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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 extracts. 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's in this 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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Table 1. Total phenolic and flavonoid content of the extracts from *P. leucophracta* (mean ± SD)*.

Sample	Phenolic content (mg GAEs/g extract) **	Flavonoid content (mg QEs/g extract) ***
n-Hexane	30.86±1.44c	nd****
Ethyl acetate	46.03±2.21b	4.93±0.30c
Methanol	43.54±0.95b	20.15±0.02b
Water	55.00±0.99a	26.09±0.14a

* Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$).
** GAEs, gallic acid equivalents.
*** QEs, quercetin equivalents.
**** nd, not determined.

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].

Table 2. Scavenging effect (%) on 1.1-diphenyl-2-picrylhydrazyl of solvent extracts from *P. leucophracta* at different concentrations (mean ± SD)*.

Sample	Sample concentration (mg/mL)		
	0.40	1.00	2.00
n-Hexane	3.22±0.24e	8.80±0.79c	21.52±0.50d
Ethyl acetate	15.71±1.19d	32.26±2.71b	58.81±0.58c
Methanol	35.30±2.27c	85.43±1.00a	94.54±0.08a
Water	61.57±1.32b	90.03±0.18a	89.14±0.08b
BHA	95.30±0.10a	-	-
BHT	94.11±0.05a	-	-

* Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$). – not tested.

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].

Table 3. Reducing power (absorbance at 700 nm) of solvent extracts from *P. leucophracta* at different concentrations (mean ± SD)*.

Sample	Sample concentration (mg/mL)		
	0.20	0.40	1.00
n-Hexane	0.032±0.002e	0.071±0.004c	0.163±0.010c
Ethyl acetate	0.135±0.013d	0.293±0.002b	0.667±0.018b
Methanol	0.312±0.010c	0.625±0.021a	1.495±0.071a
Water	0.341±0.024c	0.671±0.020a	1.418±0.004a
BHA	2.282±0.004a	-	-
BHT	1.441±0.004b	-	-

* Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$). – not tested.

β-carotene/linoleic acid system was performed to determine the capacity of the extracts for linoleic acid oxidation. The results were summarized in Table 4. Interestingly, the hexane extract exhibited remarkable activity in the test system as well as the water extract. Apparently, these results showed that antioxidant effects depend mainly on the types of solvent used. The results obtained by β-carotene-linoleic acid bleaching inhibition method were different from those of the radical scavenging and reducing power assays. Also, similar observations were reported by several researchers [24, 25].

Table 4. Antioxidant activity (%) of solvent extracts from *P. leucophracta* at different concentrations measured by β-carotene–linoleic acid method (mean ± SD)*.

Sample	Sample concentration (mg/mL)		
	0.40	1.00	2.00
n-Hexane	90.56±1.57a	93.12±0.40a	94.35±1.16a
Ethyl acetate	79.02±2.78a	87.91±0.31b	91.08±0.31b
Methanol	50.88±13.35b	71.32±2.32c	84.10±1.08c
Water	83.20±3.68a	91.44±0.78ab	94.46±0.46a
BHA	-	-	95.77±0.08a
BHT	-	-	96.99±0.09a

* Data marked with different letters within the same column indicate significant difference statistically ($p < 0.05$). – not tested.

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 followed by ethyl acetate, methanol and hexane extracts. According to Pearson correlation analysis, the strong correlation was observed between total phenolic and phosphomolybdenum activity ($p<0.01$), thus this activity may be attributed to the higher levels of total phenolic compounds (Table 6).

Table 5. Metal chelating (%), and total antioxidant (by phosphomolybdenum method) activities of the extracts from *P. leucophracta* (mean ± SD)*.

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

* 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.

Transition metals play a pro-oxidant in the lipid peroxidation and thus the chelating activity of these ions is an important way in the antioxidant mechanism. The metal chelating ability of the studied extracts was tested by ferrozine method at 0.25 mg/mL concentration. The metal chelating ability can be ranked as water>hexane>ethyl acetate>methanol (Table 5). However, EDTA is an excellent chelator. Clearly, the observed results might be related to non-phenolic chelators, such as ascorbic, citric acid and peptides. This fact was also confirmed by correlation test (Table 6). This case also supported by some researches, who reported that a negative correlation between phenolic and metal chelating assay [26-28].

Table 6. Correlation coefficients between the assays [a]

	β-Carotene	Phosphomolybdenum	DPPH	Reducing power	Chelating effect	Phenolic content
Phosphomolybdenum	-0.099					
DPPH	-0.256	0.827				
Reducing power	-0.595	0.752	0.928			
Chelating effect	0.727	0.001	0.237	-0.107		
Phenolic content	-0.182	0.994**	0.877	0.822	-0.008	
Flavonoid content	-0.466	0.729	0.971*	0.979*	0.093	0.800

[a] 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 ($\alpha = 0.05$). 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

Author contributions: Concept – M.C., C.S.; Design – A.K., C.S.; Supervision – M.C., C.S.; Resource – G.Z.; Materials – A.K.; Data Collection and/or Processing – A.K., C.S.; Analysis and/or Interpretation – A.K., C.S.; Literature Search – A.K., G.Z.; Writing – G.Z.; Critical Reviews – G.Z., C.S.

Conflict of interest statement

The authors have no conflicts of interest.

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