Evaluation of the Antioxidant Properties of Five Mentha Species

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Abstract

The aim of the study was to evaluate the antioxidant and free radical scavenging properties and to determine the phenolic content of the ethanol extract from five Mentha species [M. longifolia (L.) Huds., M. piperita L., M. pulegium L., M. rotundifolia (L.) Huds., and M. spicata L.] The antioxidant activities of the extracts were investigated with two different methods, 2,2’-diphenyl-1-picrylhydrazyl radical (DPPH•) and 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS•+). M. piperita exhibited the strongest activity as a DPPH• scavenger. On the other hand, all the extracts were active in the ABTS•+ assay and no significant difference was observed in this assay. The total phenolic content (TPC) of the extracts was determined by Folin-Ciocalteu method and M. piperita showed the highest TPC. A high correlation was found between the DPPH• scavenging potency and the total phenolic content of the extracts (r² > 0.989).

Keywords: Antioxidant activity; Labiatae; Mentha species; Total phenolic content.

Introduction

Body functions rely on oxidation reactions which in turn produce reactive oxygen species (ROS) as by products or intermediates during normal metabolism. ROS is a collective term used to include both the oxygen radicals and some nonracial reactive derivatives of oxygen. A balance between formation and removal of these molecules is required to maintain normal physiological functions (1, 2). However, prolonged high level production of the ROS can cause problems because they can react with biological molecules including DNA, proteins and lipids, leading to cell and tissue injuries and pathological events. The role of free radicals and reactive oxygen species in the etiology of many chronic diseases such as atherosclerosis, cancer, inflammation, and neurodegenerative disorders like Parkinson’s and Alzheimer’s diseases, has been documented (3, 4). One effective way to eliminate ROS is with the help of antioxidant compounds. Therefore, attention is being focused on the use of antioxidants to protect the cells from biological damages due to free radicals (5, 6). Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods and to extend their shelf-life (7).

Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and/or activators of antioxidative defense enzyme systems to suppress the radical damages in biological systems (8, 9). Most of the antioxidants in use commercially [e.g., butylated hydroxytoluene (BHT) and butylated

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hydroxyanisole (BHA)] are synthetic and some of them have been suspected of causing or promoting negative health effects; therefore, some restrictions are placed on their applications and there is a trend to substitute them with naturally occurring antioxidants (2, 3). In recent years the search for natural antioxidants especially from plants has greatly increased and in the last few years some medicinal plants have been extensively studied for antioxidant activities (5, 10, 11).

A great number of aromatic, spicy, and medicinal plants contain chemical compounds, with antioxidant properties. Several studies that have been carried out on some of these plants have resulted in the development of natural antioxidant formulations for food, cosmetic, and other applications (7, 10). For example, Salvia officinalis L. and Rosmarinus officinalis L. (Labiatae) are well known for their antioxidant properties (12). Among the natural compounds, phenolic compounds constitute one of the major groups of herbal compounds acting as radical scavengers and antioxidants (13, 14).

The Labiatae family includes about 220 genera and 3300 species which are widely used for various purposes worldwide (15). Plants belonging to the Labiatae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties (1, 12). The genus Mentha is one of the important members of the family and represents by about 6 species in the flora of Iran (16). Mentha species are generally known under the name “na’na” in Persian and commonly used as herbal tea, flavoring agent, and medicinal plant. Infusion, decoction, and distilled water of the aerial parts of Mentha species have been used for centuries as tonics, carminative, digestive, stomachic, antispasmodic, and anti-inflammatory agents in Iranian Traditional Medicine (17, 18).

The literature data on the antioxidant activities of Mentha species is frequently scattered throughout the papers and the data available is often difficult to compare because of the differences in the methodologies. (1, 5, 14, 19-28). Thus, comparison of the antioxidant activities of the species, using a similar approach is necessary.

The objectives of the research were (1) to determine and compare the antioxidant activity and total phenolic content (TPC) of five Mentha species [M. longifolia (L.) Huds., M. piperita L., M. pulegium L., M. rotundifolia (L.) Huds., and M. spicata L.] by two different methods [2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS•+) and 2,2’-diphenyl-1-picrylhydrazyl radical (DPPH•) assays]; (2) to investigate a possible correlation between activity and phenolic content.

Experimental

Chemicals

All chemicals were purchased from Sigma (USA), Fluka Chemie (Switzerland) and Merck (Germany) companies.

Plant materials

Aerial parts of Mentha longifolia (L.) Huds., M. piperita L., M. pulegium L., M. rotundifolia (L.) Huds., and M. spicata L. were collected in Tehran province during the flowering period in summer 2006 and authenticated by M. Kamalinejad. A voucher specimen for each plant was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University (M. C.), Tehran, Iran. The plants were dried in the drying room with active ventilation at ambient temperature.

Preparation of the extracts

Dried and powdered aerial parts (100 g) from each species were macerated in ethanol at room temperature for 48 h. After filtration, each macerate (extract) was concentrated under reduced pressure. The dried extracts were dissolved in ethanol to a final concentration of 500 μg/ml (sample stock solution).

Measurement of antioxidant activity

DPPH assay

One ml of 0.3 mM DPPH• solution in ethanol was added to 2.5 ml of each sample at different concentrations (500, 250, 100, 50, 25, 10, 5, 2.5 μg/ml), Test tubes were vortexed and incubated for 30 min at room temperature in the dark; then, the absorbance values were determined at 518 nm in a Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). The inhibition percentage of DPPH radical was assessed by the
following formula:

\[
I_{DPPH}^\% = \left[ \frac{A_{\text{control}} - (A_{\text{sampel}} - A_{\text{blank}})}{A_{\text{control}}} \right] \times 100
\]

Ethanol (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH\(^\cdot\) solution (1 ml) plus ethanol (2.5 ml) was used as a negative control. Also, rutin solution (at the concentrations of 200, 100, 50, 20, 10, 5, 2.5, 1 μg/ml) was used as a positive control (11).

The \(I_{DPPH}^\%\) was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC\(_{50}\) value (inhibitory concentration), which is the concentration of sample (μg/ml) necessary to decrease by 50% the absorbance of DPPH\(^\cdot\).

**ABTS\(^{\cdot+}\) assay**

The ABTS radical cation, was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate solution and the mixture was kept in the dark at room temperature for 12-16 h before use. Prior the assay, the ABTS\(^{\cdot+}\) solution was diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. An aliquot (50 μl) of each sample at different concentrations (500, 250, 100, 50, 25, 10, 5 μg/ml) was added to 5 ml of diluted ABTS\(^{\cdot+}\) solution. After 10 min, the absorbance was measured at 734 nm. The Inhibition of ABTS radical cation in percent was calculated by the following formula:

\[
I_{ABTS^{\cdot+}}^\% = \left[ \frac{A_{\text{control}} - (A_{\text{sampel}} - A_{\text{blank}})}{A_{\text{control}}} \right] \times 100
\]

Ethanol (5 ml) plus each sample solution (50 μl) was used as a blank. ABTS\(^{\cdot+}\) solution (5 ml) plus ethanol (50 μl) was used as a negative control. Also, rutin solution (at the concentrations of 200, 100, 50, 20, 10, 5 μg/ml) was used as a positive control (29).

The \(I_{ABTS^{\cdot+}}^\%\) was plotted against the sample concentration and a logarithmic regression curve established in order to calculated the IC\(_{50}\) value (inhibitory concentration), which means the concentration of sample (μg/ml) necessary to decrease the absorbance of ABTS\(^{\cdot+}\) in 50%.

**Determination of total phenolic content**

The total phenolic content (TPC) of the samples was measured by Folin-Ciocalteau reagent using rutin as the reference compound (10). One ml of rutin solution in ethanol, (at the concentrations of 150, 100, 75, 50, 25 μg/ml) was mixed with 5 ml Folin-Ciocalteau reagent (diluted 1/10) and incubated at room temperature. After10 min, 4 ml of sodium carbonate solution (75 mg/ml) was added. The final solution was mixed thoroughly and allowed to remain for 30 min in a dark place. The absorbance was measured at 765 nm with a spectrophotometer. Then a linear calibration curve (absorbance versus concentration) was developed. The plot was found to be linear across the ranged assay (150-25 μg/ml, \(r^2>0.99\)).The same procedure was carried out with 1 ml of each sample in ethanol instead of rutin. The TPC for each extract [as μg rutin equivalent (RE)/mg of extract] was determined on the basis of the standard curve.

**Statistical analysis**

All the experiments were carried out in triplicate. The IC\(_{50}\) were presented by their respective 95% confidence limits. The TPC (μg/mg) were shown as mean±SEM. One-way analysis of variance (ANOVA) followed by Tukey’s post test was used to assess significant differences (p<0.05) between extracts. All the statistical analysis were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, San Diego, CA, USA).

**Results and Discussion**

There are several methods to determine the antioxidant capacity of plant extracts. However, the chemical complexity of extracts could lead to scattered results obtained from different techniques, depending on the test employed. Therefore, an approach with multiple assays in the screening work is highly advisable (9). Among these methods, two in vitro DPPH\(^\cdot\) and ABTS\(^{\cdot+}\) assays, are used as common and useful methods to evaluate the free radical scavenging activity and antioxidant capacity of extracts. They are excellent tools for determining the antioxidant activity of hydrogen donating and
Phenolic compounds are considered as a major group of compounds that contributed to the antioxidant activities of botanical materials because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore in this study, the antioxidant capacities (IC₅₀ values) of the ethanol extracts obtained from five Mentha species were measured using two methods and their total phenolic contents were determined and compared with each other.

According to the DPPH• assay (Figure 1 and Table 1), all the extracts exhibited a noticeable concentration-dependent antiradical effect but differed in their inhibiting activities. The highest scavenging activity was observed for *M. piperita* [IC₅₀=13.32 (12.12-14.64) μg/ml], while lowest for *M. spicata* [IC₅₀ = 87.89 (81.66-94.59) μg/ml]. The IC₅₀(DPPH•) values of the extracts increased in the following order: *M. piperita* < *M. pulegium* < *M. rotundifolia* ≤ *M. longifolia* < *M. spicata*. Based on the data obtained from the ABTS•+ scavenging test, the extracts had a concentration-dependent activity on this radical but no significant difference was seen (Figure 2 and Table 1). On the other hand, the extracts possessed a weaker inhibition effect on the ABTS radical cation than the DPPH radical. The phenolic content of the tested extracts differed significantly and ranged from 150.91 to 433.60 μg/mg. *M. piperita* showed the highest TPC (433.60±19.62 μg/mg), whereas *M. spicata* had the lowest content (150.9±5.14 μg/mg). The TPC of the extracts in decreasing order was: *M. piperita* > *M. pulegium* ≥ *M. rotundifolia* > *M. longifolia* > *M. spicata* (Figure 3 and Table 1). Therefore, it can be deduced that all the tested plants are rich in phenolic compounds and they have high antioxidant properties.

![Figure 1](image1.png)

**Figure 1.** Radical scavenging and antioxidant activities of the studied Mentha species extracts using DPPH•. Each point represents the mean of three experiments and the vertical bars represent the SEM.

![Figure 2](image2.png)

**Figure 2.** Radical scavenging and antioxidant activities of the studied Mentha species extracts using ABTS•+. Each point represents the mean of three experiments and the vertical bars represent the SEM.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>IC₅₀(DPPH•) (μg/ml)</th>
<th>IC₅₀(ABTS•+) (μg/ml)</th>
<th>TPC (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. longifolia</em></td>
<td>24.07 (21.34-27.14)²</td>
<td>185.60 (164.90-209.00)³</td>
<td>288.82 ± 11.55³</td>
</tr>
<tr>
<td><em>M. piperita</em></td>
<td>13.32 (12.12-14.64)³</td>
<td>153.80 (139.90-169.00)³</td>
<td>433.60 ± 19.62³</td>
</tr>
<tr>
<td><em>M. pulegium</em></td>
<td>17.92 (16.52-19.44)³</td>
<td>152.60 (139.60-166.80)³</td>
<td>358.10 ± 15.57³</td>
</tr>
<tr>
<td><em>M. rotundifolia</em></td>
<td>21.71 (19.90-23.67)³</td>
<td>158.90 (145.40-173.60)³</td>
<td>331.31 ± 6.51³</td>
</tr>
<tr>
<td><em>M. spicata</em></td>
<td>87.89 (81.66-94.59)³</td>
<td>173.80 (146.20-206.60)³</td>
<td>150.91 ± 5.14³</td>
</tr>
</tbody>
</table>

**Table 1.** IC₅₀ values of DPPH• and ABTS•+ scavenging activities and total phenolic contents (TPCs) of the studied Mentha species extracts.

Note: The IC₅₀(DPPH•) and IC₅₀(ABTS•+) values of the positive control, rutin, were measured as 6.90 (6.61-7.20) and 79.59 (69.73-90.86) μg/ml, respectively.
²The IC₅₀ values are presented with their respective 95% confidence limits.
³The TPC values are means ± SEM of three determinations.
⁴Letters (a-d) denote homogenous subsets at p < 0.05 (Tukey’s post test).
could be due to the composition of phenolics and other compounds in the mixture that may exert a synergistic effect. Generally, Labiatae species are rich sources of terpenoids and phenolic compounds (1). Therefore, it is likely that the phenolic constituents present in the Mentha species are, at least in part, responsible for the antioxidant and free radical scavenging activities. However, other compounds without a phenolic structure may explain, in part, the antioxidant activity found for these plants.

The results of this study indicate that the genus Mentha are favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit ROS attack on biological and food systems.

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