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DPPH RADICAL SCAVENGING AND β-CAROTENE BLEACHING ACTIVITIES OF *MENTHA ROTUNDIFOLIA* EXTRACTS

Mounira Merghem[1*] Wafa Nouioua[1] and Nawel Merouani[2]

[*1]Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natur and Life Sciences, Ferhat Abbas University, Sétif-1, Algeria.
[2]Laboratory of Applied Microbiology, Faculty of Natur and Life Sciences, Ferhat Abbas University, Sétif-1, Algeria.

ABSTRACT

Mentha rotundifolia L. (Lamiaceae) is a native plant of Algeria used in traditional medicine. This study was devoted to the determination of polyphenols, flavonoids contents of *M. rotundifolia* L. extracts. Those Extracts were prepared from aerial parts by using two solvents ethanol and distilled water. Ethanolic extract (EE) from the aerial parts presented the highest contents of polyphenols (335.392 µg GAE/mg) and Flavonoids (50 µg QE/mg),in comparison with aqueous extract (307.395 µg GAE/mg) and (37.5 µg QE/mg) respectively. As well, the ethanolic extract (EE) exhibited the highest antioxidant capacity of DPPH and the β-carotene bleaching inhibition test (0.022 ± 0.001 mg/mL and 83.499 ± 4.617%, respectively), followed by aqueous extract (0.163 ± 0.005 mg/mL and 65.571 ± 5.31 %). These preliminary results could be used to justify the traditional use of this plant, and its bioactive substances could be exploited for therapeutic purposes.

Keywords: *Mentha rotundifolia* L., polyphenols, antioxidant activity, DPPH radical scavenging activity.

1. INTRODUCTION

Therapeutic properties of medicinal plants are well recognized at global level. As an estimate, over 50% of modern clinical drugs have natural products' origin. The world Health Organization has emphasized on the use of traditional medicines and reported about 80% of population from developing countries relies on medicinal plants for their primary health care. Local people use these medicinal plants for the treatment of various ailments through their indigenous knowledge. However, due to modernization, traditional medicines are only practiced in remote rural areas[1].

Plants used in traditional medicine to contain a vast array of substances that can be used to treat chronic and even infectious diseases[2].

Lamiaceae species are considered of high importance because of their use in folk medicinal, culinary, cosmetics, flavoring and production of essential oils throughout the world. The genus *Mentha* which comprises 20 species distributed all over the world is among the major genera belonging to Lamiaceae family[3].

The genus *Mentha* (Lamiaceae) includes aromatic herbs of difficult taxonomic classification due to a great variability in their morphological characters and frequent hybridisation[4]. The species of the genus *Mentha* are cultivated in several countries of the world for essential oil production[5]. According to the flora of Algeria, this genus is represented by five major species: *Mentha rotundifolia*, *Mentha longifolia*, *Mentha spicata*, *Mentha aquatica* and *Mentha pulegium*[6].

Aerial parts of *Mentha* species have been widely used for the treatment of cold, cholera, bronchitis, tuberculosis, sinusitis and for their diuretic, carminative, antiflatulent, expectorant, antitussive and antioxidant properties[3].

Mentha rotundifolia (L.) Huds. is an hybrid between *M.longifolia* (L.) L. and *M. suaveolens* Ehrh. Some authors have considered *M.rotundifolia* (L.) Huds.as a synonym of *M. suaveolens* Ehrh[4].

M. rotundifolia (L.) Huds commonly known as 'apple mint' is a wild growing perennial,

herbaceous plant. It is widely distributed in North Algeria in sub-humid areas, along rivers in plains and mountains where it is known as "Timija or Timarssat"[5].

In Algeria *M. rotundifolia* is widely used e.g. leaf decoction is made for topical application to treat furunculosis and abscesses, to reduce fever and as a mouthwash for dental pains. In addition, the plant is reported to treat bronchitis, cough, and ulcerative colitis. It is also taken as tonic, used as stimulant, stomachic, carminative, analgesic, choleritic, antispasmodic, sedative, and hypotensive as well as a common spice[5].

The study aims to determine the polyphenols, flavonoids of two extracts of the Algerian species *Mentha rotundifolia* L. collected from region of Amoucha, province of Setif, Northeast of Algeria, and to assess the antioxidant activity using two different methods DPPH assay and β-carotene/linoleic acid assay

2. MATERIALS AND METHODS

2.1. Chemicals

Folin-Ciocalteu, aluminum chloride ($AlCl_3$), gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 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).

2.2. PLANT MATERIAL

Mentha rotundifolia was collected in June, from Amoucha region, Wilaya of Sétif in Northeast of Algeria.

2.3. Preparation of plant extract

2.3.1. Aqueous extract

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

2.3.2. Ethanolic extract

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

2.4. Determination of total polyphenol content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al,[7] 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.

2.5. Determination of total flavonoids contents

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method[8]. 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.

2.6. Evaluation of antioxidant activity

2.6.1. DPPH free radical-scavenging assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl(DPPH) assay[9]. After dissolving the aqueous extract in distilled water, the ethanolic extract in ethanol, 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. The mixture was shaken vigorously and then kept in the dark for 30 minutes at room temperature. Then, the absorbance was measured at 517nm. BHT and gallic acid were used as standards. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

A_{blank}: Absorbance of the control.

A_{sample}: Absorbance of the reagent with extract.

2.6.2. β-carotene/linoleic acid assay

In this test, the antioxidant capacity of the

extracts was determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration or bleaching) by the oxidation products of the acid linoleic[10]. 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. The chloroform was evaporated using evaporator at 45°C. Then 100 mL of distilled water saturated with oxygen was added. 2.5 mL of this prepared β-carotene solution were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added before incubation for 48h at room temperature. 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 absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h. The antioxidant activity of extracts was calculated using the following equation:

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

A_{sample}: Absorbance in the presence of the Extract; A_{BHT}: Absorbance in the presence 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.

3. RESULTS AND DISCUSSION

3.1. Total polyphenols and flavonoids contents

All extracts had noticeable phenolic contents and flavonoid significantly varied among the studied species (Table 1). The uppermost amounts were observed for *M. rotundifolia* extracts AqE extract had a total phenolic content of (307.395± µg GAE/mg extract) and flavonoids (37.5 ± µg QE/mg extract). While, ethanolic extract had a total phenolic content of (335.392± µg GAE/mg extract) and flavonoids (50± µg QE/mg extract)

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

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
AqE	307.395	37.5
EE	335.392	50

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

The Polyphenols possess an ideal structural chemistry for free radical-scavenging activities, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis[11,12].

3.2. Antioxidant activity evaluation

3.2.1. DPPH radical scavenging activity

Substances which are able to donate hydrogen or an electron to DPPH•, can be considered as antioxidants and therefore radicals scavengers[13].To determine the efficacy of extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against the extract concentration. All the extracts exhibited a noticeable effect which varied significantly among species (Table 2). The results of the DPPH test showed a powerful antioxidant activity with a very similar IC_{50} for the ethanolic (IC_{50}=0.022 ± 0.001 mg/ml) and aqueous (0.163 ± 0.005 mg/ml).

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

Extracts	IC_{50}(mg/mL)
AqE	0.163 ± 0.005
EE	0.022 ± 0.001
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

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

This activity is generally dependent on total phenol content[14]. 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[15,16].

Mentha species are then a possible source for rosmarinic acid, a compound that has attracted a great deal of attention due to its reported health benefits. For the studied Mentha species, a direct correlation between total antioxidant capacity and rosmarinic acid content, indicating that this phenolic acid significantly contributes to this biological activity[17].

3.2.2. β-carotene/linoleic acid bleaching assay

In this test, the antioxidants give hydrogen molecules to the media, which stops the peroxidation of linoleic acid, the hydrogen also scavenges singlet oxygen responsible of linoleic acid peroxidation[18]. The results of the inhibition of β-carotene oxidation in the presence of extracts after 24 hours of incubation was presented in table 3. The antioxidant activity of the tow extracts in the β-carotene/linoleic acid assay was (83.499 ± 4.617%) for the ethanolic extract and (65.571 ± 5.31%) for the aqueous extract.

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

Extracts	Inhibition %
AqE	65.571 ± 5.31
EE	83.499 ± 4.617
BHT	100 ± 3.972
H2O	30.91 ± 3.864

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

The Mentha species are cited as favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit ROS attack on biological and food systems[19].

4. CONCLUSION

In the present study, we noticed that ethanolic extracts and aqueous extract of *Mentha rotundifolia* were rich in phenolics and flavonoids. Moreover, a good antioxidant activity of the extracts was confirmed by many tests. According to our results, it is clear that this plant is good for humanity.

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