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Evaluation of Antioxidant Capacity of the Ethanol Extract of Iranian *Mentha spicata*

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Abstract

Antioxidants are major ingredients that protect the quality of oils and fats by retarding oxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are widely used to prevent the oxidation of oils and fats and extend the shelf-life of lipid-containing foods. In recent years, their use in foods has suffered severe criticism, as consumers are becoming increasingly conscious of the safety of synthetic chemical additives; in addition, the use of these synthetic antioxidants is restricted because of their toxicity. This has led to an increasing interest in the search for naturally occurring antioxidants.

This study was designed to evaluate antioxidant capacity of the ethanol extract of Iranian *Mentha spicata* in the inhibition of free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ß-carotene/linoleic acid system. The extract showed weaker but close antioxidant capacity comparing to BHT, as a synthetic antioxidant. In this regard, the ethanol extract was able to reduce the stable free radical DPPH with an IC50 of 12 µg/ml and in ß-carotene/linoleic acid assay, inhibit the linoleic acid oxidation; exhibiting 61% inhibition at 2 g/l. These parameters in BHT were 5 µg/ml and 95%, respectively.

It seems that the ethanol extract of Iranian *Mentha spicata* has potent antioxidant effect which makes it as a potential antioxidant for oil and oily products.

Keywords: Antioxidant activity; Ethanol extract; Iranian *Mentha spicata*

Introduction

Lipid oxidation is a highly deteriorative process in food, as it leads to unacceptable properties for customers and loss in nutritional value. In addition, oxidation leads to health disorders such as atherosclerosis and carcinogenesis, amongst others. Oxidation of lipids occurs during raw material storage, processing, heat treatment and further storage of final products [1-3]. To retard or minimize oxidative deterioration, effective antioxidants are added to food.

Synthetic antioxidants have long been used, but their use has recently come into dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by consumers. There is, therefore, a growing interest in the identification of new, natural antioxidants that would serve as alternatives to the synthetic compounds [1-6].

Natural antioxidants, mainly in fruit and vegetables, have gained increasing interest among consumers because epidemiological studies have indicated that frequent consumption of vegetables and fruit is associated with a lower risk of cardiovascular disease and cancer [4-7].

Phenolic or polyphenolic compounds constitute one of the largest groups of plant secondary metabolites, which are therefore an integral part of the diet with significant amounts being reported in vegetables, fruit and certain beverages [8]. The antioxidant properties of polyphenols are mainly due to their redox properties. They act as free radical ferminators, hydrogen donors and metal chelators [9].

The Labiatae family includes about 220 genera and 3300 species which are widely used for various purposes [10]. Plants belonging to the Labiatae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties [11,12]. The Mentha genus is a member of this family and represents by about 6 species in the flora of Iran [13]. Mentha species are generally known under the name "na'na" and "pooneh" in Iran and commonly used as herbal tea, flavoring agent, and medicinal [14].

By considering the mentioned characteristics, the aim of the present study was to evaluate the in vitro antioxidant capacities of Iranian *M. spicata* ethanol extract.

Materials and Methods

Plant material and extraction

Aerial parts of *M. spicata* L. were collected from Gilan province (Iran) during the flowering period in spring 2010. A voucher specimen for this plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University, Tehran, Iran. The plants were dried in a dark place at room temperature. Dried leaves were powdered using an electric device and stored at refrigerator (4°C) until use.

Ethanol extract was prepared according to the method of Shyamala et al (2005) with some modifications [15]. Briefly, 15 g of dried and chopped leaves was extracted with 100 ml ethanol (HPLC grade, Merck) for 48 h with occasional shaking. The extract was filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator extractor. Ethanol extracts was kept in a closed dark glass bottle and stored at 4°C until use.

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Phytochemical screening of the extract

Phytochemical screening using thin layer chromatography (TLC) was carried out on the ethanol extract. The extract was subjected to TLC examination for group determination of the secondary materials. In this respect, Modified Dragendroff's reagent [16] for alkaloids, ferric chloride reagent (07875, Sigma) for phenolics, Naturstoff reagent [17] for flavonoids and vanilline/sulfuric acid reagent [18] for terpenoids were used. Solvent systems for developing of ready coated analytical TLC plates (Merck Silica gel 60 F254, 0.25 mm, Merck Company supplier, Tehran, Iran) were selected according to Wagner and Bladt (1996) [19].

Antioxidant activity

DPPH assay: The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH).This spectrophotometric assay was done using the stable radical DPPH as a reagent according to the method of Burits and Bucar (2000) [20]. Briefly, 50 µL of the extracts (various concentrations) were added to 5 ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical scavenging activities of the samples were calculated as percentages of inhibition according to the following equation: I% = (Ablank _ Asample/Ablank) × 100; Where Ablank is the absorbance of the control (containing all reagents except the test compound), and Asample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentages against extract concentration using PHARM/PCS-version 4. All tests were done in triplicate.

β-Carotene-linoleic acid assay: Antioxidant capacity was determined by measuring the inhibition of volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation according to the method of Dapkevicius et al. [21]. In this respect a stock solution of β -carotene-linoleic acid mixture was prepared as follows: 0.5 mg β -carotene (Merck, K15555836) was dissolved in1 ml of chloroform (HPLC grade) and 25 µl linoleic acid (Sigma, L1376-500MG) and 200 mg Tween 40(Merck, 822185) were added. After evaporation of chloroform, 100 ml oxygen-saturated distilled water was added with vigorous shaking. Then, 2500 µl aliqouts were dispensed into the test tubes, 350 µl of the extract (2 g/L) was added and the emulsion system incubated for 48 h at room temperature. The same procedure was done on both BHT (as positive control) and blank. In turn, absorbance of the mixture was measured at 490 nm. Antioxidative capacities of the extract were compared with those of BHT and blank.

Results

Ethanol extract composition

Phytochemical study showed that Flavonoid, terpenoid and phenolic compounds were major components of the *M. spicata* ethanol extract.

Free radical-scavenging activity

The ability to control free radicals was investigated by DPPH test. In the test, radicals were more strongly controlled by increasing extract concentration. The concentration of extract which caused 50% inhibition (IC50) is shown in (Table 1). The ability to control free radicals by Ethanol extract of *M. spicata*, which is 12 µg/ml, is weaker but close compared to BHT (5 µg/ml).

Inhibitory effect of the extract on lipid peroxidation

Based on available information, as shown in Figure 1, in the linoleic acid oxidation inhibition test in β-carotene – linoleic acid system, 61% controlling effect was obtained by ethanol extract of *M. spicata* in a concentration of 2 g/l. Moreover, for BHT as a synthetic antioxidant, 95% inhibitory effect was obtained in β-carotene - linoleic acid test. In this test, inhibitory effect of ethanol extract of *Mentha spicata* was lower than BHT but this extract effectively inhibited the linoleic acid oxidation compared to the control (5.3%).

Discussion

At present study, decrease in DPPH radical scavenging activity due to the ethanol (IC 50: 12 µg/mL) of *M. spicata* was higher than those reported by Sweetie et al. [22] on the ethanol extract (28.5 µg/ml) of *M. spicata* and Golluce et al. [23] on the methanol (74.4 µg/ml) *M. longifolia*.

In spite of our findings about decrease in the values of β-Carotene-linoleic acid on *M. spicata* ethanol extract (61%), Mata et al. did not show such decrease on *Mentha pulegium* extracts. In this respect, they showed that BHT was more potent than the water (about 1291%) and ethanol (about 1275%) extracts [24]. Moreover, the inhibitory effect of *M. spicata* extract on lipid oxidation in β-Carotene-linoleic acid test was much higher than Mentha longifolia extract (24%) and essential oil (36%) reported by Golluce et al. [23].

These characteristics of the ethanol extract of the *M. spicata* can be attributed to its phenolics, flavonoids and terpenoids constituents. These compounds have been shown in our phytochemical analysis. In this regard, Laximun-Ramma et al. showed a linear correlation between antioxidant activity and phenolic contents of the plant extracts, fruits and beverages [25]. Sugihara et al. [26] and Spencer et al. [27] discussed that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals, also. Moreover, Joshi et al. showed a potent antioxidant activity for terpenoids [28].

These discrepancies in the antioxidative properties of Mentha subspecies can be due to their ingredients [29]. In this regard, Duh [30]

Sample	DPPH (µg/ml)
Ethanol extract	12 ± 0.5
BHT	5 ± 0.2

Table 1: *In vitro* antioxidant activities of *M. spicata* ethanol extract and BHT in DPPH assay. Values were expressed as IC_{50}.

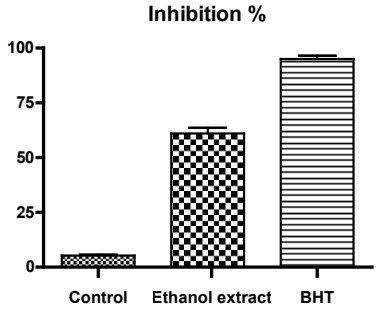

Figure 1: Antioxidant activity of *M. spicata* extract defined as inhibition percentage through β-carotene–linoleic acid assay.

discussed that the presence and synergism of different antioxidants in an extract will determine the antioxidative properties of a specific extract or essential oil.

In conclusion, it seems that ethanol extract of Iranian *Mentha spicata* can be considered as an natural antioxidant and also be purified and used for food and pharmaceutical industries.

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