Short communication

Antioxidant Activity of Five *Lathyrus* L. Species Growing in Turkey

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The antioxidant activities of five *Lathyrus* species growing in Turkey were investigated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and the contents of total phenolics in the extracts were determined spectrophotometrically according to the Folin−Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Among the *Lathyrus* species, remarkable high antioxidant activity and high total phenolic content was found in *Lathyrus aureus*. The results showed that there is a strong correlation between total polyphenol contents and antioxidant activity of the extracts.

Key words: *Lathyrus,* Antioxidant, Total phenolic compound.

Türkiye'de Yetişen Beş *Lathyrus* L. Türlerinin Antioksidan Etkileri

Bu çalışmada Türkiye'de doğal olarak yetişen beş *Lathyrus* türünün antioksidan aktivitesi 1,1- difenil-2-pikrilhidrazil (DPPH) serbest radikal süpürücü yöntemi ile, ekstrelerin total fenolik bileşik miktarları ise spektrofotometrik olarak Folin−Ciocalteu yöntemi ile gallik asit ekivalanı (GAE) olarak tayin edildi. *Lathyrus* türleri arasında en yüksek toplam fenolik madde içeriğine ve en yüksek antioksidan aktiviteye *Lathyrus aureus* türünün sahip olduğu belirlendi. Sonuçlar ekstrelerin toplam polifenol içeriği ile antioksidan aktiviteleri arasında güçlü bir ilişki olduğunu gösterdi.

Anahtar kelimeler: *Lathyrus,* Antioksidan, Toplam fenolik bileşik.

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INTRODUCTION

In recent years the plant phenolics have attracted great attention, due to antioxidant potentials of plants. There are two basic categories of antioxidants, natural and synthetic, the synthetic ones have been found to cause long-term toxicological effects, including carcinogenicity (1).

The phenolic compounds in plants are especially present in leaves, flowering tissues, and woody parts. They have an important role in the plant growth and flowers colors, defense against infection and injury (2). Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development. Plant phenolics include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans, and lignins.

The amount of phenolic compounds in plants are measured by colorimetric methods as UV/VIS spectrophotometry; These methods are easy, rapid and applicable in routine laboratory use, with low-cost. Total concentrations of phenolic hydroxyl groups in the plant extracts are measured by colorimetric assays. The Folin-Ciocalteu method is one of these assays. Polyphenols in plant extracts react with Folin-Ciocalteu reagent to form a blue complex that can be quantified by UV/VIS spectrophotometry (3).

Recently, increasing interest has been focused on antioxidant activities of plant extracts. The structural chemistry of polyphenolic compounds are known to have free radical scavenging properties. The antioxidant activity of phenolics is mainly due to their redox properties, which play a crucial roles in adsorbing and neutralizing free radicals, quenching single and triplet oxygen or decomposing peroxides. The activity of antioxidant is determined by its reduction potential ability to stabilize and delocalize the unpaired electron, reactivity with other antioxidants and transition-metal chelating potential (4).

Genus *Lathyrus* L, is a member of the Viciae tribe (family Fabaceae), consists of about 160 species and many of them are economically important and used as forage, human food, ornamental plants, soil nitrifiers and model or genetic and ecological research (5).

Some *Lathyrus* species, such as *L. latifolius* L., *L. sylvestris* L. and *L. grandiflorus* are cultivated as ornamental plants (6).

Lathyrus spices used as animal feeds and in some poor countries as food and being cultivated for nitrogen fixation and use in marginal lands as a fodder crop. Even though nitrogen is the most abundant elements on the earth, it is also the major element limiting growth of plants in many agricultural systems because of its unavailability for plants (7).

Lathyrus has many species that has a high value of proteins; but only a few of these species are cultivated as a food source. Lathyrus species as a new protein sources for use in both functional food ingredients and nutritional supplements (8).

In Turkey, *Lathyrus* is represented by 65 generaand 75 taxa (9). *Lathyrus* species contain various flavonoids, such as quercetin, kaempferol, luteolin, myricetin and their glycoside (10). They also contain fatty acids, such as linoleic and linolenic acid (11) and, as well as proanthocyanidins, cyanogenic glucosides(12), phytoecdisteroids, triterpene saponins (13).

In spite of the potential interest of *Lathyrus* species as a source of functional compounds such as antioxidant phenolics, studies on polyphenols composition in *Lathyrus* are scarce and restricted to a few species. Thus, in this study we investigate the antioxidant and total phenol contains of five *Lathyrus* species growing in Turkey.

EXPERIMENTAL

Plant material

Five species of *Lathyrus* were collected from Sivas, Turkey, during the flowering period. Voucher species were identified by Assist. Prof. Dr. Mehmet TEKİN. Plant samples deposited for future reference in Ankara University, Faculty of Pharmacy, Kamil Karamanoglu Herbarium (AEF). Investigated plants information are summarized in Table 1.

Extraction

The dried and powdered aerial parts of plants (50 g of each) were extracted with 400 mL methanol by Soxhlet extraction for 12 hrs. The residue was evaporated under the vacuum and dried and then extracted with 250 mL water. The MeOH extract was dried under reduced pressure and then partitioned consecutively between n-Hexane, chloroform, ethyl acetate and water. The whole obtained extracts were stored at 4˚C in closed vessels until analyzed.

Total soluble phenolic content assay

Total phenolic compounds of five *Lathyrus* species were measured using the Folin-Ciocalteu assay. Briefly, 5 mL of Nanopure water, 0.5-1.0 mL of sample, and 1.0 mL of Folin-Ciocalteu reagent were added to a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5−8 min at room temperature. Next, 10 mL of a 7% sodium carbonate solution was added, followed by the addition of Nanopure water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2 hrs. The absorbance of the samples was measured by spectrophotometer at 750 nm.

Total phenolic componds content was standardized against gallic acid and expressed as milligrams per liter of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5-5.0 mg/L GAE (R2= 0.9996), giving an absorbance range of 0.050−0.555 AU (14).

Table 1. Investigated plant material

Plant species	Part of plant	Collection sites	Altitude (m)	Voucher no.
L. armenus (Endemic)	Aerial	Sivas, Turkey	1261	26680
L. aureus	Aerial	Sivas, Turkey	1565	26684
L. cilicicus (Endemic)	Aerial	Sivas, Turkey	981	26681
L. laxiflorus subsp. *laxiflorus*	Aerial	Sivas, Turkey	1261	26682
L. pratensis	Aerial	Sivas, Turkey	1565	26683

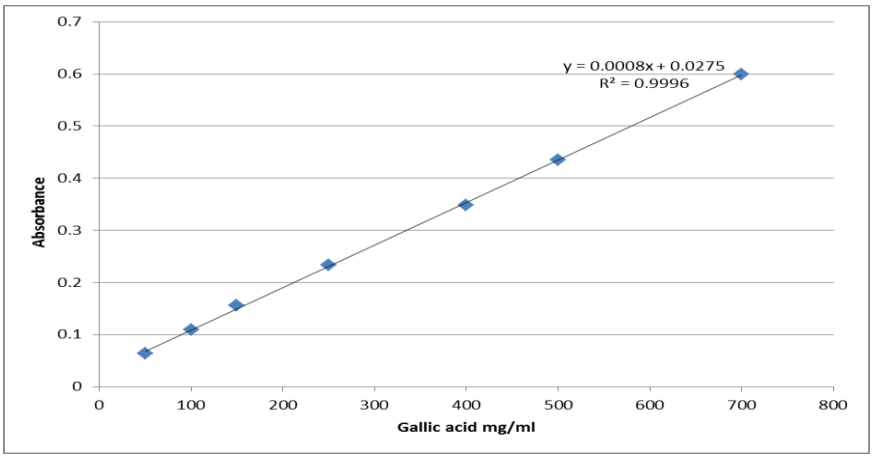


Figure 1. Calibration curve with galic acid as standard

Determination of antioxidant activity by the DPPH radical scavenging method

The antioxidant activity of plant samples was measured by using the DPPH assay. 30 µL of plant extract extract diluted 7-fold with methanol and then mixed with an aliquot of 270 µl of 6×10^{-5} M DPPH radical in methanol. Methanol was used as a control instead of extract. The reaction mixture was vortex-mixed and let to stand at 25°C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer using methanol as a blank. Various concentrations of butylated hydroxytoluene (BHT) were used as standards. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (15).

$$\text{Scavenging activity (\%)} = \{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})/\text{Abs}_{\text{Control}}\} \times 100$$

In both methods the capacity of scavenging free radicals was calculated as follows:
IC_{50} values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%. IC_{50} was calculated for all the extracts and BHT as standard based on the percentage of DPPH radicals scavenged.

RESULTS

The results of total phenolic contents of methanol extract of *Lathyrus* species are summarized in Table 2. The results of the antioxidant assays of methanol, *n*-hexane, chloroform, ethyl acetate and aqueous sub-extract of five species of *Lathyrus* genus are shown in Table 3. Inhibition percent of extracts against the concentration of methanol, *n*-hexane, chloroform, ethyl acetate and aqueous, sub-extracts are shown as graphic model in Figure 2.

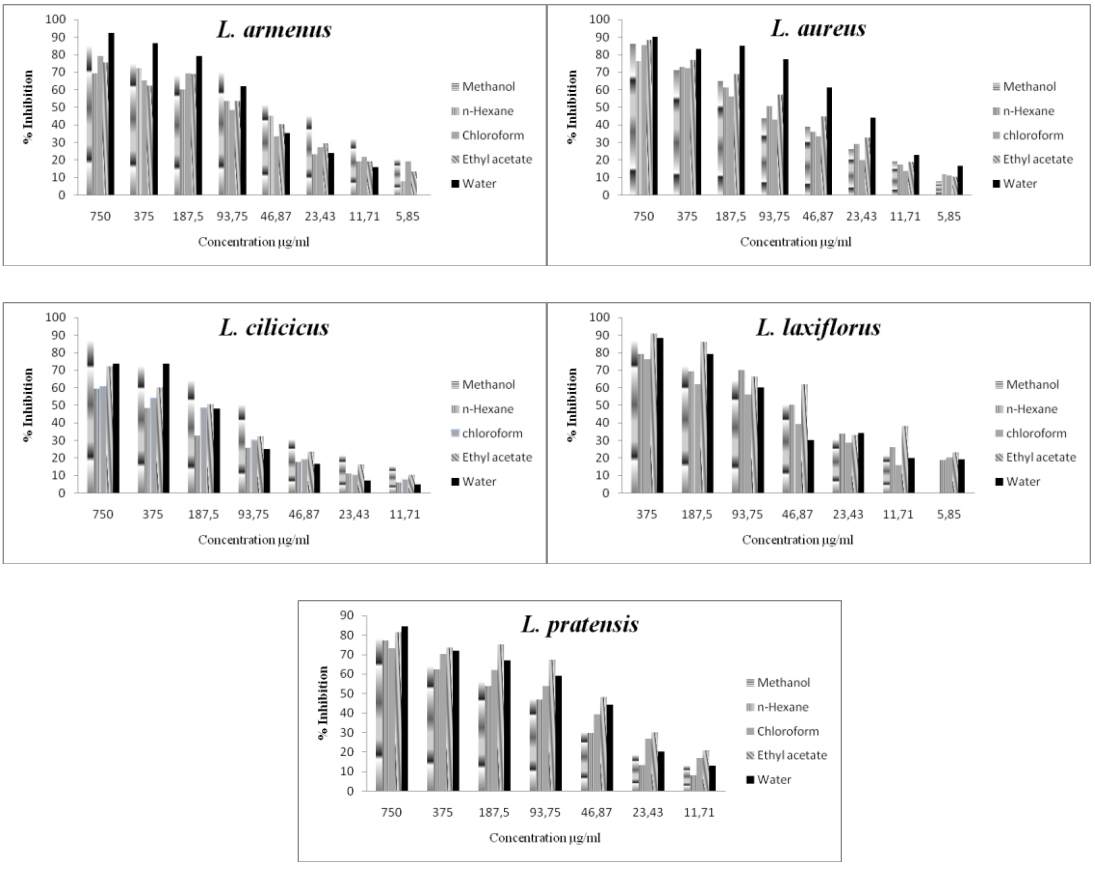
Table 2. Total polyphenolics contents of methanolic extract of *Lathyrus*

Plant species	**(mg $_{\text{gallic acid}}$/ g $_{\text{extract}}$) ± SE**
L. armenus	150.63±8.48
L. aureus	452.19±6.88
L. cilicicus	179.69±4.93
L.laxiflorus	397.00±10.96
L. pratensis	390.94±7.39

SE: Standard Error

Table 3. DPPH radical scavenging activity

	IC_{50} (µg/mL)				
Species	***n*-Hexane**	**$CHCl_3$**	**EtOAC**	**MeOH**	**Aqueous**
L. armenus	0.050	0.050	0.047	0.041	0.041
L. aureus	0.050	0.051	0.048	0.043	0.035
L. cilicicus	0.057	0.055	0.053	0.047	0.050
L. laxiflorus	0.470	0.049	0.041	0.038	0.040
L. pratensis	0.052	0.050	0.045	0.044	0.042
BHT			0.027		



Figıre 2. % Inhibition of *Lathyrus* species in DPPH radical scavenging activity assay

DISCUSSION

There were few studies on the antioxidant activities of *Lathyrus* species; such as the aerial part of *L. binatus* (16), seeds of *L. sativus* (17, 18, 21), hulls and seeds of *L. maritimus* L. (19;20), whole plant of *L. pratensis* (21), seeds of *L. hirsutus, L. filiformis, L. cicera, L. angulatus, L. sphaericus* (17), seeds and aerial part of *L. annuus* (17; 21). seeds of *L. clymenum* and *L. ochrus* (17), seeds of *L. latifolius, L. setifolius, L. tingitanus* and *L. amphicarpos* (17).

In this study the contents of phenolic compounds of *Lathyrus* methanolic extract determined by the Folin-Ciocalteu reagent. Total phenolic compound of five species of *Lathyrus* were screened; As shown in Table 2 the highest phenolic contents were observed in *L. aureus* (452,19±6,88 mg $_{\text{gallic acid}}$/ g $_{\text{extract}}$). However, the other species also had considerable amount of phenolic compounds.

The radical scavenging activity of the plant species were determined by DPPH method. As shown in Figure 2 and Table 3 all extracts and sub-extracts exerted radical scavenging activities. Although aqueous sub-extracts of *L. aureus* had shown the highest activity (0,035 IC_{50} (µg/ml)), the methanol extracts and aqueous sub-extracts of other species also have remarkable radical scavenging activities. In this study, BHT was used as a standard (IC_{50} 0,027 µg/mL). The results showed that there is a strong correlation between total polyphenol contents and antioxidant activity.

CONCLUSION

Some researchers suggest that two-thirds of the world's plant species have medicinal value and many medicinal plants have great antioxidant potentials. All methanol extracts of the plant extracts in this study had considerable phenolic compounds and all extracts and sub-extracts exhibited radical scavenging activity. Methanol extracts and aqueous sub-extracts had shown the highest radical scavenging activities. Possibly due to the extracts with high amount of phenolic contents have high antioxidant activities. Further studies in our laboratory will focus on isolation of these phenolic compounds.

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