Chemical Composition and Antioxidant Effect of Mentha rotundifolia Extracts

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ABSTRACT
Aim: This report was aimed to investigate both the chemical composition and the antioxidant activity of two extracts from Mentha rotundifolia aerial parts. Methods: Aqueous and acetonic extracts were obtained by decoction and Soxhlet apparatus, respectively. Total polyphenols contents were determined using Folin-Ciocalteu reagent whereas flavonoids were evaluated by AlCl3 method. LC-MS/MS method was carried out to reveal the phytochemical composition of extracts. Some tests were used to evaluate the in vitro antioxidant activity of extracts such as DPPH free radical scavenging assay, ABTS+ radical cation decolorization assay, OH radicals scavenging assay, ferrous ion chelating, reducing power and β-carotene/linoleic acid bleaching assay. Results: The results showed that the aqueous extract was richer on polyphenols and flavonoids comparing to the acetonic extract. The analysis by LC-MS/MS allowed to identify many phenolic compounds in extracts, predominated by rosmarinic acid. In addition, the extracts were found to possess a significant antioxidant activity. Conclusion: Accordingly, it can be concluded that M. rotundifolia is quite rich in phenolic compounds and has a good antioxidant activity.

Key words: Mentha rotundifolia, Extraction, Phenolic compounds, LC-MS/MS, Antioxidant activity.

INTRODUCTION
It is admitted that chronic diseases, as well as neurodegenerative pathologies such as cancer, cardiovascular diseases, Alzheimer's and other physiological disorders, are associated with the oxidant stress caused by free radicals and other oxidant.1 An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target, herbal plants are considered as good antioxidants since ancient times.2 Plants of the Lamiaceae family constitute one of the major sources of gastronomic and medicinal plants all over the world. One of the important genera belonging to this family is the genus Mentha which is composed of around 19 geographically widespread species and 13 named hybrids.3 M. rotundifolia is one of these species and is largely used in Algerian as well as in Mediterranean traditional medicine.4

MATERIALS AND METHODS
Plant material
The aerial parts of M. rotundifolia were collected during 2015 in the region of Djemila, Wilaya of Setif (Algeria). The collected material was identified by Pr. Laouer H from the Laboratory of Botanical Sciences, F. A. Setif University. The plant material was then air dried at room temperature and away from humidity.

Chemicals
All solvents and reagents used in this study were obtained from Sigma-Aldrich (Germany), Fluka and Merck.

 Extraction
The aqueous extract of M. rotundifolia was obtained by decoction of the grinded plant material (100g) for 15 min in distilled water, filtered and the solvent was evaporated to get the aqueous extract. The acetonic extract was obtained from 20 g of the plant material powder mixed with 200 mL of acetone, using Soxhlet extractor for 6 h. Thereafter, the extract was filtered and evaporated to dryness under vacuum at 40°C using a rotary evaporator.

Determination of total polyphenols
The amount of total phenolics in the extracts of M. rotundifolia was determined according to the method of Li et al. (2007).5 Briefly, 200 µL of plant extracts solutions (1.0 mg/mL) were mixed with 1.0 mL of Folin- Ciocalteu reagent (10.0%) for 4 mins. 800 µL of aqueous Na2CO3 (7.5%) solution were added. The mixture was kept at room temperature for 2 h and then the absorbance was measured at 765 nm. The results were expressed as µg Gallic acid equivalent (GAE)/mg of extract.
Determination of total flavonoids

Total flavonoids content in *M. rotundifolia* extracts was determined by aluminum chloride colorimetric method. A volume of 1.0 mL of each extract was mixed with 1.0 mL of AlCl₃ solution in methanol (2.0%). After incubation for 30 min at room temperature, the absorbance of the reaction mixture was measured at 430 nm. The total flavonoids contents of the extracts were expressed as μg quercetin equivalent/mg of extract.

Identification and Quantification of Individual Phenolic Compounds using Liquid Chromatography Mass Spectrometry (LC/MS/MS)

The analyses of extracts by LC-MS/MS were performed in Pharmaceutical Research Center - Jordan University of Science and Technology using an Agilent 1200 chromatography system (Agilent 1200, Agilent Technologies, Wilmington, DE, USA) equipped with mass detector API-3200. The extracts and phenolic standard were dissolved in methanol at the concentration of (1.0 mg/mL) and injected onto a C18 column Agilent zorbax 150 mm X 4.6 mm. The analysis was performed using a gradient solvent system with aqueous-formic acid (0.10%) as solvent (A) and acetonitrile (100%) as solvent (B). A five-step linear gradient elution with a total run time of 35 min was employed, the gradient elution was realized by decreasing solvent A to 10% and increasing solvent B to 90%. An injection volume of 20 μL at a constant flow rate of 1.0 mL/min was used for each analysis.

The entire flow from the High-performance Liquid Chromatography (HPLC) was directed into a triple- quadrupole mass spectrometer (API 3200; MDS Sciex, Concord, ON, Canada). The mass spectral data were acquired in negative ion mode with a capillary voltage of 4500 V, an Electrospray Ionization (ESI) ion source, a cone voltage of 70 V, a collision energy of 35 eV, a drying temperature of 650°C, N₂ as the drying gas with a flow rate of 4.0 L/ min and Analyst software version 6.

A diode-array UV detector was used to scan between 200 and 400 nm to evaluate the contents of individual phenolic compounds and the eluted samples and standards were detected at 280 nm.

Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

DPPH radical-scavenging activity of extracts was determined according to the method of Que et al. (2006). Briefly, 1.0 mL of each plant extracts at different concentrations was added to 1.0 mL DPPH solution (0.01M in methanol). The mixture was shaken and left to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm for each analysis.

The entire flow from the High-performance Liquid Chromatography (HPLC) was directed into a triple- quadrupole mass spectrometer (API 3200; MDS Sciex, Concord, ON, Canada). The mass spectral data were acquired in negative ion mode with a capillary voltage of 4500 V, an Electrospray Ionization (ESI) ion source, a cone voltage of 70 V, a collision energy of 35 eV, a drying temperature of 650°C, N₂ as the drying gas with a flow rate of 4.0 L/ min and Analyst software version 6.

A diode-array UV detector was used to scan between 200 and 400 nm to evaluate the contents of individual phenolic compounds and the eluted samples and standards were detected at 280 nm.

Metal ion chelating assay

The estimation of iron ions chelation of the extracts was performed according to Le et al. (2007). Briefly, an aliquot (500 μL) of different concentrations of extracts was mixed with 100 μL FeCl₃(0.6 mM) and 900 μL methanol. After 5 min incubation, the reaction was initiated by the addition of 100 μL ferrozine (5 mM). The mixture was shaken vigorously and after 10 min incubation period the absorbance of the solution was measured at 562 nm. EDTA was used as positive control. The ratio of inhibition of ferrozine–Fe³⁺ complex formation was calculated as follows.

\[ I(\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100. \]

Reducing power assay

The reducing power of extracts was determined according to the method cited by Beyhan et al. (2010). Briefly, an aliquot (500 μL) of different concentrations of extracts was with 2.5 mL phosphate buffer (pH 6.6) and 2.5 mL of 1.0% potassium ferricyanide [K₃Fe(CN)₆] solution. The mixture was incubated at 50°C for 20 min, 2.5 mL of 10% trichloroacetic acid was added to the mixture. After vigorous agitation, 2.5 mL of this solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl₃(0.1%), the absorbance was measured at 700 nm. The BHA was used as positive control. The EC₅₀ for this test is defined as the effective concentration at which the absorbance is 0.5.

β-Carotene/ Linoleic Acid Bleaching Assay

The ability of extracts to prevent bleaching of β-carotene was assessed as described by Miraliakbari and Shahidi (2008). A stock solution of β-carotene/linoleic acid was prepared by dissolving 0.5 mg of β-carotene in 1.0 mL of Chloroform, 25 μL of linoleic acid and 200 mg of Tween 40. The Chloroform was completely evaporated under vacuum in a rotary evaporator at 40°C, 100 mL of distilled water saturated with oxygen were added, 2500 μL of this reaction mixture were dispensed into test tubes 350 μL of the various extracts, prepared at 2.0 mg /mL concentrations were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with the positive control (BHA) and blanks (MEOH and H₂O). The covered tubes were incubated at 50°C and the absorbance was measured at 470 nm each 15 min during 120 min. The antioxidant activity was calculated as following equation.

\[ I(\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100. \]

ABTS⁺ Radical Cation(s) Decolorization Assay

The spectrophotometric analysis of ABTS radical scavenging activity was determined according to Re et al. (1999) with some modification. ABTS was dissolved in water to a 7.0 mM concentration. ABTS radical cation (ABTS⁺) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (Final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before using. The radical was found to be stable in this form and for more than two days when stored in dark at room temperature. For the study, the ABTS⁺ solution was diluted with ethanol, to an absorbance of 0.70 at 734 nm. 1.0 mL of ABTS⁺ solution was added to 50.0 μL of the extract solution at different concentrations. After 30 min, percentage inhibition was calculated for each concentration at 734 nm. Appropriate solvent blanks were used in each assay. The ABTS⁺ scavenging activity as percentage was calculated as.

\[ I(\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100. \]
Polyphenols

Gallate, cyaninchlorid, myrtillin, quercetin, rutin, caffeicacid,ellagicacid, quercetin 3-O- galactoside, luteolin, chlorogenic acid, epigallocatechin
gallate, cyaninchlorid, myrtillin, quercetin, rutin, caffeicacid,ellagicacid, ferulic acid, hydroxybenzoic acid, p-coumaric acid, rosmarinic acid,
syringic acid, transcinamaldehyde acid, vanilic acid and hypericin)

Phenolic compounds have been shown to be responsible for the antioxidant
defense system and work to protect, lipids from peroxidation by radicals. In
defense system and work to protect, lipids from peroxidation by radicals. In
order to determine the mode of action of extracts; six different assays
were used. DPPH free radical scavenging assay

Substances which are able to donate hydrogen or an electron to DPPH, can
be considered as antioxidants and therefore radicals scavengers. To
determine the efficacy of extracts concentration providing 50% inhibition
(IC50) was calculated from the plot of inhibition percentage against the extract concentration (Figure 2), noting that lower IC50 value
defines higher antioxidant activity. The results reveal that the DPPH
radical-scavenging activity of acetonic extract was more marked than
that of aqueous extract with IC50 of 15.066± 0.449 µg/mL and 97.862 ±
0.218 µg/ mL for acetic acid and aqueous extracts respectively. However,
the effect of extracts was lower (p < 0.001) than that of BHA (IC50 of 5.742±0.206 µg/mL).

ABTS® Radical Cation Scavenging Activity

The ABTS radical scavenging action is known to be one of the various
mechanisms for measuring antioxidant activity of food. This method
was not only a rapid and reliable test of antioxidant capacity but also an
advantageous assay applicable to both hydrophilic and lipophilic
antioxidants/systems. As shown in Figure 3, the extracts have a good
antioxidant effect with IC50 of 5.868± 0.487µg/mL and 22.026± 0.759 µg/mL
for aqueous and acetonic extract respectively. To evaluate the potency of
extracts, the activity was compared with the synthetic antioxidant BHT,
which presents the best potency (IC50=2.745± 0.180 µg/mL).

OH Scavenging Activity Assay

Among the Reactive Oxygen Species (ROS), hydroxyl radicals are the
most reactive and are the predominant radicals generated endogenously

RESULTS AND DISCUSSION

Phytochemical analyses

The extraction of phenolic compounds from M. rotundifolia arial parts
was realized by two methods. The aqueous extract was obtained by
decocion at a yield of 14.2 %, whereas the acetic extract obtained by
soxlet apparatus showed a yield of 3.08 %. The amount of polyphenols and
flavonoids was determined using Folin Ciocalteu and aluminium
chloride method respectively. The results shown in Table 1 indicated that
the aqueous extract was richer in polyphenols and flavonoids than the
acetic extract.

LC-MS/MS Analysis

The qualitative and quantitative analysis of phenolic compounds in aqueous
and acetonic extracts of M. rotundifolia was conducted by using LC-MS/MS analysis. Phenolic compounds were identified by
comparing retention times and spectra of each peak with those of known standards
analyzed in the same conditions.

(Apigenin, isoquercetin, catechol, epicatechin, gallic acid, procyanidin B2,
quercetin 3-O- galactoside, luteolin, chlorogenic acid, epigallocatechin-
gallate, cyaninchlorid, myrtillin, quercetin, rutin, caffeicacid,ellagicacid,
ferulic acid, hydroxybenzoic acid, p-coumaric acid, rosmarinic acid,
syringic acid, transcinamaldehyde acid, vanilic acid and hypericin) (Figure 1).

Among the 24 standards used, the analysis led to the identification of
15 and 16 phenolic compounds (Flavonols, phenolic acids etc.) in the
aqueous and the acetic extracts respectively (Table 2), the predominant
compound was rosmarinic acid at a rate of 6610 ng/mL and 6370 ng/mL
in aqueous and acetonic extract respectively. This phenolic acid was the subject of several scientific studies which made it possible to determine
its therapeutics effect as anticancer, antioxidant and anti-Alzheimer. A large
diversity of molecules was observed in these extracts as phenolic
Acids (Chlorogenic acid, ferulic Acid, vanilic Acid), flavones and flavonols
(Apigenin, luteolin, quercetin 3-O-galactoside). It is worth to note that
the quantities of these compounds are different between extracts, these
differences may be due to the method of extraction and the type of solvents.

No previous work illustrates the chemical composition of aqueous and
acetic extracts of M. rotundifolia. However, there essential oil has been
the subject of numerous phytochemical investigations belonging to
different regions in the world.6,16

Antioxidant activity

Phenolic compounds have been shown to be responsible for the antioxidant
effect of plant. The antioxidant activity of polyphenols was attributed to
their redox properties, which allows them to act as reducing agents,
hydrogen donators and singlet oxygen quenchers, some show metal
chelation properties.17,18 Antioxidants can also prevent free radicals from
forming, protect cells from free radical damage and enhance the body’s

Statistical analysis

The results were presented as the mean of three repetitions (± Standard
deivation (SD)). Statistical analysis of data was performed by using the
GraphPad Prism 5 program. The data were analyzed by one-way Analysis
of variance (ANOVA). Significant differences between means and
standards were determined by Dennett’s test, the level of significance was
set at p<0.05.

Table 1: Amounts of Total Polyphenols and Flavonoids in M. rotundifolia
Extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Polyphenols (µg GAE/mg extract)</th>
<th>Flavonoids (µg QE/mg extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>154.15±1.95</td>
<td>29.358± 2.009</td>
</tr>
<tr>
<td>Acetonic extract</td>
<td>113.7±2</td>
<td>16.275±0.375</td>
</tr>
</tbody>
</table>

AA% = A0 / A5 * 100.

A0: Absorbance of the sample at t0.
A5: Absorbance of the sample after 120 min of incubation.
Table 2: Phenolic Profile of *M. rotundifolia* Aqueous and Acetonic Extracts Identified by LC-MS/MS.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$C_1$ (ng/mL)</th>
<th>$C_2$ (ng/mL)</th>
<th>RT</th>
<th>$Q_1$</th>
<th>$Q_3$</th>
<th>MWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>/</td>
<td>/</td>
<td>1.53</td>
<td>169</td>
<td>124.6</td>
<td>170.12</td>
</tr>
<tr>
<td>Catechol</td>
<td>/</td>
<td>/</td>
<td>5.5</td>
<td>109</td>
<td>109</td>
<td>110.11</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>77.4</td>
<td>197</td>
<td>5.94</td>
<td>353.15</td>
<td>190.4/84.8/93.1</td>
<td>354.31</td>
</tr>
<tr>
<td>Vanilic acid</td>
<td>67.8</td>
<td>260</td>
<td>6.38</td>
<td>166.66</td>
<td>107.8/151.2/151.7</td>
<td>168.15</td>
</tr>
<tr>
<td>Procyanidin B2</td>
<td>/</td>
<td>/</td>
<td>6.4</td>
<td>577.1</td>
<td>407/289</td>
<td>578.52</td>
</tr>
<tr>
<td>Hydroxybenzoic acid</td>
<td>29.9</td>
<td>77.7</td>
<td>6.48</td>
<td>134.779</td>
<td>88.4/106.8/89.1</td>
<td>135.12</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>95.1</td>
<td>197</td>
<td>6.48</td>
<td>196.718</td>
<td>120.4/120.7/152.3</td>
<td>198.17</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>23.4</td>
<td>50.8</td>
<td>6.5</td>
<td>178.465</td>
<td>134.2/106.4/89.1</td>
<td>18.16</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>/</td>
<td>/</td>
<td>6.57</td>
<td>289.1</td>
<td>108.8</td>
<td>290.3</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>/</td>
<td>/</td>
<td>6.66</td>
<td>456.579</td>
<td>168.2/168.5/124.8</td>
<td>458.372</td>
</tr>
<tr>
<td>Rutin</td>
<td>64.8</td>
<td>139</td>
<td>6.84</td>
<td>609.419</td>
<td>299/299.9/270.9</td>
<td>610.52</td>
</tr>
<tr>
<td>Quercetin3-O-galactoside</td>
<td>45.6</td>
<td>32.7</td>
<td>7</td>
<td>463</td>
<td>301</td>
<td>464.379</td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>20</td>
<td>/</td>
<td>7</td>
<td>464.9</td>
<td>300</td>
<td>464.4</td>
</tr>
<tr>
<td>Myrtilin</td>
<td>0.414</td>
<td>18.1</td>
<td>7.01</td>
<td>462.178</td>
<td>299.8/270.8/254.7</td>
<td>500.8</td>
</tr>
<tr>
<td>P-Coumaric acid</td>
<td>75.8</td>
<td>262</td>
<td>7.06</td>
<td>162.756</td>
<td>118.8/118.1/92.7</td>
<td>164.16</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>93</td>
<td>99.2</td>
<td>7.2</td>
<td>192.807</td>
<td>133.9/133.4/177.8</td>
<td>194.18</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>6610</td>
<td>6370</td>
<td>7.38</td>
<td>358.319</td>
<td>160.7/161/132.7</td>
<td>360.32</td>
</tr>
<tr>
<td>Transcinamaldehyde acid</td>
<td>/</td>
<td>171</td>
<td>7.95</td>
<td>131.797</td>
<td>103.7/102.8/101.9</td>
<td>132.16</td>
</tr>
<tr>
<td>Luteolin</td>
<td>40.9</td>
<td>263</td>
<td>8</td>
<td>285</td>
<td>217</td>
<td>286.24</td>
</tr>
<tr>
<td>Cynarin chloride</td>
<td>59.9</td>
<td>438</td>
<td>8</td>
<td>286.198</td>
<td>133.4/132.8/150.6</td>
<td>287.1</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>/</td>
<td>/</td>
<td>8.01</td>
<td>300.703</td>
<td>149.7/150.6/149.9</td>
<td>302.197</td>
</tr>
<tr>
<td>Quercetin</td>
<td>/</td>
<td>8.09</td>
<td>8.01</td>
<td>300.604</td>
<td>150.4</td>
<td>302.2</td>
</tr>
<tr>
<td>Apigenin</td>
<td>9.12</td>
<td>139</td>
<td>8.4</td>
<td>269</td>
<td>151</td>
<td>270.12</td>
</tr>
<tr>
<td>Hypericin</td>
<td>/</td>
<td>/</td>
<td>10.53</td>
<td>503</td>
<td>405</td>
<td>504.45</td>
</tr>
</tbody>
</table>

*C*: Concentration of phenolic compound in aqueous extracts; *C*: Concentration of phenolic compound in acetonic extract, *Q*: Compound molecular weight, *Q*: Fragment Molecular Weight, MWT: Molecular Weight.

Figure 2: DPPH Scavenging Activity of *M. rotundifolia* Extracts Expressed as IC$_{50}$ Values, AQE: Aqueous extract, ACE: Acetonic Extract. Values are the mean ± SD (n = 3). Comparisons are made with BHA. ***: *p < 0.001.

Figure 3: ABTS+ Scavenging Activity of *M. rotundifolia* Extracts Expressed as IC$_{50}$ Values. AQE: Aqueous Extract, ACE: Acetonic Extract. Values are the mean ± SD (n= 3). Comparisons are made with BHT **p<0.01, ***: *p < 0.001.
during aerobic metabolism to initiate cell damage in vivo. The results (Figure 4) indicate that the most potent scavenger of OH Radical was observed with vitamin C (IC\textsubscript{50} = 194.868 ± 1.836 µg/mL) followed by ACE (IC\textsubscript{50} = 204.355 ± 3.925 µg/mL) and AQE (IC\textsubscript{50} = 377.232 ± 4.995 µg/mL).

**Metal Chelating Activity**

Iron is essential for life as it is required for oxygen transport, respiration and for activity of many enzymes. Chelating agents are effective as secondary antioxidants since they decrease the redox potential, thereby, stabilizing the oxidized form of the metal ion. Decrease in the red color of the complex ferrozine-Fe\textsuperscript{2+} indicates high radical scavenging activity of the compound. To compare the effect of aqueous, acetonic extracts and standard (EDTA), the values of IC\textsubscript{50} were calculated, which demonstrated that EDTA possess the most powerful chelating activity (IC\textsubscript{50} = 6.273 ± 0.288 µg/mL) followed by the aqueous extract (IC\textsubscript{50} = 128.155 ± 5.887 µg/mL). However, the acetonic extract didn’t reach 50% chelating activity and presents maximal activity of 41.433 ± 2.518 % at 468.75 µg/mL.

**Reducing Power**

The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe\textsuperscript{3+}/ferricyanide complex to the ferrous form. Therefore, Fe\textsuperscript{2+} can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. In this test, The EC\textsubscript{50} values indicated that the BHA showed the good reducing power (EC\textsubscript{50} = 13.997 ± 0.408 µg/mL), followed by the aqueous extract (EC\textsubscript{50} = 18.809 ± 0.369 µg/mL) and finely the acetonic extract (EC\textsubscript{50} = 25.83 ± 0.208 µg/mL) (Figure 5).

**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 scavenge singlet oxygen responsible of linoleic acid peroxidation. The lowest β -carotene discoloration rate exhibited the highest antioxidant activity, Figure 6 shows the lipid peroxidation inhibitory activity of M. rotundifolia extracts by the β carotene-linoleic acid assay. The inhibition ratios of the oxidation of linoleic acid by the aceton and aqueous extracts, were giving approximately the same percentage of inhibition ratio, 79.778 ± 1.812 % and 84.666 ± 0.963 % respectively. However, the activity of BHA is more effective (89.479 ± 0.055 %).

Our results demonstrate the good antioxidant effect of M. rotundifolia extracts with al tests, this effect may be due to abundance of different flavonoids and phenolic compounds. The antioxidant activity of flavonoids depends on the functional group arrangement of nuclear structures. Configurations, substitutions and amounts of hydroxyl groups substantially affect some mechanisms of antioxidant activity, a synergistic or antagonistic effect between the major and minor compounds also proposed to explain the effect of the extracts.

**CONCLUSION**

In this research we studied the chemical composition and the antioxidant activity of M. rotundifolia aqueous and aceton extracts. The LC-MS/MS technique is used to identify numerous phenolic compounds, which are
known for their therapeutic effects. Moreover, a good antioxidant activity of extracts was confirmed by many tests. According to our results, it is clear that this plant is good for human. Further studies in vitro and in vivo are necessary to identify the biological roles of individual compounds of extracts and other biological properties of this plant.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

ABBREVIATIONS
DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2’-Azino-bis(3-ethylbenzenothiazoline 6-sulfonic acid); BHT: 2,6-di-tert-butyl-4-hydroxycarboxylic acid; BHA: Butylatedhydroxy anisole.

REFERENCES