotene in 1 ml of chloroform, then 25 µl of linoleic acid and 200 mg of tween forty were added. Next, the chloroform was completely evaporated and then 100 ml of oxygen-saturated distilled water was

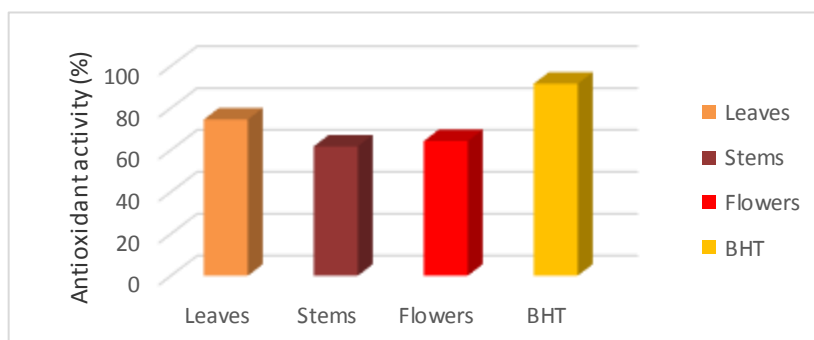
added with stirring. Methanolic extracts of different organs were prepared with methanolic solutions at concentrations of 500 µg / ml. To evaluate the antioxidant properties, 50 µl was added to 2500 µl of beta-carotene linoleic acid solution. The absorbance of the samples was read at zero moment and also after two hours of incubation at 50 ° C using the microplate reader at 470 nm; the amount of antioxidant activity of the extracts was calculated.

#### *DPPH radical scavenging activity*

DPPH assay has been extensively used for screening plant extracts because many samples can be accommodated in short period and are sensitive enough to detect active ingredients at low concentrations [26].

In this method, DPPH was used as a reagent and the activity of hydrogen atoms and electrons of the extracts was measured by discoloration of DPPH solid purple solution. From the dried extracts of different organs, concentrated solutions (0.01, 0.008, 0.006, 0.004 and 0.002) mg / ml and 0.004% solution of DPPH were prepared, then for each sample. Plant organs were tested three times with 50 µl of the extract and 1 ml of DPPH solution, then the samples were read at room temperature at 517 nm. In this method, BHT was used as a positive control.

The results of antioxidant activity in this method showed that stem essential oil with 90.25% had higher antioxidant power than leaves and flowers (78.63% and 77.44%, respectively), but the antioxidant power of BHT was slightly lower. In this study, the antioxidant activity of flowers was higher than that of stems and the order of antioxidant power of essential oils of different organs was in the form of leaves <flower <stem <BHT (Figure 4).

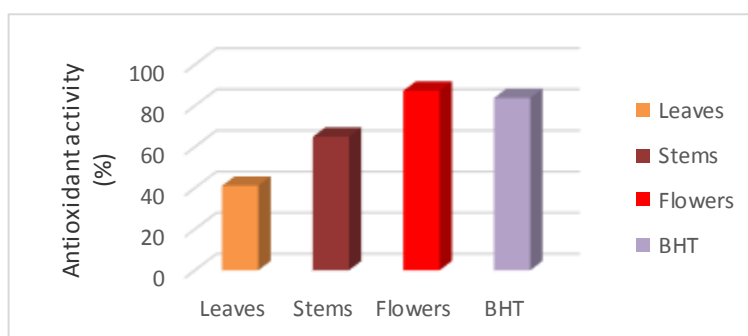


**Figure 4:** Free radical scavenging of leaves, flowers and stems methanolic extracts of *S. rechingeri* compared with BHT by DPPH assays

*Beta-carotene linoleic acid method*

The results of comparing the means showed that in this method the leaf essential oil of *S. rechingeri* had significant antioxidant power

(87.12%) so its antioxidant power was higher than BHT (83.75%). The antioxidant power of the stem was also greater than that of the leaf (stem <flower <BHT <leaf) shape (Figure 5)).



**Figure 5:** Antioxidant activity of leaves, flowers and stems methanolic extracts of *S. rechingeri* compared with BHT by β-carotene-linoleic acid assays

*Antioxidant activity of methanolic extract*

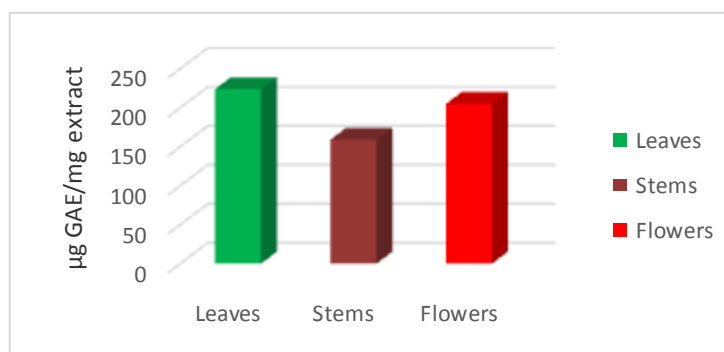
The results of antioxidant activity in this method showed that methanolic extract of *S. rechingeri* leaf had higher antioxidant power than stem and flower but its antioxidant power was less than BHT. The antioxidant power of the flower was higher than the stem, so according to Figure 6, the antioxidant power of different organs of the plant

*S. rechingeri* and the BHT standard was in the form of stem <flower <leaf <BHT (Figure 6).

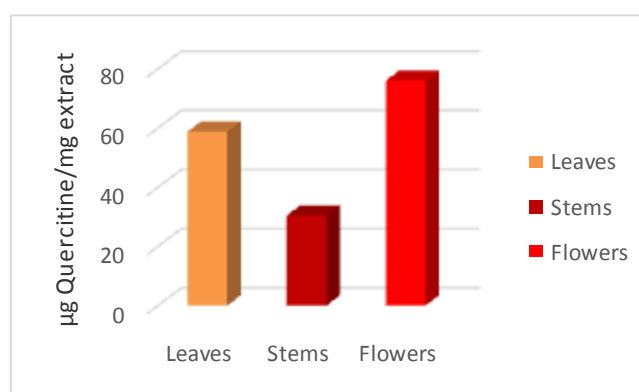
*Beta-carotene linoleic acid method*

The results of comparing the means showed that in this method, methanolic extract of flower had a very high antioxidant power, so its antioxidant activity was higher than BHT. According to Figure 7-9, the order of antioxidant power of different

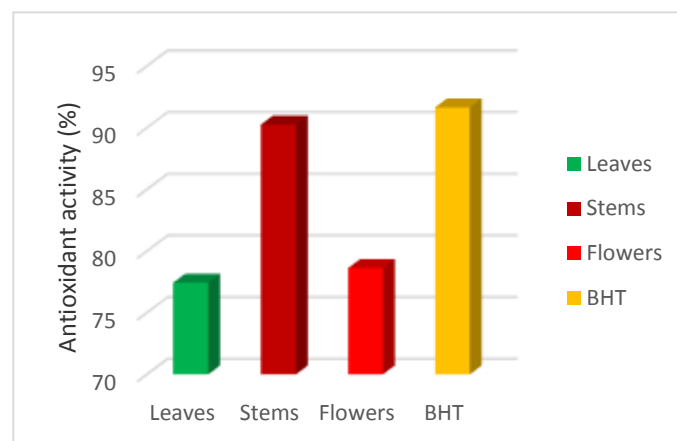
organs of *Satureja rechingeri* plant and BHT <flower.  
standard is in the form of leaf <stem <BHT



**Figure 6:** Phenolic content of different organs methanolic extracts of *S. rechingeri*



**Figure 7:** Flavonoid content of different organs methanolic extracts of *S. rechingeri*



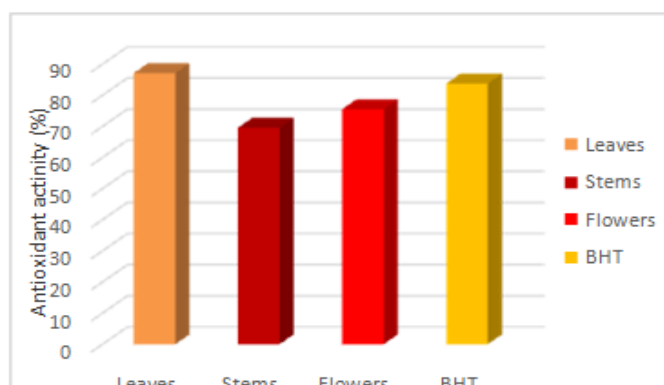
**Figure 8:** Free radical scavenging of leaves, flowers and stems essential oils of *Satureja rechingeri* compared with BHT by DPPH assays

Studies have shown that medicinal plants, due to their phytochemical, active ingredients and medicinal and antioxidant compounds, have beneficial effects on human health and have a therapeutic effect on various organs of the body and various diseases [27-37].

Nature is a rich source of phytochemicals compounds, some of which are found in medicinal

plants. Phytochemical compounds are found in various organs of medicinal plants and have biological and medicinal activities. Phytochemicals are biologically active compounds that are rich in phenols, flavonoids, tannins, anthocyanin, minerals and antioxidants and can be used for medicinal and health purposes [38-40].





**Figure 9:** Antioxidant activity of leaves, flowers and stems essential oils of *S. rechingeri* compared with BHT by  $\beta$ -carotene-linoleic acid assays

## Conclusion

Extract of *S. rechingeri* possesses remarkable antioxidant activity compared with its essential oil. Thus, the extract of *Satureja rechingeri* can prove beneficial in food and pharmaceutical industry.

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## Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

## Conflict of Interest

We have no conflicts of interest to disclose.

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