Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Antioxidant Activity of *Mentha Suaveolens* Ehrh. Extracts

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ARTICLE DETAILS

Article history:
Received on 22 April 2022
Modified on 13 June 2022
Accepted on 18 June 2022

Keywords:
Mentha Suaveolens,
Antioxidant Activity,
Polyphenols,
DPPH Scavenging,
B-Carotene.

ABSTRACT

Mentha is a genus belonging to the family of Lamiaceae, whose plants are among the most aromatic and spread in diverse environments worldwide. The aim of this study is to evaluate the total phenolics and flavonoids contents and the *in vitro* antioxidant activities of aqueous and ethanolic extract of *Mentha suaveolens*. The Folin–Ciocalteu method was used to determine the total phenols content while flavonoids were estimated according to the aluminum chloride colorimetric method. Also the antioxidant activities were determined by two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene linoleic acid. The total polyphenols content of the aqueous extract was 240.66±30.42µg GAE/ mgE and flavonoids were 18.35±0.57µg QE/mgE. The total polyphenols content of the ethanolic extract was 219.217±20.63µg GAE/mgE and flavonoids were 20.59±0.6µg QE/ mgE. In the DPPH assay the aqueous extract showed the higher scavenging capacity (IC_{50} = 0.035 ± 0.001 mg/ml), followed by ethanolic extract with IC_{50} of 0.049 ± 0.000 mg/ml. In the test of β-carotene /linoleic acid, the percentage of inhibition was 66.954 ± 2.64% of aqueous extract and 74.329 ± 2.03% of ethanolic extract.

INTRODUCTION

Reactive oxygen species (ROS) will act as damaging or signaling molecule depends on the delicate equilibrium between ROS production and scavenging. Because of the multifunctional roles of ROS, it is necessary for the cells to control the level of ROS tightly to avoid any oxidative injury and not to eliminate them completely. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of the nonenzymic as well as enzymic antioxidants. ROS are liberated by virtue of stress, and thus, an imbalance is developed in the body that damages cells in it and causes health problems [1].

Natural antioxidant compounds exhibit their antioxidant activity by various mechanisms including chain breaking by donation of hydrogen atoms or electrons that convert free radicals into more stable species and decomposing lipid peroxides into stable final products [2].

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Herbal polyphenolic compounds are secondary metabolites with a characteristic aromatic structure, which can be classified into fifteen groups according to the basic part of their molecule, as for example phenols, phenolic acid, flavonoids, anthocyanins, qui-nones, catechins and tannins, just to name a few [3]. Since ancient times, the human has been exploring the potential of plants to improve their health, often attributed to the presence of phenolic compounds with strong antioxidant properties. The antioxidant ability of phenolic components occurs mainly through a redox mechanism and allows the components to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators [4]. Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including anti oxidant activity [5].

The *Lamiaceae* family includes about 220 genera and 3300 species which are widely used for various purposes worldwide [6]. Plants belonging to the Labiatae family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties [7, 8]. The genus *Mentha* is an important member of the

family. It includes eighteen species and eleven hybrids, among which several species have economic importance due to their high-valued oil and good taste [9, 10]. *Mentha Suaveolens* (*Lamiaceae* family) locally is traditionally used to treat cold, digestive system ailment, hemorrhoid, fever, intestinal swelling, flu against chill, and as carminative and conception and fertility booster [11 - 14]. This study aims to determine the phenol and flavonoid contents of *Mentha Suaveolens* extracts and evaluate the antioxidant activity.

MATERIALS AND METHODS

1. Chemicals

Folin-Ciocalteu, aluminum chloride ($AlCl_3$), gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid and tween 40 were purchased from Sigma Chemical Co. (St. Louis, MO). Linoleic acid, β-carotene and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical Co. (Buchs, Switzerland).

2. Plant Material

Mentha suaveolens plant material was collected from Bougaa région, Wilaya of Sétif Northeast of Algeria.

3. Preparation of Plant Extract

3.1. Aqueous Extract

The aerial parts of plant material were cleaned with tap water, dried in the shade at room temperature for 2 weeks and ground into powder using an electric grinder. The aqueous extract was prepared by boiling 100g of *Mentha suaveolens* powder in distilled water for 15 minutes, The resulting mixture was filtered using Whattman filter paper and then evaporated in rotary vacuum evaporator at 45°C.

3.2. Ethanolic Extract

The ethanolic extract was obtained by maceration in water/ethanol mixture (20:80) for 24 h. The resultant extract was filtered through Whattman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C.

4. Determination of Total Polyphenol Content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al, [15] with slight modifications. A volume of 100 µL of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µL of sodium carbonate solution Na_2CO_3 (75 g/L) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/L) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

5. Determination of Total Flavonoids Contents

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method [16]. Briefly, 1 mL of 2% $AlCl_3$ in methanol was mixed with 1 mL of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/L) were used as standards for calibration curve and the total flavonoids content was expressed as micrograms quercetin equivalents (QE) per milligram of extract.

6. Evaluation of Antioxidant Activity

6.1. DPPH Free Radical-Scavenging Assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay [17]. After dissolving the extracts, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration and kept in the dark for 30 minutes at room temperature. Then, the absorbance of this solution was measured at 517nm. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

$$\text{RSA}\ (\%) = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100$$

Where,
RSA: Radical Scavenging Activity
A_{blank}: Absorbance of the control.
A_{sample}: Absorbance of extract.

6.2. β-Carotene/Linoleic Acid Assay

In this test, the antioxidant capacity of the extracts was determined according to the method described by Kartal et al. [18]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform, one milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added in the foil and the chloroform was evaporated

using evaporator. After evaporation, 100 mL of distilled water saturated with oxygen was added. Then 2.5 mL of this mixture were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added and the absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h respectively. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and with distilled water and methanol as a negative control. The antioxidant activity of extracts was calculated using the following equation:

$$AA\% = \frac{A_{sample}}{A_{BHT}} \times 100$$

Where,
A_{sample}: Absorbance of the Extract.
A_{BHT}: Absorbance of positive control BHT.

Statistical Analyses
The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

RESULTS AND DISCUSSION

1. Total Polyphenols and Flavonoids Contents
Total polyphenols compounds, total flavonoids of *Mentha suaveolens* extracts were estimated spectrophotometrically. The results are shown in Table 1, and are expressed as µg of gallic acid or quercetin equivalent per miligram extract, respectively, the aqueous extract is richer on polyphenols (240.66±30.42 µg GAE/mg extract) compared to ethanolic extract (219.217±20.63 µg GAE/mg extract). However, the ethanolic extract showed a high total flavonoids with value of (20.59±0.6 µg QE/mg extract), followed by the aqueous extract (18.35±0.57 µg QE/mg extract).

Table 1: Total polyphenols and flavonoids content of *Mentha suaveolens* extracts.

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	240.66±30.42	18.35±0.57
EE	219.217±20.63	20.59±0.6

AqE : aqueous extract, EE : ethanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent. Each value represents the mean ± SD (n = 3).

2. Antioxidant Activity Evaluation
Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress [19].

Natural antioxidant compounds exhibit their antioxidant activity by various mechanisms including chain breaking by donation of hydrogen atoms or electrons that convert free radicals into more stable species and decomposing lipid peroxides into stable final products [20].

The antioxidant activities of *Mentha suaveolens* extracts were determined by DPPH scavenging activity assay and the β-carotene bleaching inhibition. DPPH radical scavenging assay is the most popular and frequently used for the determination of antioxidant activity of plant extracts. Bleaching ability of β-carotene in linoleic acid system is another simple, reproducible, and time efficient method for rapid evaluation of antioxidant properties [21].

β-carotene undergoes rapid decolorization in the absence of an antioxidant. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing linoleate free radicals and other radicals formed in the system [22].

2.1. DPPH Radical Scavenging Activity
In DPPH, the sample was able to reduce the stable violet DPPH radical to the yellow DPPH-H, the results were expressed as IC_{50} values (Table 2). Aquous extract of *Mentha suaveolens* revealed higher antioxidant capacities with the value of (0.035 ± 0.001 mg/mL) than ethanolic extract (0.**0**49 ± 0.0**0**0 mg/mL).

Table 2: DPPH scavenging activity of *Mentha suaveolens* extracts and standards

Extracts	IC_{50}(mg/mL)
AqE	0.035 ± 0.001
EE	0.**0**49 ± 0.0**0**0
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

#: µg/ml. Each value represents the mean ± SD (n = 3).

2.2. β-carotene/Linoleic Acid Bleaching Assay
The highest antioxidant capacity was observed in the ethanolic extract (74.329 ± 2.03%) folewed by aquous extract (66.954 ± 2.64%) (Table 3).

The DPPH radical-scavenging capacity of the extracts could be explained by the presence of phenolic components [23]. The high correlation between the values of phenols concentration in plant extracts and antioxidant activity is well documented [24]. Sugihara *et al* (1999), discussed

that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals [25].

Table 3: Antioxidant activities of *Mentha suaveolens* extracts at 24 hours of incubation measured by β-carotene bleaching method

Extracts	Inhibition %
AqE	66.954 ± 2.64
EE	74.329 ± 2.03
BHT	100 ± 3.972
H2O	30.91 ± 3.864

Each value represents the mean ± SD (n = 3).

Different *Mentha* species have been reported to contain a range of components, including cinnamic acids, flavonoids, and their derivatives [26 - 28]. M. suaveolens extracts contain several phenolic compounds including rosmarinic acid, salvianolic acid, quercetin, caffeic acid, luteolin and apigenin, whose radical scavenging properties have been demonstrated. M. suaveolens manifested the best antiradical activity and this effect could be explained with the presence of the salvianolic acids, potent antioxidant compounds, detected exclusively in this species [29]. Indeed, Miller (1996) showed that the luteolin is a significantly more potent antioxidant than the synthetic antioxidant butylated hydroxytoluene (BHT) [30].

CONCLUSION

Extracts of *Mentha suaveolens* exhibited strong antioxidative activities including DPPH free radicals-scavenging activity and protection from β-carotene bleaching.

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