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Antioxidant Activity of Methanol and Ethanol Extracts of *Satureja hortensis* L. in Soybean Oil

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Abstract

Background: Considering the wide consumption of some synthetic antioxidants and their probable negative health effects, in this study, the protective effects of *Satureja hortensis* L. extracts in stabilizing soybean oil against oxidation at different concentrations (200 and 400 ppm) were tested.

Methods: The aerial parts of *S. hortensis* L. were collected from Isfahan province and extracted with 100 ml methanol and ethanol. Soybean oil was selected due to its unsaturated fatty acids variety. Antioxidant activity of the extract was evaluated using 2, 2-diphenyl-1-picrylhydrazyl test. A β-carotene-linoleic acid assay was also applied for the antioxidant capacity. Total phenolic and flavonoid content, peroxide value and thiobarbituricacid-reactive substances (TBARs) were measured for the soybean oil samples. Experiments were carried out in triplicates and data were processed with ANOVA test by SPSS software (16.0).

Results: *S. hortensis* L. extracts had significantly ($p \leq 0.05$) lower peroxide and thiobarbituric acid value of samples during storage at 60 °C. The half maximal inhibitory concentration (IC_{50}) values for methanol and ethanol extracts were 31.5±0.7 and 37±0 µg/ml, respectively. In the β-carotene/linoleic acid system, methanol and ethanol extracts exhibited 87.5%±1.41 and 74%±2.25 inhibitions against linoleic acid oxidation. The total phenolic and flavonoid contents of methanol and ethanol extracts were (101.58±0.26 mg/g) and (96±0.027 mg/g), (44.91±0.14 mg/g) and (14.3±0.12 mg/g) expressed in gallic acid and quercetin equivalents, respectively.

Conclusion: Methanol and ethanol extracts of *S. hortensis* could be prepared and added to the commercial vegetable oils as natural antioxidant and so they may be suitable alternative for some synthetic antioxidants.

Introduction

Vegetable oils and fats provide essential fatty acids, which play an important role in hormones such as prostaglandins. Furthermore, many physiological factors are affected by fats such as blood pressure, cholesterol level, and the reproductive system (Iqbal and Bhanger, 2007; Naz et al., 2005). Vegetable oils with higher contents of unsaturated fatty acids are more susceptible to the oxidation (Mohdaly et al., 2010). The problem is worsens if the oil is exposed to the factors such as oxygen, light, high temperatures or trace metals (Sikwese and Duodu, 2007). Lipids not only contribute to the color, odor, flavor and consistency, but also confer a feeling of satiety and palatability to the foods. Lipids oxidation during storage or food processing result in organoleptic property changes, the loss of product shelf life, nutritional value, sensory and other physiological properties (Iqbal and Bhanger, 2007). Due to the mentioned changes,

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consumers do not accept oxidized products and industries lose their market. Thus, it is an important concern for the oil industry (Mohdaly et al., 2010). Both natural and synthetic antioxidants are widely used (Frutos and Hernandez-Herrero, 2005).

The most common synthetic antioxidants in food, namely butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ), have been suspected to cause or promote harmful effects to the health (Ozcan and Arslan, 2011). Thus, over the last few years, this has led to an increase in the use of natural antioxidants found in numerous plant sources such as oil seeds, vegetables, cereal crops, fruits, leaves, spices and herbs (Abdalla et al., 2007). Phenolic compounds may act as both radical scavengers and metal chelators (Sikwese and Duodu, 2007). A large number of vegetables are known to be rich sources in antioxidants (Frutos and Hernandez-Herrero, 2005; Kamkar, 2009; Shyamala et al., 2005).

The Labiates family includes about 220 genera and 3300 species which are widely used for various purposes (Evans, 1996). Plants belonging to the Labiates family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties (Tepe et al., 2006). *Satureja hortensis* L., known as medicinal plant, is widely distributed in different parts of Iran. In addition to its usual use in food industry as an aromatic and flavoring agent, it has considerable anti-inflammatory effect (Hajhashemi et al., 2002), antioxidant (Güllüce et al., 2003), antibacterial and antifungal activities (Boyraz and Ozcan, 2006)

The purpose of this study was to evaluate the antioxidant effectiveness of methanol and ethanol extracts of *S. hortensis* L. during accelerated oxidation of soybean oil by measuring both primary and secondary oxidation products and compare its antioxidant activity with commercially antioxidant to understand its potential use as an antioxidant in the food industries.

Materials and methods

Plant material and extraction

The aerial parts of *S. hortensis* L. were collected from Isfahan province, Iran and approved by an experienced herbalist. Then, it was dried in the shade, ground in a grinder and a portion (15 g) of dried plant material was extracted with 100 ml of methanol and ethanol (HPLC grade) using an electrical shaker for 6 h.

The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40 °C using a rotary evaporator extractor. Extracts were kept in the dark at 4 °C until used.

Samples and reagents

Chemicals including BHT, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, quercetin (Sigma-Aldrich; Milwaukee, USA) methanol, HCl, petroleum ether, $FeCl_3$, NH_3, β-carotene, chloroform, glacial acetic acid, KI, thiobarbituric acid, malon di aldehyde, folin-Ciocalteu reagent, potassium carbonate, gallic acid, potassium acetate (Merck; Gernsheim, Germany) were provided. Refined, bleached and deodorized soybean oil without any antioxidants was taken from an oil company (Behshahr Vegetable Oil Factory, Tehran, Iran).

In vitro antioxidant activity

-Scavenging effect on DPPH radicals

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of purple-colored methanol solution of DPPH. This spectrophotometer assay uses stable radical DPPH as a reagent (Burits and Bucar, 2000). Aliquots (50 µl) of various concentrations of the extract in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After 30 min incubation at room temperature the absorption was read against a blank at 517 nm. Inhibition of free radical of DPPH in percentage terms (I%) was calculated in the following way:

$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$

Where A_{blank} is the absorption of the control reaction (containing all reagents except the sample) and A_{sample} indicates the absorption of the sample.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract. All tests were done in triplicate. Values (mean±SD) of the extracts were compared with those values of BHT using student's t-test.

-β-carotene-linoleic acid assay

Antioxidant capacity was defined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acidoxidation (Dapkevicius et al., 1998). A stock of β-carotene-linoleic acid mixture was prepared by dissolving 0.5 mg of β-carotene in 1 ml chloroform (HPLC grade); 25 µl linoleic acid and 200 mg tween 40 were added, too. Chloroform was thoroughly evaporated by a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min), was added with proper shaking. Amount of 2500 µl of this reaction mixture were dispensed in test tubes and 350 µl portions of the extracts with the concentration of 2 g/l were added. The emulsion was incubated for 48 h at the room temperature. The same treatment was performed for the positive control using a synthetic antioxidant (BHT)

and also for the blank. After incubation, absorption of the mixtures was measured at 490 nm. Antioxidant capacity of the extracts, BHT and the blank were compared together.

-Determination of total phenolic content

The total phenolic contents of extracts were determined using the Folin–Denis method (Swain and Hills, 1959) with a little modification. This method is based on the reducing power of the phenolic hydroxyl groups; 0.1 ml of extract (0.1 mg/ml in ethanol) and 1 ml folin–ciocalteu reagent (1:1 with water) were mixed. After 5 min, 1 ml of 10% sodium carbonate (Na_2CO_3) was added and the volume was accurately adjusted to 25 ml by ethanol 96%. Then the mixture was kept at room temperature for 60 min. The absorption rate was measured with a spectrophotometer (Shimadzu UV- 2550; Shimadzu, Kyoto, Japan) at 765 nm against a blank. The determination was repeated three times. Total phenolic contents were calculated from standard curve of gallic acid, and then expressed as mg/g sample.

-Determination of total flavonoid

Total flavonoid content was determined by the method of Kumaranand Karunakaran (2007). Two ml of 2% $AlCl_3$ and 3 ml (50 g/l) sodium acetate solutions were added to 2 ml of extract solution. The absorption was recorded at 440 nm after 2.5 h at 20 °C. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid contents were calculated from standard curve of quercetin, and then expressed as mg/g sample.

Oxidative stability experiment

-Selection of oil

The selection of tested oil was based on the presence of unsaturated fatty acids composition. Soybean oil is rich in linolenic acid and linoleic acid. Schaal oven test as described by Economou et al. (1991) was conducted to evaluate the effect of antioxidants against oxidation during the accelerated oxidative storage of oils.

-Sample preparation

Antioxidant effects of the extracts on lipid peroxidation were evaluated in the soybean oil. Each sample (50 ml) was transferred to series of dark capped glass test bottles. Then *S. hortensis* L. extracts (200, 400 ppm) and BHT (200 ppm) were added to the dark capped glass test bottles and put in oven at 60 °C. The oxidation stability of oil was evaluated per week over a 7-week period by analyzing the peroxide values (PVs) and thiobarbituric acid-reactive substances (TBARs) levels. At least three samples of each category were reanalyzed to fulfill the requirements for statistical analyses.

-Peroxide value

PVs were measured by AOCS cd 8-53 official method (1990). For this purpose, 3 g of the substance was dissolved in glacial acetic acid (30 ml) and chloroform (20 ml). Then saturated KI solution (1 ml) was added to the mixture and it was stored for 15 min in the darkness. After adding distilled water (50 ml), the mixture was titrated by sodium thiosulfate (0.02 N) using starch as an indicator. A blank titration was done parallel to treatment and PVs (meq of oxygen/kg) was calculated using the following formula:

Peroxide value = 1000 S×N/W

When, S is the sodium thiosulfate solution volume (blank corrected) in ml; N is the sodium thiosulfate solution normality (0.02 N) and W is the oil sample weight (g).

-Thiobarbituric acid-reactive substances

TBARs were determined weekly, using the method of AOCS (1998). Oil sample (50 mg) was solved in 10 ml 1-butanol, mixed with 10 ml 0.2% thiobarbituric acid (TBA) in 1-butanol, incubated for 2 h in a 95 °C adjusted water bath and cooled for 10 min under tap water. The absorption was measured at 532 nm compared with a corresponding blank (reaction with all the reagents and treatments except the oil). The standard curve was determined by the TBARs of a series of aliquots (0.1-1 ml) of 0.2 mM 1, 1, 3, 3-tetraethoxypropane (Merck, S4258497) prepared in 1-butanol. The results were expressed as μmol malonaldehyde (MDA)/g of oil (n=3).

Statistical analysis

Oxidation experiments were carried out in triplicates. Results were averaged and statistically analyzed with one-way ANOVA using SPSS 16.0.

Results

The scavenging model of stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. It was found that the radical scavenging activities of all extracts rose with increasing concentration. IC_{50} values for the two extracts and BHT in DPPH assay are shown in Fig. 1. The methanol and ethanol extracts of *S. hortensis* L. have IC_{50} values of 31.5±0.7 and 37±0 μg/ml, respectively, that is inversely related to its antioxidant ability. This study showed that among the methanol and ethanol extracts of *S. hortensis* L., at the same concentration, the methanol extract possess significant antioxidant activities ($p \leq 0.05$). Overall antioxidant activity of the methanol and ethanol extracts was comparable to BHT ($p \leq 0.05$).

Fig. 2 shows inhibition of lipid peroxidation in response to extracts. Methanol and ethanol extracts and BHT inhibited

the linoleic acid oxidation as much as 87.5%, 74%, and 88.86%, respectively. In addition, at the same concentration methanol extract showed higher inhibition effect compared to the ethanol extract and to the control which was 6.55% ($p \leq 0.05$).

The total phenolic and flavonoid contents in methanol and ethanol extracts of *S. hortensis* L. are indicated in Table 1. These contents in the methanol extract were significantly higher ($p \leq 0.05$) than those of the ethanol extract.

The PVs levels during the storage of soybean oil over 49 days in the presence of various concentrations of *S. hortensis* L. methanol and ethanol extracts are illustrated in Fig. 3. The initial PVs levels in the oil were 2.4 meq/kg. The methanol and ethanol extracts reduced PVs at different concentrations (200, 400 ppm) compared to the control (0 ppm). However, for all samples PVs showed an increasing trend by the end of experiment. Considering control sample, the PV amounts increase significantly from day 7 to the end of the storage period ($p \leq 0.05$) even higher than amounts for samples with different extracts and BHT.

Fig. 4 shows the values of TBARs in BHT (200 ppm) and methanol and ethanol extracts (200, 400 ppm). TBARs values of the samples with different treatments were lower than that of the control during days even to 49th day ($p \leq 0.05$). TBARs values of oil samples containing methanol extract (200, 400 ppm) were significantly lower than the oil sample containing ethanol extract ($p \leq 0.05$). In this respect, we found that TBARs values of oil treated with methanol extract (400 ppm) and BHT starting from the 7th day to the end of storage period was comparable ($p > 0.05$).

It was found out that PVs and TBARs values of oil treated with all concentrations of the methanol and ethanol extracts and BHT were lower than the control over the 49 days of storage ($p \leq 0.05$). However, the methanol extract decreased the TBARs levels more effectively than the ethanol extract during whole of storage period ($p \leq 0.05$).

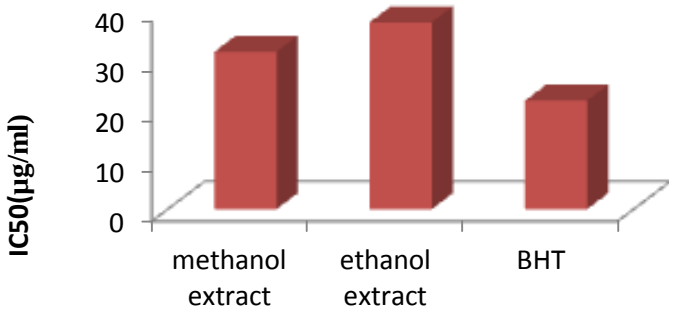

Fig. 1: Antioxidant activity of methanol and ethanol extracts of *S. hortensis* L. and BHT in DPPH assay

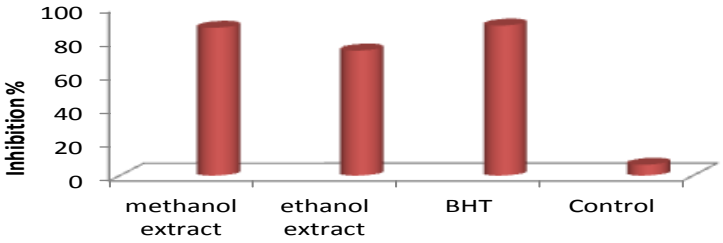

Fig. 2: Antioxidant activity of *S. hortensis* L. extracts defined as inhibition percentage through β-carotene–linoleic acid assay

Table 1: Total phenolic compounds and flavonoids amounts [a] in the methanol and ethanol extracts of *S. hortensis* L.

Extract type	Phenolic content (mgGAs/g extract)[b]	Flavonoid content (mg QEs/g extract)[c]
Methanol extract	101.58±0. 26	44.91 ±0.14
Ethanol extract	96±0.027	14.3 ±0.12

[a] Results are means of three different experiments
[b] GAs: Gallic acid equivalents
[c] QEs: Quercetin equivalents

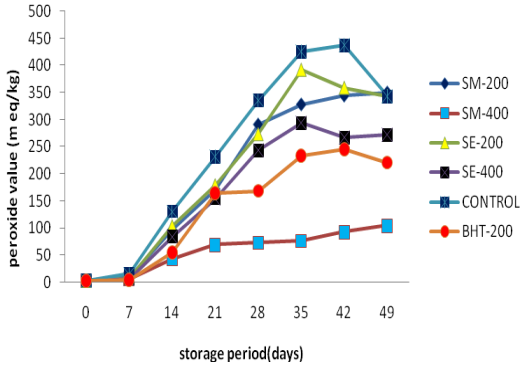

Fig. 3: Effect of ethanol extract (SE) and methanol extract (SM) of *S. hortensis* L. on peroxide formation in comparison with control and BHT in refined-bleached soybean oil at 60 °C up to 49 days

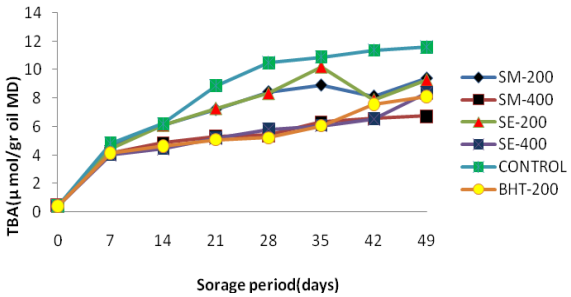

Fig 4: Effect of ethanol extract **(**SE**)** and methanol extract **(**SM**)** of *S. hortensis* L. on TBA formation in comparison with control and BHT in refined-bleached soybean oil at 60 °C up to 49 days

Discussion

In the present study, antioxidant activity of extracts and their abilities to inhibit lipid oxidations were different according to the type of extracts. The DPPH assay, which measures the ability of compounds to transfer labile H-atoms to radicals, is the most common method of antioxidant activity evaluation (Brand-Williams et al., 1995). Sarikurkcu et al. (2008) showed that antioxidant activity of the extract of *Marrubium globosum* was higher than all samples tested with an IC_{50} value of 157.26±1.12 µg/ml (Sarikurkcu et al., 2008). As shown in Fig. 2, the inhibition capacity percentage of the polar fraction of methanol extract (87.5%) was higher than ethanol one (74%) with less polar fraction, which is almost equal to the inhibition capacity of the positive control BHT (88.86%).

The researchers also found a decrease in the values of β-carotene–linoleic acid on both organic extracts; they reported that BHT was more potent than the plant extracts (Sarikurkcu et al., 2008; Tosun et al., 2009; Wu et al., 2009). According to Sarikurkcu et al. (2008), the inhibitory capacity percent of the methanol extract of *M. globosum* subsp. *globosum* (97.39%) was found higher than all samples, and almost equal to the inhibition capacity of the positive control BHT (97.44%). Essential oil of *M. globosum* subsp. *globosum* showed the weakest activity. In our study, antioxidant activity of the ethanol and methanol

extracts of *S. hortensis* can be attributed to their phenolic, flavonoids constituents (Pietta, 2000). Farombi et al. (2003) reported a potent antioxidant activity for terpenoids, so the difference in the antioxidative properties of plants can be due to their secondary metabolites. Moreover, the presence and synergism of different antioxidants in an extract determine the antioxidative properties of a specific extract (Duh, 1999).

In this study, we showed that the ethanol and methanol extracts of *S. hortensis* are able to inhibit both primary and secondary oxidation of soybean oil during storage. While PVs and TBARs levels of the soybean oil in the control group increased after incubating for seven days, a slight increase was observed in the samples contained ethanol and methanol extracts. Anwar et al. (2007) showed that BHT and BHA made higher inhibition to primary oxidation of oil than the plant extracts. Antioxidant activity of sorghum extract in the sunflower oil in presence of ferric ions showed that TBHQ made a higher inhibitory effect on the primary oxidation of the oil than the extract. Nevertheless their abilities to inhibit the secondary oxidation were similar (Sikwese and Duodu, 2007). Duh (1999) showed a higher inhibition in both primary and secondary oxidations for the water extract of Harng Jyur (*Chrysanthemum morifolium*) varieties in comparison with tocopherol and BHA in soybean oil. Such results have been attributed to the presence of several antioxidants with a range of solubility. Monfared et al. (2011) showed that the stability effects of the water extracts of *Urtica dioica* L. (post and pre flowering) with concentrations above 200 ppm on heated sunflower oil was higher than the BHT. This feature can be due to the presence of water soluble active ingredients such as flavonoids in the polar extract of plants (Arumugam et al., 2006; Rahmat et al., 2003). Such pattern was shown by Kamkar et al. (2010) on both water and methanol extracts of *Mentha pulegium* compared to BHT when added to the sunflower oil emulsion. Marinova and Yanishlieva (1997) reported that ethanol extract of *S. hortensis* L. had strong antioxidative activity during oxidation of sunflower oil at 100 °C.

Results of the colorimetric analysis of total phenolics are given in Table 1. Data obtained from the total phenolic assay supports the key role of phenolic compounds in free radical scavenging and/or antioxidant systems. As expected, amount of the total phenolic and total flavonoid were highest in polar fraction. There is a positive correlation between antioxidant activity potential and amount of phenolic compounds of the extracts. Sarikurkcu et al. (2008) showed that amount of the total phenolics was the highest in polar sub-fraction. Another study showed that the total phenolic concentrations of the *Astragalus sinicus* L. seed extracts were dependent on the solvent (Lim et al., 2011). The acetone extract had the most antioxidant effects, followed by the extracts from ethanol, hexane, and finally water. When acetone was used as extracting agent, the total phenolic concentration was approximately 4.1 times higher than that of the water extract. The acetone extract also contained higher amounts of total flavonoid than those of ethanol, hexane, and water extract. This result indicated that the total phenol and flavonoid concentration of *A. sinicus* L. seed extract was strongly affected by extractant. Several reports have shown a close relationship between antioxidant activity and the amount of total phenolics or total flavonoid (Cuvelier et al., 1992). Phenolic compounds are commonly found in both edible and non edible plants. They have been reported to have many effects such as antioxidant activity. Crude extracts of plant materials rich in phenols, retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The benefit of the antioxidant effects of plant materials on health, prevention of coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers in a trend that is moving towards functional foods with specific health effects (Löliger, 1991).

Conclusion

We have shown that *S. hortensis* extracts can be considered as an antioxidant for edible oils. Methanol and ethanol extracts showed antioxidant potency in a descending order when added to soybean oil. The protective effect of the *S. hortensis* extracts was comparable with widely used synthetic antioxidant BHT. Thus, methanol and ethanol extracts of *S. hortensis* could be prepared and added to the commercial vegetable oils as natural antioxidant and suitable alternative for some synthetic antioxidants.

Conflicts of interest

There is no conflict of interest.

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