Comparison in vitro of Antioxidant Activity between Fifteen Campanula Species (Bellflower) from Palestinian Flora

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ABSTRACT

Background: The natural antioxidant products in the plant kingdom play an important role in the healthy lifestyle and reduce the risk for various chronic diseases. Objective: The objective of this study was to investigate different antioxidant pharmacological property of methanol extract for fifteen species of Campanula plant from Palestinian flora. Methods: The antioxidant activity of fifteen Campanula species growing wildly in Palestine were studied using 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity and their antioxidant activity was compared to Trolox antioxidant activity. Results: The results clearly demonstrate a very high antioxidant activity of the Campanula sulphurea and Campanula sidoniensis and they showed almost the same antioxidant activity of Trolox. The other twelve species extracts also exhibited excellent antioxidant activity in DPPH radical scavenging activity in comparison with trolox standard. Conclusion: Methanol extract of Campanula can be valuable for treatment of different diseases and could be used as a possible new source of natural antioxidants in the food, nutraceuticals, pharmaceuticals and cosmetic industry.

Key words: Antioxidant, Bellflower, Campanula species, Campanulaceae, DPPH radical scavenging activity.

SUMMARY

• The results show a difference in the antioxidant activity in the fifteen Campanula subgenera.
• The results clearly demonstrate a high antioxidant activity of the Campanula sulphurea and Campanula sidoniensis which show the same antioxidant activity of Trolox.

INTRODUCTION

Palestine is unique and rich land in its natural flora diversity due to its geographical location as jointing point of Asia continent, Africa continent, and Europe continent, in addition to that it has mountains, hills, valleys, coastal plains, desert, Mediterranean Sea, Dead Sea and Rift Valley. Different climatic, phytogeographic and zoogeographic zones converges Palestine, creating great biological multi-diversity. In many developed and developed countries, a huge section of the population relies on traditional herbal healers and their armamentarium of medicinal and non medicinal plants in order to provide health care needs. Any therapeutic treatment or prevention of diseases began along ago with the utilization of plants, as well as the methods of preparations of folklore traditional healings throughout the world commonly used plants as part of their traditions and cultures. The plant genus Campanula L. (Bellflower) belonged to the Campanulaceae family and contains about 300 species distributed widely in temperate regions of the northern part of Hemisphere. It includes perennial or annual. This plant is widely used for several ethano-medicinal purposes by tribal peoples and traditional practitioners.

Most of all Campanula species grow wildly in the West and Central Asia, Black Sea, Mediterranean Sea, Eurasian Artic and North America regions. Campanula roots are fleshy and have a lot of fibers, the stems are simple, erect, stiff, slender, more or less covered with white stiff hairs, which disappear when cultivated with 2 to 3 feet high. The leaves are oval or oblong and slightly crenate on long stalks, 1 to 3 inches long, the leaves on the stem are narrow, obscurely toothed or mostly entire. The flowers have purple, reddish purple, blue or white colors forming long, simple or slightly branched panicles on short peduncles which bloomed in July and August. The genus Campanula as represented in the Palestinian territory is divided into fifteen subgenera. All Campanula subgenera show a moderate antioxidant activity. The Campanula species could be used as a possible new source of natural antioxidants.
• Campanula sulphurea Boiss.

Campanula plant genus contains flavonoids secondary metabolic compounds as kaempferol-3-O-glucoside, quercetin-3-O-glucoside, rutin, lobetol, coniferin, 4'-O-(p-hydroxybenzoyl)-isorhamnetin-3,7-di-O-b-D-glucopyranoside, lobetol, and quercetin-3-O-rutinoside and other phytochemical compounds as p-hydroxybenzoic acid, ethyl docosanoate, bis(2-ethylhexyl) adipate, sitosterolb-D-glucoside. Bellflower different species have been used as a traditional medicine in form of decoctions for treatment of various diseases such as laryngitis, constipation, warts, tonsillitis and bronchitis for a long time in addition to their use as ornamental plants, also as emetics, spasmyloytic, antiinflammatory, antioxidant, antimicrobial, antiviral and antiphlogistic properties, as well as they possess refreshing and stimulant properties. The roots have been chewed fresh for treatment of lung and heart problems while the roots infusion has been used as ear drops for ears inflammations. A decoction of the entire plant has been used as a wash in the treatment of sore eyes.

METHODOLOGY

Materials and reagents
Trolox ((S)-(--)6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. Methanol was of analytical grade. All other chemical reagents that are used in the research were purchased from reliable commercial sources.

Instrumentation
The following instrumentations were used: Shaker device (LabTech Shaking Incubator), rotatory evaporator (Heidolph VV2000), heater and stirrer [Heidolph OB2000], Spectrophotometer (Jenway 6505 UV/Vis Spectrophotometer).

Plant material
Campanula’s fifteen species were collected from different regions in Palestine during the spring session (June- August, 2013 and 2014) and authenticated by Dr. Nidal A. Jaradat. A voucher specimen was deposited in the Herbarium of the Laboratory of Pharmacognosy at An-Najah National University as presented in (Table 1).

The plants species under study were washed twice with distilled water, dried in the shade at an average temperature of 20-30°C, for 72 hours and stored in a dry place.

Preparing of plants extracts
For evaluating the antioxidant capacity of studied fifteen Campanula species plants, the entire plants were powdered separately using a grinder. The extraction was performed at room temperature. About 100 g of the each Campanula species were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per minute for 72 hours and stored in refrigerator for 4 days. The extracts were then filtered using filter papers. The extract was then concentrated under vacuum on a rotatory evaporator. The crude extract was stored at 4°C for further use, and this procedure repeated for all fifteen Campanula species.

Antioxidant activity
Trolox standard and plant working solutions
A stock solution of a concentration of 1mg/1ml in methanol was firstly prepared for all samples of plant extracts and the standard trolox. The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/ml) were prepared by suitable dilution with methanol from the stock solution.

Spectrophotometric measurements
2, 2-diphenylpicrylhydrazyl (DPPH) was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above prepared working concentration in a ratio of 1:1:1 respectively. The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only.

The solutions were incubated in dark place for 30 minute at room temperature before the absorbance readings were recorded at 517 nm.

Percentage of inhibition of DPPH activity
The percentage of antioxidant activity of the fifteen Campanula species and the Trolox standard were calculated using the following formula:

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

Where \( A_{\text{control}} \) is the absorbance of the blank control (containing all reagents except the extract solution) and \( A_{\text{sample}} \) is the absorbance of the test sample. The antioxidant half maximal inhibitory concentration (IC\(_{50}\)) for all plant samples and the standard were calculated using Bio Data Fit edition 1.2 (data fit for biologist).

Data analysis
The antioxidant activity was reported as a percentage of DPPH reduction. The inhibition of the Campanula plants and Trolox standard at different concentration were plotted and tabulated and the IC\(_{50}\) for each of them was calculated using the Bio Data Fit fitting program. The best fit for the data was the adapted model to calculate the IC\(_{50}\).

RESULTS AND DISCUSSION

Antioxidant activity
There are a lot of clinical studies suggesting that the antioxidant compounds in the plants leaves, fruits and vegetables, are the main factors for the observed efficacy of these products in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported. The free radical scavenging activity of the
methylene extract of the entire fifteen *Campanula* species have been tested by DPPH radical method using Trolox as a reference standard. The concentration ranged from 1–100 μg/ml. The zero inhibition was considered for the solution which contained only DPPH without any plant extract.

The results show a difference in the antioxidant activity for all samples. The more potent activity was for *Campanula sulphurea* extract was comparatively relative with IC₅₀ of trolox standard. Moreover, the antioxidant activity for the other plants were comparative with slight difference in the antioxidant activity a summary of the antioxidant activity is present in (Table 2).

The results clearly demonstrate a high antioxidant activity of the *Campanula sulphurea* and *Campanula sidoniensis* which show the same antioxidant activity of Trolox. *Campanula cymbalaria, Campanula rapunculus* have a high antioxidant activity that is equal to about half the antioxidant activity of trolox. Moreover, *Campanula strigosa, Campanula stellaris Campanula peregrina* and *Campanula erinus* have an antioxidant activity that is three fold less than the Trolox antioxidant activity. All the other plants show a moderate antioxidant activity that is less than 25 μg/ml with an exceptional to only one plant namely *Campanula camplostiada* which have an antioxidant activity of about 62 μg/ml.

**CONCLUSION**

The results clearly show that the *Campanula* species in general have high free radical scavenging activity of antioxidants. The *Campanula sulphurea* in comparison with the other twelve *Campanula* species showed the highest antioxidant activity which was almost equal to the Trolox. Thus *Campanula* species could be used as a possible new source of natural antioxidants in the food, nutraceuticals, pharmaceuticals and cosmetic industry.

**ACKNOWLEDGEMENT**

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**Table 2: Maximum inhibitory concentration of the fifteen Campanula species and trolox standard and ±SD**

<table>
<thead>
<tr>
<th>Campanula species founded in Palestine</th>
<th>IC₅₀ (μg/ml)</th>
<th>±SD (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trolox (As Standard)</td>
<td>1.91</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Campanula camplostiada</em></td>
<td>61.98</td>
<td>16.06</td>
</tr>
<tr>
<td><em>Campanula cymbalaria</em></td>
<td>3.09</td>
<td>0.68</td>
</tr>
<tr>
<td><em>Campanula damascene</em></td>
<td>23.30</td>
<td>0.56</td>
</tr>
<tr>
<td><em>Campanula erinus</em></td>
<td>6.96</td>
<td>2.44</td>
</tr>
<tr>
<td><em>Campanula hierosolymitana</em></td>
<td>25.69</td>
<td>7.13</td>
</tr>
<tr>
<td><em>Campanula kotschyiyna</em></td>
<td>3.01</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Campanula peregrine</em></td>
<td>5.99</td>
<td>1.35</td>
</tr>
<tr>
<td><em>Campanula Phrygia</em></td>
<td>17.41</td>
<td>3.01</td>
</tr>
<tr>
<td><em>Campanula rapunculus</em></td>
<td>3.78</td>
<td>0.85</td>
</tr>
<tr>
<td><em>Campanula retrorsa</em></td>
<td>14.18</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Campanula sidoniensis</em></td>
<td>2.15</td>
<td>0.77</td>
</tr>
<tr>
<td><em>Campanula stellaris</em></td>
<td>5.56</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Campanula stricta</em></td>
<td>5.70</td>
<td>1.36</td>
</tr>
<tr>
<td><em>Campanula strigosa</em></td>
<td>6.54</td>
<td>2.06</td>
</tr>
<tr>
<td><em>Campanula sulphurea</em></td>
<td>1.80</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**CONFLICT OF INTEREST**

The authors report no conflict of interest.

**REFERENCES**

NIDAL AMIN JARADAT and MURAD ABUALHASAN: Antioxidant Activity of Campanula species


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