Determination of antioxidant activities of solvent extracts from an endemic plant: *Phlomis leucophracta*

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**ABSTRACT:** The members of the genus *Phlomis* have been traditionally used for therapeutic purposes in Turkey. In this study, the antioxidant properties of different extracts from *P. leucophracta* were investigated. Antioxidant properties were evaluated by different assays including free radical scavenging (DPPH assay), reducing power (potassium ferricyanide method), β-carotene/linoleic acid, metal chelating and phosphomolybdenum. Moreover, total phenolic and flavonoid contents were detected for each extract. Total phenolic and flavonoid contents were detected as 30.86-55.00 mg GAE/g extract and 4.93-26.09 mg QE/g extract, respectively. The methanol and water extracts exhibited higher DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and reducing power abilities as compared to ethyl acetate and hexane extracts. The best activity was observed by the hexane extract in β-carotene/linoleic acid assay (94.35% at 2 mg/mL). In metal chelating ability, those samples exhibited the following order (at 0.25 mg/mL concentration): Water (73.90%)>Hexane(64.87%)>Ethyl acetate(4.88%)>Methanol (2.28%). Based on our results, *P. leucophracta* may be utilized as a natural source of antioxidant compounds in food and pharmaceutical areas.

**KEYWORDS:** *Phlomis leucophracta*; phenolics; DPPH; antioxidant activity; reducing power.

1. INTRODUCTION

Natural products have formed the basis of modern medicines for thousands of years. In recent years, many natural compounds have been reported as antioxidant, antimicrobial and anticancer agents [1-3]. From this point, the discovery of new biologically-active compounds is gaining interest in the scientific area. As an example of these, artemisinin from *Artemisia annua* was awarded in Nobel Prize at 2015 to treat malaria. Moreover, several plant species could be suggested by some researchers as potential raw materials for preparation functional ingredients. Within this framework, uninvestigated plants could be considered as valuable candidates for discovering novel bioactive compounds [4-7].

The genus *Phlomis* is belonging to Lamiaceae family and it represented more than 100 species in Turkey. The members of this genus are known as “çalba or ballıkotu” in Anatolia [8]. This genus has great potential in terms of traditional usages in different countries including Turkey. Some members of this genus such as *P. russeliana*, *P. bourgaei* and *P. lycia* are used as stimulants, tonics, diuretics and also for the treatment of ulcer, hemorrhoids and wound [9-13]. At this point, new studies on uninvestigated *Phlomis* species could provide valuable information in the pool for the genus *Phlomis*. From this point, several papers focused on the biological activities of the genus *Phlomis* and its phytochemical profiles [14-20]. To the best of our knowledge, this is the first study carried out on *P. leucophracta*. Within this mind, we aimed to detect antioxidant properties of different extracts (hexane, ethyl acetate, methanol and water) from *P. leucophracta*. Therefore, data obtained here could be assumed as new insights to the literature.

3. RESULTS AND DISCUSSION

Total phenolic content in the studied extracts was determined by Folin-Ciocalteu method. The water extract had the highest phenolic content (55.00 mg GAES/g extract), followed by ethyl acetate (46.03 mg GAES/g extract), methanol (43.54 mg GAES/g extract) and hexane extracts (30.86 mg GAES/g extract). However, the water (26.09 mg QEs/g extract) and methanol extracts (20.15 mg QEs/g extract) contained the higher level of flavonoids (p<0.05) (Table 1). However, total flavonoid content was not detected in the hexane. In accordance with our results, the water and methanol extracts were reported as the richest extracts in terms of total bioactive compounds [17, 18].

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Antioxidant capacity of the studied extracts was tested by different methods. DPPH is a stable radical and it is widely used to radical scavenging ability of plant extracts. As can be seen in Table 2, the DPPH radical scavenging abilities of the extract showed in a concentration-dependent manner. The methanol and water extract exhibited remarkable radical scavenging abilities, while the hexane extract has the lowest ability. The observed results could be explained with the higher level of phenolics in the water and methanol extracts. This fact was supported by several researchers [21, 22].

Reducing power is an important indicator of antioxidant effects. For this purpose, potassium ferricyanide assay was performed. From Table 3, the reducing power of the studied extracts exerted in a dose-dependent manner. Similar to DPPH assay, the methanol and water extracts exhibited stronger reduction abilities compared to ethyl acetate and hexane extracts (Table 3). The results might be related to higher level of total bioactive compounds. In this sense, several researchers were reported a positive correlation between total bioactive components and reducing power [21, 23].

The phosphomolybdenum assay is based on the reduction of Mo (VI) to Mo (V) by antioxidants, forming subsequently a green phosphate/Mo (V) complex at acid pH. As can be seen in Table 5, the water extract exhibited the strongest activity was observed between total phenolic and phosphomolybdenum content of the extracts. This fact was supported by several researchers [24, 25].
Table 5. Metal chelating (%), and total antioxidant (by phosphomolybdenum method) activities of the extracts from *P. leucophracta* (mean ± SD).\(^*\).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phosphomolybdenum (mmol TEs/g extract)</th>
<th>Chelating effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>0.73±0.05c</td>
<td>64.87±0.67c</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.72±0.14b</td>
<td>4.88±1.61d</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.40±0.08b</td>
<td>2.28±0.62d</td>
</tr>
<tr>
<td>Water</td>
<td>2.18±0.06a</td>
<td>73.90±2.96b</td>
</tr>
<tr>
<td>EDTA</td>
<td>-</td>
<td>99.10±0.05a</td>
</tr>
</tbody>
</table>

\(^*\) Data marked with different letters within the same column indicate significant difference statistically (\(p < 0.05\)).

\(^{**}\) TEs, trolox equivalents.

\(^{***}\) At 0.25 mg/mL concentration.

– not tested.

Table 6. Correlation coefficients between the assays *.

<table>
<thead>
<tr>
<th></th>
<th>β-Carotene</th>
<th>Phosphomolybdenum</th>
<th>DPPH</th>
<th>Reducing power</th>
<th>Chelating effect</th>
<th>Phenolic content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphomolybdenum</td>
<td>-0.099</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>-0.256</td>
<td>0.827</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Reducing power</td>
<td>-0.595</td>
<td>0.752</td>
<td>0.928</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelating effect</td>
<td>0.727</td>
<td>0.001</td>
<td>0.237</td>
<td>-0.107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolic content</td>
<td>-0.182</td>
<td>0.994*</td>
<td>0.877</td>
<td>0.822</td>
<td>-0.008</td>
<td></td>
</tr>
<tr>
<td>Flavonoid content</td>
<td>-0.466</td>
<td>0.729</td>
<td>0.971</td>
<td>0.979*</td>
<td>0.093</td>
<td>0.800</td>
</tr>
</tbody>
</table>

\(^{*}\) Data represents Pearson Correlation Coefficient R.

\(^{*}\) indicates \(p < 0.05\)

\(^{**}\) indicates \(p < 0.01\)

4. CONCLUSION

In summary, the antioxidant properties of different extracts from *Phlomis leucophracta* were detected by different antioxidant methods as well as total bioactive components. Generally, the water and methanol extracts exerted considerable antioxidant properties compared to hexane and ethyl acetate extracts. These results suggested that *Phlomis leucophracta* could be utilized as source of natural antioxidants in food and pharmacological area. Further studies are needed to identify bioactive compounds in the studied extracts.

5. MATERIALS AND METHODS

Plant material

*Phlomis leucophracta* P. H. Davis et Hub.-Mor. plant was collected in 2015 from Bolvadin-Afyonkarahisar, Turkey (during flowering season). Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr. Olcay Ceylan, in Department of Biology, Mugla Sitki Kocman University. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Mugla Sitki Kocman University, Mugla, Turkey (1020 m, 38° 43’ 46.06”N 31° 02’ 47.72”E, Voucher No: OC 1009).

Preparation of the extracts

Four different solvents (n-hexane, ethyl acetate, methanol, and water) were used to fractionate the soluble compounds from *P. leucophracta* in ascending polarity. The air-dried samples (20 g) were sequentially extracted by using a Soxhlet extractor for 5 h, including n-hexane, ethyl acetate, and methanol under reflux conditions (250 mL for each solvent). The residues were then extracted by boiling water (300 mL). n-Hexane, ethyl acetate and methanol were then removed by using a rotary evaporator. Then, the water extract was freeze-dried. All extracts were stored at +4 °C until analyzed.
Assay for total phenolic and flavonoids

Total phenolic and flavonoid constituent of the extracts were determined by employing the methods given in the literature [29].

Antioxidant activity

Antioxidant capacity of the extracts was tested by different assays including scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) [29], chelating effects on ferrous ions [15], reducing power [30], and total antioxidant activity by β-carotene-linoleic acid method [15] and phosphomolybdenum methods [25] according to the procedures given in literature.

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean and standard deviation values (mean ± SD). Statistical differences between the extracts were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post hoc test (α = 0.05). Correlation analyses were performed by using a two-tailed Pearson’s correlation test. All the analyses were carried out by using SPSS v22.0 software.

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Authorship statement


Conflict of interest statement

The authors have no conflicts of interest.

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