# A COMPARISION OF ANTIOXIDANT CAPACITIES OF ETHANOL EXTRACTS OF *SATUREJA HORTENSIS* AND *ARTEMISIA DRACUNCULUS* LEAVES

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**Summary**

*Satureja hortensis* (Lamiaceae family) and *Artemisia dracunculus* (Asteraceae family) are both used as home remedies in Iran. Due to the fact that some species of Asteraceae family have proven to contain potential antioxidant capacities, present study was designed to evaluate and compare the antioxidant capacities of ethanolic extracts of *S. hortensis* and *A. dracunculus* leaves using in vitro assays such as DPPH and ABTS along with ferric reducing antioxidant power (FRAP). In addition, total flavonoid and phenolic contents of both extracts were measured. Results showed that *S. hortensis* has the ability of scavenging ABTS and DPPH radicals as well as reducing ability for ferric iron ions in a dose dependent manner much better than *A. dracunculus.* Based on our data, it can be concluded that *S. hortensis,* as a natural rich source of antioxidants, could be evaluated for probable pharmaceutical application in prevention of many free radical-associated diseases.

**Key words:** *Satureja hortensis*; *Artemisia dracunculus*; antioxidant activity; ABTS; Ferric reducing power.

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## Introduction

Oxidative stress is caused by the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological condition but they might be harmful if they are not eliminated by the endogenous antioxidant machinary. Different environmental stress factors like pollution, drought, temperature, excessive light intensities, and nutritional limitation are able to increase the production of ROS [1]. Biological systems have several antioxidant defense mechanisms which help them to prevent the destructive effects of ROS. These defense mechanisms include antioxidative enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase and small molecules such as glutathione and vitamins C and E [2]. The efficiency of the antioxidant defense system is altered under pathological conditions and therefore, the ineffective scavenging and/or overproduction of free radicals may play a crucial role in free radical tissue damages [3].

Although there are some synthetic antioxidant compounds such as BHT and butylated hydroxyanisole (BHA), which are commonly used in food processings, it has been reported that these synthetic antioxidants are not devoid of biological side effects and their consumption may lead to carcinogenecity and liver damages [4]. The use of spices and/or herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants. Polyphenolic compounds constitute one of the largest and ubiquitously distributed groups of plant secondary metabolites and are therefore an integral part of the diet [5]. Based on various investigations, these compounds can act as antioxidants, by inhibiting biomolecules from undergoing oxidative damage through free radicals mediated reactions. *S. hortensis* is an aromatic and herbaceous plant belonging to the family labiatae native to southern Europe and naturalized to sections of North America which is composed of species with exploitable antioxidant activity [6-10]. This herb has been used as a condiment and also a table vegetable in Iran. The leaves, flowers and stems are used as herbal tea and folk medicine to treat various diseases especially gastrointestinal and infectious ones [11]. Extracts from *S. hortensis* has been shown to have antibacterial, antifungal, antioxidant, antispasmodic, anti diarrhea and sedative properties [11-14]. *A. dracunculus* or tarragon is a small shrubby perennial herb belonging to Asteraceae family. Tarragon leaves, used as an anticoagulator and antihyperlipidaemic agent, are commonly used fresh or dried as seasoning on salads and soups in Iranian food. The extract of *A. dracunculus* leaves appears potentially useful for decreasing the incidence of coronary diseases in human [15] and used for treatment of headaches, dizziness [16] and epilepsy [17]. In the present study, we evaluated and compared the antioxidant activities of both crude extracts of *S. hortensis* and

*A. dracunculus*, employing various in vitro assay systems, such as the 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays as well as ferric reducing ability potential (FRAP) assay.

## Materials and methods

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tripyridyl-S-triazine (TPTZ) were obtained from BDH chemicals Ltd (England). Gallic acid, ascorbic acid and ferric chloride were obtained from Sigma (St. Louis, MO, USA). Nitroblue tetrazolium (NBT), 5,5-dithiobisnitro benzoic acid (DTNB), thiobarbituric acid (TBA) were obtained from Merck (Germany). All other chemicals used were analytical grade and were used as such without further purifications.

### Plant material

Aerial parts of *S. hortensis* and *A. dracunculus* were collected from suburb of Arak at the end of spring (2006). They were air-dried, protected from direct sunlight, and finely ground. The powder was kept in a closed container at 4 $^{o}$C.

### Extraction

50 grams of each botanic powder (*S. hortensis* and *A. dracunculus*), were seperately extracted four times (overnight) with 1 L of mixture of ethanol: water (7:3 ratios) at room temperature. Ethanol was completely removed by rotary vacuum evaporator at 75 $^{o}$C. Both concentrated extracts were distinctly filtered for 2 times, frozen at −70 $^{o}$C and then lyophilized. The residue was stored at -20 oC for further investigation.

### Determination of total polyphenol content

Total phenolic content of each lyophilized extract was determined with the Folin – ciocaltued method [24]. We mixed 2.5 ml fresh Folin reagent with 0.5 ml of different concentrations of each extract and immediately added 2 ml $Na_2CO_3$ (7.5%) to each one and left for 90 min at 30 $^{o}$C .The absorbance was measured at 765 nm. Results were expressed as Gallic acid equivalents (mg Gallic acid/g dried extract).

### Determination of total flavonoid content

The total flavonoid content of each plant extract was estimated by Zhishen et al method [25]. Based on this method, each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%). After 6 min, 0.15 ml of an $AlCl_3$ solution (10%) was added and allowed to stand for 6

min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was then determined at 510 nm versus the water blank. Results were expressed as catechin equivalents (mg catechin/ g dried extract).

**DPPH radical scavenging activity**

DPPH radical scavenging activity of each plant extract was determined according to the method of Blois [26]. Briefly, 1ml of each lyophilyzed crude extract of *S. hortensis* and/or *A. dracunculus,* at variable concentrations (25-400 µg/ml), was added to 1ml of DPPH (1, 1 diphenyl2 picrylhydrazyl) solution (0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30 minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C was used as the positive control. The DPPH Radical Scavenging Activity was calculated using the fallowing formula:

DPPH Radical Scavenging Activity (%) = $[(A_0 - A_1 / A_0) \times 100]$, where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of extract or standard sample.

**ABTS radical cation scavenging activity**

The ABTS radical cation scavenging activity was determined according to Re et al [27]. A solution of $ABTS^{\bullet+}$ (7.4 mM) was prepared in 100 mM phosphate buffer saline (PBS) pH 7.4, 0.15 M sodium chloride and oxidized using potassium persulfate (2.45 mM) for at least 12-16 h in dark. The $ABTS^{\bullet+}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm with ethanol, pH 7.4. The $ABTS^{\bullet+}$ solution was used within 2 h because the absorbance of the radical dissipates with time. For measuring antioxidant capacity, 200 µl of the sample was mixed with 2.8 ml of $ABTS^{\bullet+}$ solution. The absorbance of the above mixture was measured at 734 nm after 6 min. Appropriate blank measurements were carried out and the values recorded. Trolox was also tested under the same conditions as a standard antioxidant compound. The decrease in absorption at 734 nm was used for calculating inhibition values relative to the control sample.

**Ferric reducing antioxidant power (FRAP) assay**

The reducing power was determined by using FRAP assay described by Benzie and Strain [28], with some modifications. Briefly, the FRAP reagent contain 2.5 mL of 10 mM tripyridyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer, pH 3.6, was freshly prepared. Both extracts dissolved in water at a concentration of 1 mg/mL and diluted to 10, 20, 30, 50 and 100µg/mL. Aliquots (0.1 mL) of each diluted extract mixed with 2.9 mL of FRAP reagent. The absorption of the reaction mixture was measured at 595 nm. Ethanolic solutions of known $FeSO_4$ (II) concentration, in the range of

20–500 μM ($FeSO_4$), were used for obtaining the calibration curve. In order to make comparison, Trolox was also tested under the same conditions as a standard antioxidant compound. The FRAP value was defined as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1μM $FeSO_4$.

## Results and discussion

By now, it is well established that fruits and vegetables are rich sources for natural occurring antioxidants. Among these substances, the phenolic compounds have the ability to scavenge free radicals, superoxide and hydroxyl radicals through single-electron transfer reactions [29-32]. In this study, we evaluated and compared the antioxidant activity of crude extracts of *S. hortensis* and *A. dracunculus*. The total phenolic and flavonoid contents of both crude extracts were determined and expressed in terms of gallic acid and catechin equivalents (Table 1). Based on our results, *S. hortensi* contains higher phenolic and flavonoid contents (128 mg gallic acid equivalents/g dried extract and 88.5 mg catechin equivalents/g dried extract, respectively). As shown in table 1, A. *dracunculus* has less phenolic and flavonoid content.consequently we expect that *A. dracunculus* to posses lower antioxidant activity relative to *S. hortensis.*

**Table1.** Total phenolic and flavonoid contents of *S. hortensis* and *A. dracunculus* crude extracts.

| *sample* | Total phenol[a] | Total flavonoid[b] |
|---|---|---|
| *S. hortensis* | 128 ± 5.6 | 88.5 ± 6.6 |
| *A. dracunculus* | 49 ± 4.2 | 23 ± 2.2 |

[a]. Total phenolic content was expressed as mg gallic acid equivalents/g dried extract.

[b]. Total flavonoid content was expressed as mg catechin equivalents/g dried extract.

In order to get more information about these botanic extracts, we managed to measure and compare the antioxidant activity of each plant extract. There are several assays commonly used for the measurement of antioxidant activity of plant extract, including DPPH and ABTS radical scavenging and FRAP assays.
The approach of scavenging the stable DPPH radicals is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods. DPPH is a stable electron containing free radical which is used for detecting radical scavenging activity in chemical analyses.

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [33-34]. The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant . Fig. 1 shows that both plant extracts scavenged DPPH radicals in a dose dependent manner, though by different capabilities. These scavenging activities might be due to the presence of different phenolic contents in each extract. In addition, the antioxidant ability of each crude extract in scavenging the blue-green colored $ABTS^{\bullet+}$ was measured relative to the radical scavenging ability of Trolox. 2, 2′-azinobis 3-ethylbenzothiazoline-6-sulfonate ($ABTS^{+}$) radical scavenging method is a common spectrophotometric procedure for determining the antioxidant capacities of plant components.The ABTS method is easy to use, has high sensitivity, and allows for rapid analyses of the antioxidant activity of a larger number of samples. Unlike the reactions with DPPH radical, which involves proton transfer, the reaction with ABTS radicals involves electron transfer processes. Fig. 2 depicts a decrease in the absorbance of ABTS radicals at 734 nm in the presence of each extract and/or trolox as the positive standard at different concentrations (10-200µg/ml). As shown in Fig.2, both extracts show antioxidant activities in a dose dependent manner and at lower doses (<300µg/ml) the antioxidant activity of *S. hortensis* is more significant than that of *A. dracunculus.*

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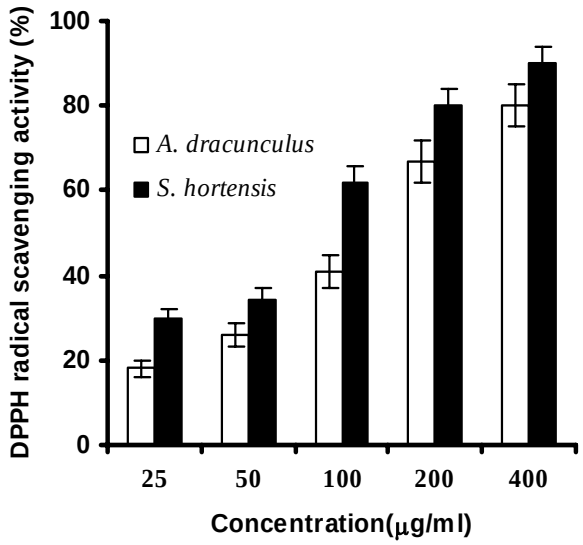


**Figure 1.** DPPH radical scavenging activities of *S. hortensis* and *A. dracunculus* crude extracts. Each value represents the mean ± SD (n = 3).
$EC_{50}$ of Vitamin C was found to be 3.5 µg/ml.

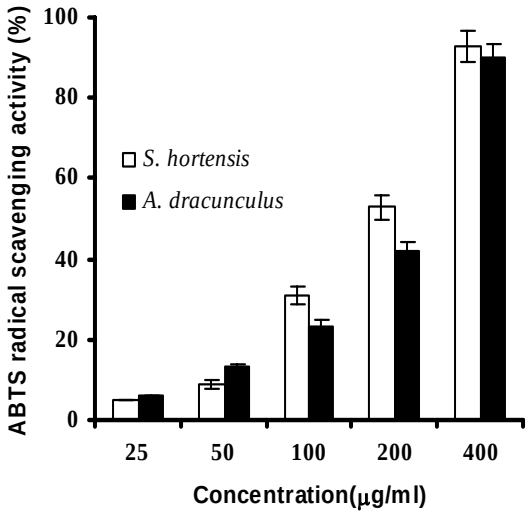


**Figure 2.** ABTS radical scavenging activities of *S. hortensis* and *A. dracunculus* crude extracts. Each value represents the mean ± SD (n = 3).
$EC_{50}$ of Trolox was found to be 9.2 µg/ml.

In still another approach, the antioxidant power of each crude extract of the plants was evaluated based on FRAP method and compared with trolox as a reference antioxidant. In this method, the reduction of ferric-tripyridyltriazine complex to its ferrous form by antioxidant compound is measured [28]. At low pH, ferric-tripyridyltriazine ($Fe^{3+}$-TPTZ) complex is reduced to the ferrous ($Fe^{2+}$) blue colour complex with an absorption maximum at 593 nm. Test conditions favor reduction of the complex and, thereby, color development, provided that an antioxidant is present. The FRAP values have been calculated by comparing the absorbance change at 593 nm in test samples with those containing ferrous ions at known concentrations ranging from 20 to 500 µM. Based on results, both crude extracts and trolox, as a positive control, have the ability of reducing $Fe^{3+}$ to $Fe^{2+}$ by FRAP value of 645 and 135 µM/mg dried extract of *S. hortensis* and *A. dracunculus,* respectively and 1650 µM/mg trolox (Table 2). These results might represent the presence of polyphenols and flavonoids in these plants.

**Table 2.** Antioxidant activities of *S. hortensis*, *A. dracunculus* crude extracts and Trolox using FRAP assay.

| Sample | FRAP value(μM)[a] |
|---|---|
| *S. hortensis* | 645.3 ± 20 |
| *A. dracunculus* | 135.2 ± 5 |
| Trolox | 1650 ± 52 |

[a] FRAP value expressed concentration of antioxidant having a ferric reducing ability equivalent to that of 1 μM $FeSO_4$.
Trolox was the positive control, it is a known antioxidant**.**

## Conclusion

The results of this study clearly indicate that *S. hortensis* extract has powerful *in vitro* antioxidant activity.The antioxidant activity of *S. hortensis* might be due to its effective free radical scavenging activity as well as its high reductive capability determined in this study. The relatively good free radical-scavenging property of this plant is probably the basis for using it as a foodstuff as well as a traditional medicine. Additional studies are needed to characterize the bioactive compounds responsible for the observed activities. Therefore, *S. hortensis* can be used as an accessible source of natural antioxidants and a possible food supplement.

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