DPPH Free Radical Scavenging Activity of Ethanolic Extracts of Twenty two Medicinal Species From South Algeria (Laghouat region)

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ABSTRACT

Free radicals scavenging Activity, total phenolic and flavonoids contents of Twenty two ethanolic extracts, from the botanical families Asteraceae, Lamiaceae, Amaranthaceae, Chenopodiaceae, Cupressaceae, Ericaceae and Rhamnaceae, collected from the Laghouat region (Algeria Sahara) were investigated. The 2,2-diphenyl-1-picrylhydrazyl radical assay was used to determine the antioxidant activity of the plant extracts, while the Folin–Ciocalteu method was used to determine the total phenolic content and flavonoids using AlCl3 method. The antioxidant capacity expressed as IC50 values ranged from 20 µg/ml for O. basilicum to 650 ± 8.60 µg/ml for A. iva. The total phenolic content ranged from 2.72 to 87.11 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 1.48 to 12.59 mg/g, expressed as rutin equivalents. The results of this study showed that there is no significant correlation between antioxidant activity and phenolic content of the studied plant materials and phenolic content could not be a good indicator of antioxidant capacity.

Keywords: Medicinal plants; Phenolic content; flavonoid content; Antioxidant; DPPH.
INTRODUCTION

Medicinal plants are a very important natural resource whose valuation requires a perfect knowledge of the properties to develop. The medicinal activities of herbal depend on the presence of various bioactive agents belonging to different chemical classes (Ouraïni et al., 2005). In many African and Asian countries, herbal medicine continues to be widely used. All these figures show that people are turning back to traditional medicine and medicinal plants mainly (Muthu et al., 2006). In Algeria, the empirical use of plants continues to maintain a high popularity [Benmerabet and Abed, 1982; Boulos, 1983]. The Algerian people are sometimes preying of quackery ignorant and dangerous for patients. Many plants are known for their therapeutic properties, especially for their antiseptic, antibacterial and antioxidant effect, as Rosemary, Sage, Thyme, Chamomile, Eucalyptus, Grenadier, etc... (Ali-Delille, 2013).

The evaluation of free radical scavenging activity of plant extracts have been extensively performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995). DPPH is a purple colored radical that, at er being reduced by an antioxidant turns into a yellow product.

The aim of this study was to gather information about medicinal plants used traditionally in the region of Laghouat and and we determined the antioxidant activity, total phenol and flavonoid contents of selected medicinal plants.

MATERIALS AND METHODS

CHEMICALS

Folin-Ciocalteu’s phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethyl alcohol, methanol, sodium carbonate, aluminium chloride, gallic acid, rutin, ascorbic acid and butylated hydroxy toluene (BHT) were purchased from Sigma Aldrich-Fluka (Germany).

PLANT SAMPLES

The medicinal plants were selected based on information regarding their traditional uses in Algerian folk medicine (Table1). Asteraceae and Lamiaceae were the most representative families with 6 and 11 tested species respectively. Plant materials were collected between March and April 2016 in the area of the Algerian Saharan Atlas (Laghouat region). Collected plants parts were different following the species. Fresh plant samples were cleaned and air-dried in darkness at room temperature. Dried plant parts were then powdered and stored in the dark at a dry place until further use.

EXTRACTION PROCEDURE

one gram of each powdered plant material was soaked in 10 mL of ethanol at room temperature overnight. The solvents were decanted and residues macerated two more days with the same solvent. The pooled solvents were combined and filtered. The obtained extracts were used for antioxidant activity, total phenols and flavonoids determination.

Table 1. List of plants used in the study with their traditional uses.

<table>
<thead>
<tr>
<th>Family; Species</th>
<th>Local name</th>
<th>Traditional uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asteraceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemisia campestris</td>
<td>Dgouft</td>
<td>Antidiabetic and antihypertensive</td>
<td>Boudjeial et al., 2013</td>
</tr>
<tr>
<td>Anvillea radiata</td>
<td>Nougdt</td>
<td>diabetes and Indigestion</td>
<td>Djellouli et al., 2013</td>
</tr>
<tr>
<td>Cotula Cinerea</td>
<td>Gartoufa</td>
<td>Asthma, Cough and Allergy</td>
<td>Djellouli et al., 2013</td>
</tr>
<tr>
<td>Anthemis arvensis</td>
<td>Babounj</td>
<td>anthelmintic and analgesic rules</td>
<td>Sanri et al., 2014</td>
</tr>
<tr>
<td>Artemisia absinthum</td>
<td>Chehalba</td>
<td>Antidiabetic and diuretic</td>
<td>Meddour et al., 2015</td>
</tr>
<tr>
<td>Artemisia herba alba</td>
<td>Chih</td>
<td>Antidiabetic and eczema</td>
<td>Sanri et al., 2014</td>
</tr>
</tbody>
</table>
2.2. Determination of total Phenolic Content
The total phenolic content is estimated using Folin-Ciocalteu’s method (Slinkard and Singleton 1977), with some modifications. 200 µl of sample was dissolved in 1000 µl of Folin-Ciocalteu reagent (1/10 dilution). The solution were mixed and incubated at room temperature during 5 mn. Then, 800 µl of saturated sodium carbonate (7.5%) was added. The final mixture was mixed and then incubated for one hour in the dark at room temperature. The absorbance of all samples was measured at 760 nm and the results are expressed in milligrams of gallic acid equivalents per gram dried weight (mg GAE/g DW).

2.3. Determination of total Flavonoid Content
Total flavonoid contents were determined by the method of (Ahn et al., 2007). Each sample (1.5 ml) was added to1.5 ml of aluminum chloride (AlCl₃) solution (2%) and allowed to stand for 15 at room temperature. The absorbance of the mixture was determined at 430 nm against the same mixture without the sample as a blank. Total flavonoid content was expressed as Rutin equivalent per gram dried weight (mg RE /g DW).

2.4. Determination of DPPH radical scavenging capacity
The DPPH assay was performed according to the method described by Brand-Williams et al., 1995 with modifications. A 50 µl aliquot of extract was mixed with 1950 µl of ethanolic solution of DPPH in concentration of 60 µM. The reaction mixture was shaken vigorously.
and incubated 30 min at room temperature. Then the absorbance at 517 nm was taken against a blank (DPPH solution without extract). The decrease in absorbance indicates the free radical scavenging effect of the tested sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged according to the following formula: \([(A_0-A_1)/A_0]\) \times 100, where \(A_0\) is the absorbance of the control, and \(A_1\) is the absorbance of the extract/standard. The inhibition curves were prepared and IC\(_{50}\) values were obtained.

### 2.5 Statistical analysis

Total phenolic content, favonoid content and Antioxidant activity reported as the mean ± Standard Deviation (SD). Significant differences for multiple comparisons were determined using one way Analysis Of Variance (ANOVA). Tukey’s multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Difference at \(P<0.05\) were considered statistically significant.

### Results and Discussions

#### Total phenolic and favonoid content

The total phenolic content was found to be 2.72 ± 0.12 mg GAE/ g DW in A. iva extract and 87.11± 0.71 mg GAE/g DW in A. unedo extract. The total favonoid content was found to be 1.48 ± 0.01 mg RE/g DW in A. absinthum extract and 12.59 ± 0.06 mg RE/ g DW in A. campestris extract (Table 2). Considering the broad range of variation of the results, the phenolic contents were also categorized into three groups: high (> 50 mg), moderate (20-50 mg) and low (< 20 mg). The extracts of A. unedo, M. spicata, J. phoenicea, A. campestris and O. basilicum have high value of phenolic and favonoid content exhibited the greatest antioxidant activity.

The total phenolics content of these plant extracts are compared to the plant extracts of some previously studied plants (Djeridane et al., 2006; Djidel et al., 2009; Bakchiche et al., 2013; Khacheba et al., 2014). Total phenolics of some previously studied plant extracts was found as M. vulgare (1.36±0.07 mg GAE/gdw), A. iva (3.16±0.016), A. arboresens (3.42±0.50), A. arvensis (3.94±0.05), A. herba halba (4.93±0.0036), H. scoparium (26.71±0.15), S. officinalis (7.78±0.0041), M. pulegium (16.34±0.011), M. spicata (19.65±0.001), O. basilicum (13.1±0.021) T. polium (8.29±0.0064), A. campestris (18.96±0.0079), J. phoenicea (46.61±4.59), T. algeriensis (18.73±4.59), A. unedo (104.98±4.59), and Z. lotus (36.30±4.59). The result showed that the plant extracts studied in this work showed the potent sources of secondary metabolites and could be used as the sources to isolate the active ingredient.

#### DPPH radical-scavenging activity

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. The dosage of extract is expressed in μg of dry weight of the extract per mL of the assay mixture. IC\(_{50}\) value represents the concentration of test extract where the inhibition of test activity reached 50 %. The IC\(_{50}\) values of all the 22 different plant extracts have been furnished in the Table 2. Highest scavenging was observed with O. basilicum extract with an IC\(_{50}\) value of 20 μg/ml as opposed to the IC\(_{50}\) value of Ascorbic acid 16 μg/ml, which is a well known antioxidant Scavenging of DPPH radical was found to rise with increasing concentration of the extracts (Figure 1). Plant extracts of A. campestris, A. unedo, L. officinalis, S. officinalis and T. capitus, ranked as the top five most active plant extracts, exhibited strong activity on scavenging DPPH radicals with the determined IC\(_{50}\) values 37 ± 1.25, 51 ± 0.42, 65 ± 0.85, 70 ± 1.42, 78 ± 0.88 μg/mL, respectively. Another plant extracts of T. algeriensis, H. scoparium, M. pulegium, T. polium, R. officinalis, A. radiata, A. arvensis, M.
vulgare and Z. lotus also possessed significant activity and their IC50 values were between 100-500 μg/mL. Little antioxidant activity (>500μg/mL) was observed for the rest of extracts. Based on these results of investigation, it could be concluded that O. basilicum is a rich source of phenolic compounds as natural antioxidants of high value.

**Relationship between flavonoid content and antioxidant activity**

Attempts to correlate the level of flavonoid content of these medicinal plants with their antioxidant activity were not successful. No significant correlation (R²=0.37) was observed between flavonoid content and IC50 values when all plant materials were included in the calculation (Figure 2).

**Table 2 : Total phenolic content, flavonoid content and free radical scavenging (IC50)**

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Total phenolics (mg GAE/g DW)</th>
<th>Total flavonoids (mg RE/ g DW)</th>
<th>DPPH IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. campestris</td>
<td>57.82±0.12c</td>
<td>12.59±0.06a</td>
<td>37±1.25o</td>
</tr>
<tr>
<td>A. radiata</td>
<td>9.45±0.3l</td>
<td>6.57±0.1cd</td>
<td>177±3.22l</td>
</tr>
<tr>
<td>C. Cinerea</td>
<td>10.98±0.92kl</td>
<td>5.07±0.02elg</td>
<td>494±5.55p</td>
</tr>
<tr>
<td>A. arvensis</td>
<td>9.69±0.15l</td>
<td>3.56±0.03hij</td>
<td>242±2.22e</td>
</tr>
<tr>
<td>A. absinthum</td>
<td>12.62±0.09jk</td>
<td>1.48±0.01i</td>
<td>145±1.18l</td>
</tr>
<tr>
<td>A. herba alba</td>
<td>22.41±0.08ij</td>
<td>2.39±0.13jkl</td>
<td>103±1.22k</td>
</tr>
<tr>
<td>T. polium</td>
<td>14.21±0.27i</td>
<td>4.95±0.09g</td>
<td>171±1.32l</td>
</tr>
<tr>
<td>M. pulegium</td>
<td>13.43±0.89i</td>
<td>5.60±0.22def</td>
<td>161±1.45ph</td>
</tr>
<tr>
<td>L. officinalis</td>
<td>7.01±0.83M</td>
<td>2.72±0.01ijk</td>
<td>65±0.85lm</td>
</tr>
<tr>
<td>T. algeriensis</td>
<td>10.95±0.85kl</td>
<td>3.67±0.05hi</td>
<td>111±1.12k</td>
</tr>
<tr>
<td>S. officinalis</td>
<td>23.50±0.47hi</td>
<td>5.63±0.16def</td>
<td>70±1.42l</td>
</tr>
<tr>
<td>R. officinalis</td>
<td>39.54±0.95i</td>
<td>6.12±0.09def</td>
<td>176±2.35g</td>
</tr>
<tr>
<td>M. spicata</td>
<td>64.18±0.36b</td>
<td>10.75±0.2b</td>
<td>98±1.24k</td>
</tr>
<tr>
<td>T. capitatus</td>
<td>41.99±0.21e</td>
<td>6.98±0.06c</td>
<td>78±0.88l</td>
</tr>
<tr>
<td>O. basilicum</td>
<td>50.86±0.92d</td>
<td>9.62±0.32b</td>
<td>20±0.71o</td>
</tr>
<tr>
<td>M. vulgare</td>
<td>6.05±0.05M</td>
<td>4.19±0.22gh</td>
<td>381±5. 26d</td>
</tr>
<tr>
<td>A. iva</td>
<td>2.72±0.12N</td>
<td>2.15±0.01kl</td>
<td>650±8.60a</td>
</tr>
<tr>
<td>H. scoparium</td>
<td>25.84±0.24b</td>
<td>3.15±0.59hijk</td>
<td>114±1.50l</td>
</tr>
<tr>
<td>A. halimus L</td>
<td>3.51±0.3h</td>
<td>2.03±0.01kl</td>
<td>156±2. 85hi</td>
</tr>
<tr>
<td>J. phoenieca</td>
<td>63.46±0.71b</td>
<td>9.59±0.63b</td>
<td>98±1.45k</td>
</tr>
<tr>
<td>A. unedo</td>
<td>87.11±0.71a</td>
<td>9.23±0.06ode</td>
<td>51±0.42mn</td>
</tr>
<tr>
<td>Z. lotus</td>
<td>31.19±0.86g</td>
<td>3.96±0.02ghsi</td>
<td>464±4.45c</td>
</tr>
<tr>
<td>Ascorbique acid</td>
<td>-</td>
<td>-</td>
<td>16±1.22e</td>
</tr>
</tbody>
</table>

In the column different lettres mean significant differences by the Tukey’s multiple range test (P<0.05)
The main objective of our study was to determine the total phenolics compounds as well as to evaluate the antioxidant activity of 22 plants from Algerian Sahara, which are used in traditional medicines. Many plants contain high amounts of phenolics and exhibited high antioxidant capacity. The DPPH radical scavenging assay shows that *Ocimum basilicum* shows a very good scavenging activity among all the plants. The results obtained showed that this plant is very important from medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents showing antioxidant activity. The present study will help the researchers as basic data for future research in exploiting the hidden potential of this important plant which has not been explored so far.

**ACKNOWLEDGEMENT**

The authors would like to acknowledge Department Process Engineering, faculty of Technology Laghouat *University* for providing laboratory facilities.

**References**


