

## Research Article



## Antioxidant and Antimicrobial Activity of Aqueous and Methanolic Extracts of *Mentha rotundifolia* L. from Algeria

Meryem Seladji\*, Nacéra Belmekki, Chahrazed Bekhechi, Nassima Bendimerad

Université de Tlemcen, Laboratoire des Produits Naturels (LAPRONA), Faculté des SNV-STU, Département de Biologie, Nouveau pôle la Rocade 2 Mansourah, BP119, Tlemcen 13000, Algérie.

\*Corresponding author's E-mail: mery\_seladji@yahoo.fr

Accepted on: 06-03-2014; Finalized on: 30-04-2014.

### ABSTRACT

Plants are an essential and integral part of complementary and alternative medicine due to their ability to generate secondary metabolites that are used to restore health and treat many diseases. The aim of the present study was to determine the antioxidant and antimicrobial activities of methanolic and aqueous extract of stems and leaves of *Mentha rotundifolia* L. from Algeria. The amounts of total phenolics solvent extracts (methanol and water extract) for the two parts of plant were determined spectrometrically. From the analyses, leaves aqueous extract had the highest total phenolic content ( $20.75 \pm 0.643$  mg GAE/g). The highest total flavonoids content was measured in methanolic leaves extract ( $1.97 \pm 0.035$  mg CAE/g). However the methanolic leaves extract had the highest DPPH scavenging ability with the lowest  $IC_{50}$  value ( $0.7 \pm 0.028$  mg/ml), the same tendency was observed with ferric reducing power. Concerning  $\beta$ -carotene bleaching assays results showed that the stems aqueous extract exhibited the highest antioxidant ability with an  $IC_{50}$  near than standards ( $0.52 \pm 0.024$  mg/ml). The antimicrobial activity was studied; all extracts were tested against five bacteria and three fungal species.

**Keywords:** Aqueous extract, Antioxidant activity, Antimicrobial activity, *Mentha rotundifolia* L., Methanolic extract.

### INTRODUCTION

The medicinal plants have been main source for drugs over many centuries in many countries, in both developed and developing world. Traditional medicines products are not officially recognized in many countries, and the European Union presently developing regulatory laws for quality traditional medicines. It is estimated that at least 25% of all modern medicines are derived either directly or indirectly from medicinal plants. Traditional medicines play important role in world health treating millions of people.<sup>1</sup> The medicinal property of herbs is due to the presence of different complex chemical substance as secondary metabolites, which are exclusively accumulated in different parts of the plants.<sup>2</sup>

Medicinal plants are rich sources of antimicrobial agents. Many infectious diseases have been known to be treated with herbal extracts which are known to have a vast potentiality. The clinical efficacy of many antibiotics is being threatened by the emergence of multidrug-resistant pathogens.<sup>3</sup> As an alternate source to the existing antibiotics; there is an urgent need to discover new antimicrobial compounds from various medicinal plants which can be used to treat many infectious diseases.

Several reports indicate that the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which including flavonoids which are important in plant defense mechanisms against invading bacteria and other types of environmental stress.<sup>4-7</sup> Flavonoids have long been recognized to possess anti-inflammatory, anti-allergic, antiviral and anti-proliferative activities.<sup>4,8</sup> These compounds are of great

value in preventing the onset and / or progression of many human diseases.<sup>9</sup> The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species.<sup>6</sup> Antioxidants are compounds that help delay and inhibit lipid oxidation and when added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life.<sup>10</sup>

Lamiaceae species are considered of high importance because of their use in folk medicine, 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.<sup>11</sup> It comprises herbaceous, perennial plants, common in temperate climates in Mediterranean region, Australia and South Africa.<sup>12</sup> Aerial parts from *Mentha* species have been widely used for the treatment of cold, cholera, bronchitis, tuberculosis, sinusitis and for their diuretic, carminative, anti flatulent, expectorant, anti tussive and antioxidant properties.<sup>11,13</sup>

Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components.<sup>14</sup> 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.<sup>15</sup> In Algeria the genus is represented by five species namely: *M. rotundifolia*, *M. longifolia*, *M. spicata*, *M. aquatica* and *M. pulegium*.<sup>16</sup>



*Mentha rotundifolia* L. is a long-lived plant, with a strong apple odor, drawn up stems, small or average (80 cm maximum), always covered with a thick sleeping bag with a ramified rhizome.<sup>17</sup> *Mentha rotundifolia* (L.) Huds. is a hybrid between *M. longifolia* (L.) and *M. suaveolens* Ehrh.,<sup>18</sup> but, some authors considered *M. rotundifolia* (L.) Huds. as a synonym of *M. suaveolens* Ehrh.,<sup>19</sup> which is always used as condiment. It has been applied in the traditional medicine for a wide range of actions: tonic, stomachic, carminative, analgesic, antispasmodic, anti-inflammatory, sedative, hypotensive and insecticidal.<sup>20</sup> However many studies have been carried on the chemical composition and biological activities of essential oils of *M. rotundifolia*.<sup>14,21-23</sup> Until the investigations of *M. rotundifolia* extracts are still limited to an extract on the corrosion of steel.<sup>24</sup>

To the best of our knowledge, there are no previous reports concerning *in vitro* antioxidant and antimicrobial activities of these plant parts extracts. The purpose of this study was to determine the total phenolic and the total flavonoid contents of methanol and aqueous extract of stems and leaves of *Mentha rotundifolia* L. from Algeria, to evaluate their antioxidant activities and to study the antimicrobial activities of those extracts on various bacterial and fungal species.

## MATERIALS AND METHODS

### Plant material

Plant materials were collected in April 2013 at Tlemcen, Algeria. Botanical identification of the plant was conducted by Professor Noury Benabadiji, "Laboratoire d'Ecologie et Gestion des Ecosystèmes", Abou Bekr Belkaid University, Tlemcen (Algeria). A Voucher specimen of the plant was deposited in the Herbarium of this laboratory. Plant samples were dried in the shade and conserved for future use.

### Preparation of extracts

#### Aqueous extract

10g of powder dissolved in 150 ml of water leaves distilled water were heated to reflux for 2 hours, after cold filtration, the filtrate was then evaporated to dryness under 65°C at reduced pressure using a rotary evaporator (Büchi Rotavapor R-200). The residues were then dissolved in 3 ml of methanol.<sup>25</sup>

#### Methanolic extract

A sample of 2.5 g of powder sheets was macerated in 25 ml of absolute methanol in magnetic stirring for 30 min. The extract then was stored at 4 °C for 24 h, filtered and the solvent evaporated. Dry under reduced pressure at 50°C at pressure using an evaporator rotary (Büchi Rotavapor R- 200). The resulting solutions were evaporated under vacuum at 50°C. The residues were then dissolved in 3 ml of methanol.<sup>26</sup>

### Determination of total phenolic content

The total phenolic in leaves and stems aqueous and methanolic extracts content was determined by spectrometry using "Folin-Ciocalteu" reagent assay.<sup>27</sup> A volume of 200 ml of the extract was mixed with 1 ml of Folin- Ciocalteu reagent diluted 10 times with water and 0.8 ml of a 7.5% sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

### Determination of total flavonoid

The total flavonoid content in leaves and stems of methanolic and aqueous extracts was determined by a colorimetric assay.<sup>28</sup> 500 µL of catechin standard solution with different concentrations or methanolic extracts was mixed with 1500 µL of distilled water in a test tube, followed by addition of 150 µL of a 5% (w/v) NaNO<sub>2</sub> solution, at time t 0. After 5 min, 150 µL of AlCl<sub>3</sub> at 10% (m/v) was added. After 6 minutes of incubation, at room temperature, 500 µL of NaOH (1 M) were added. The mixture was homogenized immediately after the end of the addition. The absorbance of the solution was measured at 510 nm against the blank. Results were expressed as milligram catechin equivalents of dry weight (mg CE/ g DW).

### DPPH Free-Radical-Scavenging Assay

The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of the stable 2,2-diphenyl-2 picrylhydrazyl (DPPH) radical.<sup>29</sup> Fifty microliter of various concentrations of the extracts in methanol were added to 1950 ml of a 0.025 g/l methanol solution DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

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

Where a blank is the absorbance of the control reaction (containing all reagents except the test compound), a sample is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid (AA) methanol solution and tert-butyl-4-hydroxyanisole (BHA) methanol solution were used as positive control.

### Reducing power assay

Reducing power of all the extracts of *M. rotundifolia* was determined as described by Oyaizu.<sup>30</sup> Different concentrations of plant extract solutions were mixed with



phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. After incubation, 2.5ml of 10% trichloroacetic acid was added and the mixture was then centrifuged at 3000 rpm for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and 0.1% ferric chloride (0.5ml). The absorbance of the mixture was measured at 700 nm. A higher absorbance indicates a higher reducing power. The assays were carried out in triplicate and the results are expressed as mean  $\pm$  standard deviation. The extract concentration providing absorbance of 0.5 (IC<sub>50</sub>) was calculated from the graph of absorbance at 700 nm against extract concentration, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) and tert-butyl-4-hydroxyanisole (BHA) were used as standards.

### $\beta$ Carotene bleaching inhibition capacity

Antioxidant activity based on the  $\beta$ -carotene/ linoleic acid method was evaluated by measuring the inhibition of the bleaching of  $\beta$ -carotene by the peroxides generated during the oxidation of linoleic acid.<sup>31</sup> Two mg of  $\beta$ -carotene were dissolved in 20 ml chloroform, and 4 ml of this solution were added to linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated water were added. An emulsion was obtained by vigorously shaken, an aliquot (150 ml) of which was distributed in 96 well microtitre plate and methanolic solutions of the test samples (10 ml) were added. Twice replicates were prepared for each extract concentration. The microplate was incubated at 50 °C for 120 min, and the absorbance was measured at 470 nm using a EAR 400 microtitre reader (Multiskan MS, Labsystems). Readings were performed both immediately (t 0 min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in terms of bleaching inhibition of the  $\beta$  carotene using the following formula:

$$\beta \text{ carotene bleaching inhibition (\%)} = [(S - A_{120}) / (A_0 - A_{120}) \times 100]$$

Where A<sub>0</sub> and A<sub>120</sub> are the absorbances of the control at 0 and 120 min, respectively, and S the sample absorbance at 120 min. The results were expressed as IC<sub>50</sub> value (mg/ml).

### Antimicrobial activity

#### Microbial strains

The extracts were individually tested against a panel of 8 microorganisms including six bacteria species, two Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876), three Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603), yeast (*Candida albicans* ATCC 10231) and two filamentous fungi (*Aspergillus flavus* MNHN 994294 and *Aspergillus fumigatus* MNHN 566).

### Inhibition zone determination by disc diffusion assay

Antimicrobial activity of extracts was screened for their inhibitory zone by the agar disc-diffusion method. The inoculums for the assays were prepared by diluting cell mass in 0.9% NaCl solution, adjusted to 0.5 McFarland scale, confirmed by spectrophotometrical reading on Specord 200 Analytikjena (Germany) at 625nm ( $\lambda = 0.08 - 0.1$ , corresponding to 10<sup>8</sup> CFU/ml) for bacteria and 530nm ( $\lambda = 0.12 - 0.15$ , corresponding to 1-5 x 10<sup>6</sup> CFU/ml) for yeasts. For filamentous fungi, spore suspensions were prepared from 1 week old cultures on PDA plates at 25°C and standardized by adjusting the transmittance to 68 to 82% at 530nm.<sup>32</sup> One milliliter of standardized suspension of the tested microorganisms (10<sup>6</sup> CFU/ml for yeasts and bacteria except, *S. aureus* at 10<sup>7</sup> CFU/ml and filamentous fungi at 10<sup>4</sup> spores/ml) was spread on the solid media plates, using Mueller–Hinton agar (Sigma, India) for Bacteria, Sabouraud Dextrose Agar (Sigma, India) for yeasts and PDA (Sigma, Spain) for filamentous fungi. They were “flood-inoculated” onto the surface of the solid media plates. After drying, a sterile paper discs (6mm in diameter) impregnated with 10  $\mu$ l of the plant extracts dissolved in pure dimethyl sulfoxide (DMSO) at a final concentration of 30 mg/ml (300 $\mu$ g/disc) were applied in the Petri dish. A disc prepared in the same condition with only the corresponding volume of DMSO (Sigma, France) was used as a negative control. The activity was determined by measuring the inhibitory zone diameter in mm after incubation at 37°C/24h for bacteria, at 30°C/48h for yeasts and at 25°C/72h for filamentous fungi. Nystatin (30 $\mu$ g/disc) was used as reference antifungal against yeasts and filamentous fungi and Cephalexin (30 $\mu$ g/disc), Amoxicillin (25 $\mu$ g/disc) and Vancomycin (30 $\mu$ g/disc) were used as positive controls against bacteria. The data used was the mean of two replicates.

### Statistical analysis

All experiments were performed at least in triplicate and the results are presented as mean  $\pm$  standard deviation (SD).

## RESULTS AND DISCUSSION

### Total phenolic and total flavonoid content

The total phenol and total flavonoid contents of extracts are shown in Table 1. The results obtained for our extracts show that the total phenolics ranged from 3.96 $\pm$ 0.35 mg GAE/g to 20.75 $\pm$ 0.64 mg GAE/g (Table 1). The extracts having extracted the highest total phenolics are: aqueous extract leaves (20.75 $\pm$ 0.64 mg GAE/g) and methanolic extract leaves (13.46 $\pm$  0.97 mg GAE/g). The lowest contents of total phenolics were obtained with the methanolic extract stems (3.96 $\pm$ 0.35 mg GAE/g), followed by aqueous extract stems (10.40 $\pm$ 0.61 mg GAE/g). For the total flavonoid the leaves extracts were higher than stems content with 2.19 $\pm$ 0.08 mg CAE/g for methanolic extract and 1.97 $\pm$ 0.03 mg CAE/g for aqueous extract.



However the flavonoid content of stems methanolic and aqueous extracts were  $1.6 \pm 0.01$  mg CAE/g and  $0.59 \pm 0.03$  mg CAE/g respectively. As can be seen, high phenol content was not always accompanied by high flavonoid content concentration, but the percentage contribution

of flavonoid to total phenol varied between aqueous leaves extracts (9.4 %) and the others which are similar for aqueous, methanol stems and leaves methanol extracts.

**Table 1:** Total phenolic and flavonoid contents of *Mentha rotundifolia* methanolic and aqueous extracts of leaves and stems

| Parameter            | Leaves           |                  | Stems            |                 |
|----------------------|------------------|------------------|------------------|-----------------|
|                      | Aqueous          | Methanol         | Aqueous          | Methanol        |
| Phenol content*      | $20.75 \pm 0.64$ | $13.46 \pm 0.97$ | $10.40 \pm 0.61$ | $3.96 \pm 0.35$ |
| Total flavonoid **   | $1.97 \pm 0.035$ | $2.19 \pm 0.08$  | $1.6 \pm 0.01$   | $0.59 \pm 0.03$ |
| Flavonoid/Phenol (%) | 9.4              | 16               | 15               | 14.8            |

\*Expressed as mg GAE/g of dry plant material; \*\* Expressed as mg CE/g of dry plant material; The data are displayed with mean  $\pm$  standard deviation of twice replications; Mean values followed by different superscript in a column are significantly different ( $p < 0.05$ ).

### Antioxidant activity

In the present study, three commonly used antioxidant evaluation methods such as DPPH radical scavenging activity, reducing power assay and  $\beta$ -Carotene bleaching inhibition capacity were chosen to determine the antioxidant potential of the methanolic and aqueous extracts of leaves and stems of *Mentha rotundifolia*. All the extracts tested showed an antioxidant activity.

### DPPH Free-Radical-Scavenging Assay

The identification of antioxidants from medicinal plants is a fast-growing field of research, and many antioxidants have been investigated by several methods. The DPPH assay is a quick, reliable, and low-cost method that has frequently been used to evaluate the antioxidative potential of various natural compounds.<sup>33</sup>

IC<sub>50</sub> value was determined from plotted graph of scavenging activity against the different concentrations of *M. rotundifolia* extracts, ascorbic acid, and BHA. The scavenging activity was expressed by the percentage of DPPH reduction after 30 min of reaction. The measurements were duplicate and their scavenging effects were calculated based on the percentage of DPPH scavenged.<sup>34,35</sup>

The obtained results are summarized in table 2 and the methanolic extract leaves ( $0.7 \pm 0.03$  mg/ml) exhibited scavenging activity against DPPH radicals in vitro of all studied extracts of *M. rotundifolia*. For methanolic extract stems ( $1.38 \pm 0.02$  mg/ml), aqueous extract leaves ( $1.01 \pm 0.01$  mg/ml) and aqueous extract stems ( $1.07 \pm 0.01$  mg/ml). These capacities of all extracts were less than the synthetic antioxidant ascorbic acid ( $0.12 \pm 0.08$  mg/ml) and BHA ( $0.09 \pm 0.03$  mg/ml), also determined in parallel experiments.

### Reducing antioxidant power assay (FRAP)

The antioxidant capacity of leaf extracts was evaluated by FRAP assay because it also showed high reproducibility.<sup>36</sup> Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of

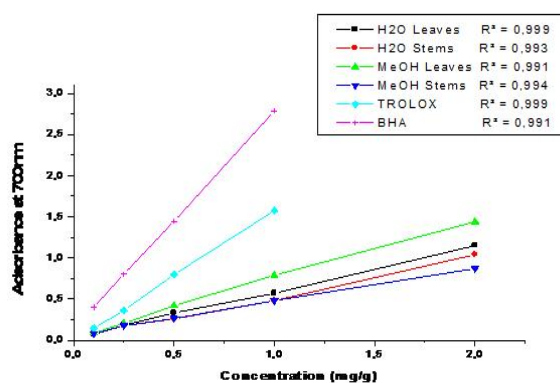
$Fe^{3+}$  to  $Fe^{2+}$  by donating an electron, Therefore, the  $Fe^{2+}$  can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.<sup>37,38</sup>

Reducing power of methanolic and aqueous extracts of *Mentha rotundifolia* and standards (BHA and TROLOX) using the potassium ferricyanide reduction method were described in Figure 1.

**Table 2:** IC<sub>50</sub> (mg/ml) values of different methanolic and aqueous extracts of *M. rotundifolia*.

| Extracts        | DPPH IC <sub>50</sub> (mg/ml) |
|-----------------|-------------------------------|
| Methanol leaves | $0.7 \pm 0.03$                |
| Aqueous leaves  | $1.01 \pm 0.01$               |
| Methanol stems  | $1.38 \pm 0.02$               |
| Aqueous stems   | $1.07 \pm 0.01$               |
| Ascorbic acid   | $0.12 \pm 0.08$               |
| BHA             | $0.09 \pm 0.03$               |

The data are displayed with mean  $\pm$  standard deviation of twice replications; Mean values followed by different superscript in a column are significantly different ( $p < 0.05$ ).



**Figure 1:** Total reducing power of methanolic and aqueous extracts of *M. rotundifolia*.

Methanolic leaves extracts of *M. rotundifolia* showed strong ferrous iron chelating activity in a dose dependent manner with an IC<sub>50</sub> value of 0.6 mg/ml. However, methanol stems extract displayed little reducing power under the experimental concentrations.

### β-Carotene-linoleic acid assay

In this model system, β-Carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. This is due to the oxidation of linoleic acid that generates free radicals that attacks the highly unsaturated β-Carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs the β-carotene molecule loses its conjugation and, as a consequence, the characteristic orange color disappears. The presence of antioxidant avoids the destruction of the β-carotene conjugate system and the orange color is maintained. The obtained results are summarized in table 3.

**Table 3:** IC<sub>50</sub> (mg/ml) values of β-carotene-linoleic acid assay of methanolic and aqueous extracts of *M. rotundifolia*.

| Extracts        | β-carotene IC <sub>50</sub> (mg/ml) |
|-----------------|-------------------------------------|
| Methanol leaves | 1.72± 0.03                          |
| Aqueous leaves  | 0.97 ±0.03                          |
| Methanol stems  | 0.62 ± 0.02                         |
| Aqueous stems   | 0.52± 0.02                          |
| Gallic acid     | 0.435 ± 0.003                       |
| Trolox          | 0.242 ± 0.003                       |

The data are displayed with mean ± standard deviation of twice replications; Mean values followed by different superscript in a column are significantly different (p<0.05).

Oxidation of the linoleic acid was effectively inhibited by the parts extracts of *M. rotundifolia*.

**Table 4:** Antimicrobial activity of different extracts of *M. rotundifolia* using disc diffusion method (inhibition zones, mm)

| Microorganism                 | Leaves   |          | Stems     |          | Controls          |         |          |          | Negative Control |
|-------------------------------|----------|----------|-----------|----------|-------------------|---------|----------|----------|------------------|
|                               | Extracts |          |           |          | Positive Controls |         |          |          |                  |
|                               | Aqueous  | Methanol | Aqueous   | Methanol | AMX               | VA      | CN       | NY       |                  |
| <i>Staphylococcus aureus</i>  | 6.5±0.7  | 9.5±0.7  | 6.3±0.4   | 6.1±0.2  | 26.0±0.0          | 16.0±0  | 28.0±0.0 | nt       | na               |
| <i>Bacillus cereus</i>        | na       | 07.0±0.0 | 6.11±0.17 | na       | 25.0±0.0          | 15±0.4  | na       | nt       | na               |
| <i>Escherichia coli</i>       | 9.9±0.1  | 10.4±0.8 | 6.12±0.2  | 7.5±0.7  | 12.0±0.2          | 8.0±0.0 | 18.0±0.1 | nt       | na               |
| <i>Pseudomonas aeruginosa</i> | 6.1±0.1  | 9.0±0.0  | na        | 6.5±0.7  | na                | na      | na       | nt       | na               |
| <i>Klebsiella pneumoniae</i>  | na       | 6.1±0.2  | 6.12±0.2  | 7.0±0.0  | na                | na      | 10.0±0.7 | nt       | na               |
| <i>Candida albicans</i>       | 8.2±0.3  | 10.0±0.0 | na        | 6.5±0.7  | na                | nt      | nt       | 19.0±0.8 | na               |
| <i>Aspergillus flavus</i>     | na       | na       | na        | na       | na                | nt      | nt       | 22.0±0.2 | na               |
| <i>Aspergillus fumigatus</i>  | na       | na       | na        | na       | na                | nt      | nt       | 34.0±0.7 | na               |

Nystatin (Ny. 30µg/disc), Cephalixin (CN. 30µg/disc), Amoxicillin (AMX. 25µg/disc) and Vancomycin (VA. 30µg/disc); Mean values of the growth inhibition zones, in mm, including the disc diameter of 6 mm; Antimicrobial activity = Average zone of inhibition ± SD Na, no antimicrobial activity; Nt, not tested

The results show that methanolic extract stems (0.62 ± 0.02 mg/ml), aqueous extract stems (0.52± 0.02 mg/ml), were the most potent of all studied extracts of *M. rotundifolia* and are most similar to Gallic acid (0.43±0.003 mg/ml). Aqueous extract leaves (0.97 ±0.03 mg/ml) and methanol extract leaves (1.72± 0.03 mg/ml) are lower than the activity of standards.

### Antimicrobial activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay.<sup>39</sup> The antimicrobial potential of both the experimental plant was evaluated according

to their zone of inhibition against various microorganisms and the results (Zone of inhibition) were compared with the activity of the controls (Table 4).

The antimicrobial activity of *M. rotundifolia* extracts was determined against five bacteria, one yeast and two filamentous fungi and their potency was qualitatively assessed by the presence or absence of inhibition zones, zone diameters. Diameters of inhibition zone less than 7 mm were recorded as non-active, between 7 and 10 mm were recorded as weakly active, more than 10 mm and less than 15 mm were recorded as moderately active and significantly active when diameters of growth inhibition were more than 16 mm.<sup>40</sup>



There is no previous report on evaluation of this plant extracts against these set of microorganisms. The results showed a little or no antimicrobial activity against all strains tested, with inhibition zones between 6.1 and 10.4 mm. However, the methanol and aqueous extracts of *M. rotundifolia* leaves were found to be most effective against *E. coli* and *C. albicans*. In contrast, the most resistant microbial strains were the filamentous fungi. Otherwise, these results showed low antimicrobial activity in comparison with the diameter inhibition of positive controls.

## CONCLUSION

In this work, the phenol and flavonoid contents of Algerian *M. rotundifolia* leaves and stems extracts and their related antioxidant activity with three different *in vitro* testing systems, and antimicrobial activity are demonstrated for the first time. Among various extracts tested, the methanolic leaves extract showed the best antioxidant activity. Further, although its antioxidant activity may be due to the presence of high flavonoid content. Although antimicrobial activity of the mentioned extracts were lower than standard reference, this need to be fully clarified by further assay methods. Further studies are also warranted to isolate and characterize active ingredients of this plant extracts that are responsible for the antioxidant activity, and to explore the existence of synergism, if any, among the compounds.

## REFERENCES

- Robinson MM, Zhang X, The World Medicines Situation 2011 Medicines: Global Situation, Issues and Challenges WHO, Geneva, 2011, WHO/EMP/MIE/2.3.
- Koochak H, Seyyednejad SM, Motamedi H, Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran), Asian Pacific Journal of Tropical Medicine, 3(3), 2010, 180-184.
- Balandrin MF, Kjocke AJ, Wurtele E, Natural plant chemicals: sources of industrial and mechanical materials, Science, 228, 1985, 1154-1160.
- Ndhala AR, Kasiyamhuru A, Mupure C, Chitindingu K, Benhura MA, Muchuweti M, Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *sclerocarya birrea*, Food Chemistry, 103, 2007, 82-87.
- Wallace G, Fry SC, Phenolic components of the plant cell wall, International Review of Cytology, 151, 1994, 229-267.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna, Stocker P, Vidal N, Antioxidant of some Algerian medicinal plants extracts containing phenolic compounds, Food Chemistry 97, 2006, 654-660.
- Wong C, Li H, Cheng K, Feng CA, Systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay, Food Chemistry, 97, 2006, 705-711.
- Sharma S, Stutzman J, Kellof G, Steele V, Screening of potential chemo-preventive agents using biochemical markers of carcinogenesis, Cancer Research, 54, 1994, 5848-5855.
- Kim D, Ock K, Young J, Hae-Yeon M, Chang YL, Quantification of polyphenolics and their antioxidant capacity in fresh plums, Journal Agricultural and Food Chemistry, 51, 2003, 6509-6515.
- Fukumoto LR, Mazza G, Assessing antioxidant and prooxidant activities and phenolic Compounds, Journal of Agricultural and Food Chemistry, 48(8), 2000, 3597-604.
- McKay DL, Blumberg JB, A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.), Phytotherapy Research, 20, 2006, 519-530.
- Lange B, Croteau R, Genetic engineering of essential oil production in mint. Curr. Opin. Plant Biology, 2, 1999, 139-144.
- Kamkar A, Jebelli Javan A, Asadi F, Kamalinejad M, The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil, Food and Chemical Toxicology, 48, 2010, 1796-1800.
- Riahi L, Elferchichi M, Ghazghazi H, Jebali J, Ziadi S, Aouadhi C, Chograni H, Zaouali Y, Zoghalmi N, Mliki A, Phytochemistry, antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia, Industrial Crops and Products, 49, 2013, 883- 889.
- Nickavar B, Alinaghi A, Kamalinejad M, Evaluation of the antioxidant properties of five Mentha species, Iran Journal of Pharm Research, 7 (3), 2008, 203-209.
- Quézel P, Santa S, Nouvelle flore de l'Algérie et des régions désertiques méridionales, Ed CNRS, Tome II, Paris, 1963.
- Bézanger-Beauquesne L, Pinkas M, Torck M, Trotin F, Plantes Médicinales des Régions Tempérées, Ed Maloine, Paris, 1990, 294-295.
- Lorenzo D, Paz D, Dellacassa E, Davies P, Vila R, Canigual S, Essential Oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay, Brazilian Archives of Biology and Technology, 45, 2002, 519-524.
- Hendriks H, Van Os FHL, Essential oils of two chemotypes of *Mentha suaveolens* during ontogenesis, Phytochemistry, 15, 1976, 1127-1130.
- Moreno L, Bello R, Primo-Yufera E, Esplugues J, Pharmacological properties of the methanol extract from *Mentha suaveolens* Ehrh, Phytotherapy Research, 16, 2002, 10-13.
- Sutour S, Bradesi P, De Rocca-Serra D, Casanova J, Tomi F, Chemical composition and antibacterial activity of the essential oil from *Mentha suaveolens* ssp. *Insularis* (Req.) Greuter Flavour Fragrance Journal, 23, 2008, 107-114.
- Sutour S, Bradesi P, Casanova J, Tomi F, Composition and chemical variability of *Mentha suaveolens* ssp. *suaveolens* and *M. suaveolens* ssp. *Insularis* from Corsica, Chemistry and Biodiversity, 7, 2010, 1002-1008.
- Ladjel S, Gherraf N, Hamada D, Antimicrobial effect of essential oils from the Algerian medicinal plant *Mentha rotundifolia* L., Journal of Applied Sciences Research, 7(11), 2011, 1665-1667.
- Khadraoui A, Khelifa A, Hamitouche H, Mehdaoui R, Inhibitive effect by extract of *Mentha rotundifolia* leaves on the corrosion of steel in 1 M HCl solution, Research on Chemical Intermediates, 40, 2014, 961-972.



25. Majhenic L, kergert MS, Knez Z, Antioxidant and antimicrobial activity of guarana seed extracts, Food Chemistry, 104, 2007, 1258-1268.
26. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M and Abdelly C, Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities, Comptes Rendu de Biologie, 331, 2008, 372-379.
27. Singleton CP, Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture, 16, 1965, 144.
28. Zhishen J, Mengcheng T, Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 64(4), 1999, 555-559.
29. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F, A procedure to measure the antiradical efficiency of polyphenols, Journal of the Science of Food and Agriculture, 76, 1998, 270-276.
30. Oyaizu M, Studies on product of browning reaction prepared from glucose amine, Japanese Journal of Nutrition, 44, 1986, 307-315.
31. Koleva II, Teris AB, Jozef PH, Linssen, AG, Lyuba NE, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, Phytochemical Analyses, 13 (1), 2002, 8-17.
32. Pfaller MA, Messer SA, Karlsson Å, Bolmström A, Evaluation of the Etest method for determining fluconazole susceptibilities of 402 clinical yeast isolates by using three different agar media, Journal of Clinical Microbiology, 36, 1998, 2586-2589.
33. Molyneux P, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin Journal of Science and Technology, 26(2), 2004, 211-219.
34. Blois MS, Antioxidant determination by the use of a stable free radical, Nature, 29, 1958, 1199-1200.
35. Singh R, Singh N, Saini BS, Rao HS, In vitro antioxidant activity of pet ether extracts of black pepper, Indian Journal of Pharmacology, 40, 2008, 147-151.
36. Thaipong K, Boonprakoba U, Crosby K, Cisneros-Zevallos L, Hawkins BD, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, Journal of Food Composition and Analysis, 19, 2006, 669-675.
37. Diplock AT, Will the good fairies please prove us that vitamin E lessens human degenerative disease, Free Radical Research, 27, 1997, 511-532.
38. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V, Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf. Ex. D.C.) Sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts, Journal of Agricultural and Food Chemistry, 48, 2000, 5030-5034.
39. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ, Antiamoebic and phytochemical screening of some Congolese medicinal plants, Journal of Ethnopharmacology, 61 (1), 1998, 57-65.
40. Tekwu EM, Pieme AC, Beng VP, Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance, Journal of Ethnopharmacology, 142, 2012, 265-273.

Source of Support: Nil, Conflict of Interest: None.

