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Article

A comparative study on biological activities of different solvent extracts from whole seed, seed coat and cotyledon of two *Lathyrus* species

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The present study was conducted to assess the phenolic content, and antibacterial and antioxidant activities of *Lathyrus* L. species. The extraction of phenolic compounds from whole seeds, seed coat and cotyledon of *Lathyrus hierosolymitanus* Boiss. and *Lathyrus annuus* L. seeds was performed employing different solvents. Total phenolic content (TPC) was measured by Folin-Ciocalteau assay, while the antioxidant activity was determined by DPPH radical scavenging activity, and reducing power assay. It was found that TPC of extracts ranged from 0.12 mg to 6.53 mg GAE/gdw. For each solvent, seed coat extracts were generally observed to render higher TPC and antioxidant activities. There was a correlation between TPC and antioxidant activity. In addition, all extracts were also examined for their antimicrobial activity against *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Methanol extracts showed the highest antibacterial activity which is consistent with TPC, but there was no correlation between TPC and antibacterial activity. Solvents were observed to have effects on gallic acid, caffeic acid, and epicatechin extractions. HPLC analysis results of extracts confirmed methanol and ethanol as preferred solvents for phenolic extraction from *Lathyrus* sp. Phenolic content in the extracts could be suggested to contribute to their antioxidant and antibacterial activity.

Keywords: Antioxidant activity. Antibacterial activity. *Lathyrus*. Phenolic content. HPLC.

INTRODUCTION

Oxidative stress is the disruption of the balance between antioxidants and pro-oxidants. If there is an increase in favor of pro-oxidants, it causes a significant change in DNA biomolecules due to their high reactivity. The body has an endogenous defense system that can reduce the effects of these free radicals. The contribution of exogenous antioxidants may be required if this defense system is insufficient to neutralize free radicals (Sokamte, 2019).

Plants, as suppliers of these exogenous antioxidants, have become important sources of medicine as well as food. Most of the therapeutic effects of plants are attributed to their high antioxidant content and their phenolic composition (Singh *et al*., 2017a). Phenolic compounds found widely in plants are crucial sources of pharmaceutical products (Khan *et al*., 2009). They have biological properties including antioxidant, anti-inflammatory, anti-atherogenic and antimicrobial effects (Singh *et al*., 2017a).

The protective effect of consumption of phenolic-rich grains, and legumes against some chronic diseases was assumed (Zhao *et al*., 2014; Zhang *et al*., 2015; Fratianni *et al*., 2014; Parikh, Patel, 2018). Legumes are essential sources of macronutrients and micronutrients, as well as

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being rich in proteins, lipids, vitamins, carbohydrates, and polyphenols (Zhang *et al*., 2015; Fratianni *et al*., 2014; Zhao *et al*., 2014). Many studies have demonstrated the phenolic content of legumes and their antioxidant activity (Amarowicz *et al*., 2004; Pastor-Cavada *et al*., 2009; Xu, Chang, 2010). The rich phenolic content of legume seeds also makes them the main source of antioxidant activity in food (Zhao *et al*., 2014).

The legume seed coat, often called hull or testa, is the outer layer of seeds. They act as a protective barrier for the cotyledon which essentially contains proteins and carbohydrates (Zhong *et al*., 2018; Singh, *et al*., 2017a; Parikh, Patel, 2018). The seed coats are the major contributors to the phytochemical content of whole seeds (Boudjou *et al*., 2013; Zhong *et al*., 2018). They show high antioxidant potential owing to large amounts of phenolic compounds (Singh *et al*., 2017a; Singh *et al*., 2017b). For example, lentil seed coat contains a more abundant and diverse type of polyphenols compared to cotyledon (Singh *et al*., 2017a).

Genus *Lathyrus* L. is characterized by the presence of leguminous species, belonging to the *Fabeae* tribe of the Fabaceae family and is represented by more than 200 species worldwide. Genus *Lathyrus* contains 79 taxa in the species, subspecies, and variety levels in Turkey and 25 of them are endemic (Davis *et al*., 1970; Genç, Şahin, 2011; Güneş, 2014). Genus *Lathyrus* is used in different areas including nutrition, agriculture, industry, and ornament (Vaz-Patto, Rubiales, 2014). In addition, *Lathyrus* species have been used in traditional medical treatments such as analgesic, and anti-rheumatism (Altundag, Ozturk, 2011).

In the literature, several studies have reported that the extracts from *Lathyrus* species have antioxidant activities, and inhibit enzymes such as cholinesterases, α-amylase, and α-glucosidase (Llorent-Martínez *et al*., 2017a; Llorent-Martínez *et al*., 2017b). *Lathyrus* species have been suggested to be a new source of natural phenolic antioxidants and high-quality proteins for human and animal nutrition (Pastor-Cavada *et al*., 2009). However, the data on their antioxidant and antimicrobial activities are limited. Furthermore, the differences in phenolic compounds in the seed coat and cotyledon of *Lathyrus* taxa are not known. As it is known, *Lathyrus* species have variations in nutrient contents and antinutritional factors in the seeds as well as morphological traits (Grela *et al*., 2012). Therefore, the studies on wild *Lathyrus* species are crucial to determine their properties and to benefit from them.

In our previous study, *L. hierosolymitanus* and *L. annuus* were found to be excellent protein sources with low β-ODAP (β-N-oxalyl-L-α,β-diaminopropionic) content and to contain natural antioxidants (Yazici Ozbek *et al*., 2020). These species are very similar in taxonomic terms. Therefore, it is important to thoroughly investigate the biological activities of each of these species.

The aim of this study is to determine the best solvent for the extraction of phenolic compounds from different parts of *L. hierosolymitanus* and *L. annuus* seeds growing in Turkey, as well as to compare the distribution of phenolic compounds in seed parts and their antioxidant and antimicrobial activities.

MATERIAL AND METHODS

Material

The chemical materials to be used in this study are Folin–Ciocalteau reagent, gallic acid, DPPH (2,2-diphenyl-1-picryl-hydrazyl), BHA (butylated hydroxyanisole). They were all purchased from Sigma-Adrich.

Plant Material

The seeds of *Lathyrus* taxa were collected from their natural habitats in 2014. Their taxonomic identification was confirmed by Genç and Yıldırım. Taxa and their localities are given below:

L. annuus	Mugla, Yatagan, around Stratonikeia ancient city, Genç-3002, 2015.
L. hierosolymitanus	Mugla, Dalyan, around Eskiköy, Genç-3001, 2015.

Extraction procedure

The three samples including whole seed, seed coat, and cotyledon obtained from *Lathyrus* seeds

were extracted to carry out the study. Seed coats were removed manually from their cotyledons. The seeds and their parts were dried at room temperature. After that, the samples were pulverized and were firstly defatted with hexane for 2 h. The defatted samples were shaken at 300 rpm at ambient temperature on an orbital shaker within the solutions of absolute ethanol, absolute methanol, and water for 3 h to extract phenolic compounds. After 3 h, the supernatants were separated by centrifugation at 3000 x g for 10 min. Each supernatant was filtered through filter paper and the extracts were concentrated using a rotary evaporator and stored at + 4 ˚C in dark until use.

Total Phenolic Content (TPC)

In the extracts, TPC was determined using Folin–Ciocalteu method as previously described (Yazici Ozbek *et al.,* 2020). Gallic acid was taken as standard for comparison and the results were expressed in milligrams of gallic acid equivalent (GAE) per gram dry weight *Lathyrus* seed (mg GAE/gdw).

Radical Scavenging Assay

The antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) the radical-scavenging method as previously described (Yazici Ozbek *et al.,* 2020). Antioxidant activities of the extracts were expressed as IC_{50}, defined as the concentration of the extract required to cause a 50% decrease in the initial $DPPH^{\bullet}$ concentration.

Ferric reducing power assay

The modified Oyaizu method (1986) was used to determine the ferric reducing power. Substances which have reduction potential react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form a ferric-ferrous complex. Absorbance was measured at 700 nm. The EC_{50} value (the effective concentration at which the absorbance was 0.5) was calculated from the graph of absorbance at 700 nm against extract concentration.

HPLC-DAD analyses of phenolic compounds

The analytical HPLC system employed comprised of a Shimadzu Prominence high-performance liquid chromatography coupled with a 20A CBM (HPLC System Controller), DAD (diode array detector) (SPD-M20A, Tokyo, Japan), an SIL 20ACHT automatic sampler, a CTO10ASVp column oven, and an LC20 AT pump. LC Solution data processing system was used to evaluate the analytical data. The separation was achieved on Agilent ZORBAX Eclipse plus C18, 4.6 × 250 mm, 5 µm column at 25 C. m. The mobile phase used was 2% formic acid in (A) water and (B) methanol. The elution gradient applied at a flow rate of 1 ml min^{-1} was: 95% A/5% B for 3 min, 80% A/20% B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, 100%B in 10 min until the end of the run (Caponio, Alloggio, Gomes, 1999). The quantification was performed by using the standards, and the levels were expressed as µg phenolic compounds/g dry seed weight (gdw).

Antibacterial Activity of Extracts

Antibacterial activity of the extracts of samples was carried out by the standard broth micro-dilution assay against *Bacillus cereus* ATCC11778, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC35032.

Initially, extracts were solved in the water, and the extract concentrations were adjusted (50, 100 and 2000 µg/ml). The following inocula were produced using bacterial colonies with incubation times, not superior to 24 h, adjusted to the McFarland standard solution of 0.5 of bacterial turbidity, in a concentration of 1.5×10^8 CFU/ml (colonies forming unities for milliliter) and, following, the concentration of 5×10^5 CFU/ml with caso soy broth (CSB).

For the determination of the MIC, 1 ml of test solutions was poured in each test tube (in the specified concentrations) as well as 1 ml of the bacterial suspension, except in the negative control tube. The tubes were incubated at 35 ± 2 ºC for 20 h, and afterward the turbidity (bacterial growth) was evaluated by absorbance measurement at 620 nm. The minimum inhibitory concentration (MIC) value for each sample was

determined by testing each concentration in triplicate. The MIC value was the lowest concentration of the extracts (mg/ml) capable of inhibiting the complete growth of the bacterium being tested. CSB without the inoculum was used as negative control tubes. The CSB broth and just bacterial growth was used as positive control tubes.

Statistical analyses

Statistical analyses were carried out with SPSS 20. Data were expressed as the mean ± SD. The independent t-test was used to analyze the differences between the extracts from *Lathyrus* species while one-way ANOVA was used to analyze the differences among *Lathyrus* seed parts extracts obtained with different solvents. As the data demonstrated, the differences were significant ($p < 0.05$). Correlations among variables were performed with Pearson's correlation test.

RESULTS AND DISCUSSION

Phenolic Content and Antioxidant Activity of Seed Extracts

Natural phenolics show mainly their beneficial effects on health through their antioxidant activities (Xu, Chang, 2007). Responses of phenolic compounds vary in the Folin–Ciocalteu method depending on the phenolic groups in plant samples (Parikh, Patel, 2018). In the present study, total phenolic content, antioxidant activities of the extracts of seeds and their parts were determined and are shown in Table I. All the extracts evaluated in the study were identified to have a significant total phenolic content ranged from 0.12 ± 0.01 to 7.50 ± 0.6 mg GAE/gdw. The total phenolic content of frequently consumed legumes was reported to vary between 0.325 – 6.378 mg GAE/g (Marathe *et al*., 2011).

TABLE I - Total phenolic content (TPC), DPPH radical scavenging activity (IC_{50}), and Ferric reducing antioxidant activity (EC_{50}) values of extracts from *Lathyrus* seeds

Species	Parts	Solvents	TPC (mg GAE /gdw)	DPPH IC_{50} (mg/ml)	Ferric reducing power EC_{50} (mg/ml)
L. annuus	Seed coat	Water	1.02 ± 0.12^{cA}	3.57 ± 0.5^{aB}	6.2 ± 0.2^{aB}
		Ethanol	3.05 ± 0.45^{bA}	1.65 ± 0.22^{cB}	1.33 ± 0.11^{bB}
		Methanol	5.95 ± 0.61^{aA}	1.35 ± 0.42^{bB}	1.43 ± 0.1^{bB}
	Cotyledon	Water	0.12 ± 0.01^{bC}	6.01 ± 0.9^{aA}	11.7 ± 0.6^{aC}
		Ethanol	0.84 ± 0.34^{aB}	3.4 ± 0.6^{bA}	4.97 ± 0.67^{bB}
		Methanol	0.59 ± 0.14^{aC}	4.23 ± 0.52^{bA}	6.19 ± 0.9^{bB}
	Whole seed	Water	0.53 ± 0.17^{bB}	3.4 ± 0.4^{aB}	3.73 ± 0.19^{aC}
		Ethanol	1.01 ± 0.11^{bB}	2.6 ± 0.6^{aB}	1.55 ± 0.22^{bB}
		Methanol	2.78 ± 0.68^{aB}	1.7 ± 0.7^{bB}	1.55 ± 0.4^{bB}

TABLE I - Total phenolic content (TPC), DPPH radical scavenging activity (IC_{50}), and Ferric reducing antioxidant activity (EC_{50}) values of extracts from *Lathyrus* seeds

Species	Parts	Solvents	TPC (mg GAE /gdw)	DPPH IC_{50} (mg/ml)	Ferric reducing power EC_{50} (mg/ml)
L. hierosolymitanus	Seed coat	Water	$1.61 \pm 0.^{19cA}$	3.0 ± 0.5^{aB}	$2.56 \pm {}^{b}.1^{aC}$
		Ethanol	4.02 ± 0.38^{bA}	1.3 ± 0.2^{bB}	1.32 ± 0.22^{bB}
		Methanol	7.50 ± 0.6^{aA}	0.73 ± 0.19^{cB}	0.81 ± 0.1^{cC}
	Cotyledon	Water	0.24 ± 0.1^{cC}	6.84 ± 0.81^{aA}	10.7 ± 0.2^{aA}
		Ethanol	0.56 ± 0.7^{bC}	3.6 ± 0.5^{bA}	2.56 ± 0.25^{bA}
		Methanol	0.94 ± 0.25^{aC}	2.45 ± 0.35^{bA}	2.79 ± 0.49^{bA}
	Whole seed	Water	0.75 ± 0.14^{cB}	3.5 ± 0.34^{aB}	4.34 ± 0.62^{aB}
		Ethanol	1.78 ± 0.15^{bB}	1.44 ± 0.39^{bB}	1.9 ± 0.62^{bAB}
		Methanol	3.81 ± 0.2^{aB}	0.87 ± 0.14^{bB}	1.73 ± 0.13^{bB}

*Values are mean a standard deviation. Different letter within the same column show differences of means among different solvents for each part of each plant species ($p < 0.05$). Different caps letter shows differences between extracts from different part of the plant in the same solvent for *Lathyrus* species ($p < 0.05$).

The high phenolic content found in the leguminous seeds makes them an important source of antioxidants. However, the total phenolic content varies significantly among legumes (Zhao *et al*., 2014). This difference in phenolic content of legumes can be explained by environmental factors as well as genetic factors. In this study, TPC in extracts from *L. hierosolymitanus* was higher than that of *L. annuus,* but the difference between the total phenolic content of *Lathyrus* species was not found to be significant ($p > 0.05$). The result can be attributed to similar environmental conditions. However, there was a significant difference among the TPC of the extracts from different parts of *Lathyrus* seeds ($p < 0.05$). In particular, the TPC of seed coat extracts was higher than that of both whole seed and cotyledon extracts ($p < 0.05$) (Table I). The results imply that the amount of total phenolic content of seed parts (cotyledon, coat, and whole seed) significantly differed. Similarly, mung bean hull extracts were found to have significantly higher TPC than whole seed and cotyledon (Singh *et al*., 2017b). It is reported that the phenolic compounds in legumes are observed to concentrate in their seed coats due to their protective function in the plants (Marathe *et al*., 2011; Singh *et al*., 2017a). The present study also confirmed the availability of high antioxidant content in the legume seed coat.

Some parameters such as the solvent used, the interaction between phenolics and extraction time were observed to affect the extractability of the phenolic compounds (Marathe *et al*., 2011). The choice of solvents used for extraction is critical because the polarities of the solvents have a direct effect on the amounts and types of recovered phenolic compounds. Several solvents have been used to achieve optimal extraction. Furthermore, the polarity of solvent has an important role in the increasing solubility of phenolic

compounds (Haminiuk *et al*., 2014). In addition, it is known that phenolic compounds are generally associated with biomolecules in the plant, including inorganic compounds, polysaccharides, proteins, terpenes, chlorophyll (Iloki-Assanga *et al.,* 2015).

Methanol and ethanol are some of the most common solvents used for extraction (Gomes, Torres, 2016). However, the use of water for bioactive compounds extraction may be a safer option for pharmaceutical and food applications (Shelembe *et al*., 2012). Polyphenols are known to be often most soluble in organic solvents which are less polar than water (Haminiuk *et al*., 2014). For the mentioned reasons, these solvents were preferred in this study. There is a lack of literature to compare the effects of different solvents on the phenolic extraction and activity of antioxidants in *Lathyrus* materials. Table I shows the effect of different solvents on the extraction of total phenolic from the extracts. These different TPC results can be explained by the polarity of the solvent. In terms of polarity, the solvents used in this research can be put in order according to their dielectric constant as follows: ethanol < methanol < water. In the study, the methanol extracts have mostly higher TPC. The seed coat methanol extract from *L. hierosolymitanus* also found to contain the highest TPC ($p < 0.05$) followed by the seed coat methanol extract from *L. annuus*. ANOVA coupled with post hoc Tukey tests showed significant differences in the TPC of methanol extracts when compared those of extracts obtained with other solvents ($p < 0.05$). The lowest TPC was obtained by water. In a work with similar solvents, Haminiuk *et al*. (2014) found that 100% methanol which is not the most or the least polar solvent was the most effective one for the extraction of phenolic compounds.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in neutralizing and absorbing free radicals (Borra, Gurumurthy, Mahendra, 2013). In this study, the antioxidant activities of all tested extracts were evaluated with reducing power and DPPH assays. The lowest EC_{50} or IC_{50} means had the highest antioxidant capacity. Significant differences were detected between antioxidant activities of water extracts and other extracts ($p < 0.05$). For the IC_{50} values, the seed coat extracts showed higher values between 0.73 ± 0.19 and 3.57 ± 0.5 mg/ml, while the values in the cotyledon ranged from 2.45 ± 0.35 to 6.84 ± 0.81 mg/ml. The seed coat methanol extract, whole seed methanol extract, and seed coat ethanol extract from *L. hierosolymitanus* are found to have the highest DPPH radical scavenging activity ($p < 0.05$). In a previous study, the IC_{50} values of extracts from *L. czeczottianus* Bässler and *L. nissolia* L. were found as 1.42 mg/ml and 3.19 ± 0.11 mg/ml, respectively, for DPPH assay (Llorent-Martínez *et al*., 2017a). In the present study, EC_{50} values of ferric reducing power of the extracts also varied from 0.81 ± 0.1 to 11.7 ± 0.6 mg/ml. Similarly, the reducing power of the methanol extracts and seed coat extracts of *Lathyrus* species was mostly higher. The seed coat methanol extract from *L. hierosolymitanus* has the highest reducing power ($p < 0.05$). The extracts from cotyledon and extracts obtained by water exhibited significantly lesser antioxidant activity when compared with the extracts from whole seed and seed coat obtained with both ethanol and methanol ($p < 0.05$).

Phenolic components contribute to all antioxidant activities of the plant (Xu, Chang, 2007). Previous studies found the correlation between TPC with DPPH scavenging activity and reducing power capacity (Noor Atiqah, Maisarah, Asmah, 2014; Fidrianny, Natalia, Insanu, 2015; Krishnappa *et al*., 2017). In the present study, Pearson's correlation coefficient between TPC in the extracts and antioxidant activities of extracts showed that TPC had a negative correlation with IC_{50} of DPPH scavenging activities ($r = -0.733$) and EC_{50} of reducing power ($r = -0.594$). Phenolic content in the extracts from *Lathyrus* seed could be suggested to contribute to their DPPH scavenging activity and reducing power activity.

As a conclusion, the TPC, DPPH scavenging activity and reducing power of extracts from seed parts showed high variability, while the variability between the species was lower. The methanol and ethanol extracts from the seed coat and whole seed also showed the strongest antioxidant activity. According to these results, it was found that seed coat has the strongest contribution to the antioxidant capacity of the tested *Lathyrus* species.

Solvents affect the rate and aggregate quantity of extraction of phenolics (Khan, Bakht, 2016). Therefore, the determination of a suitable solvent provides the

stability of extracted phenolic compounds and it is very important for an efficient extraction procedure. The other aim of our study was to investigate the influence of the solvents on some individual phenolic compounds as well as on TPC. In order to identify this, the concentrations of gallic acid, caffeic acid, and epicatechin in the extracts were evaluated by using HPLC. The phenolic compositions of the extracts are shown in Figure 1-3.

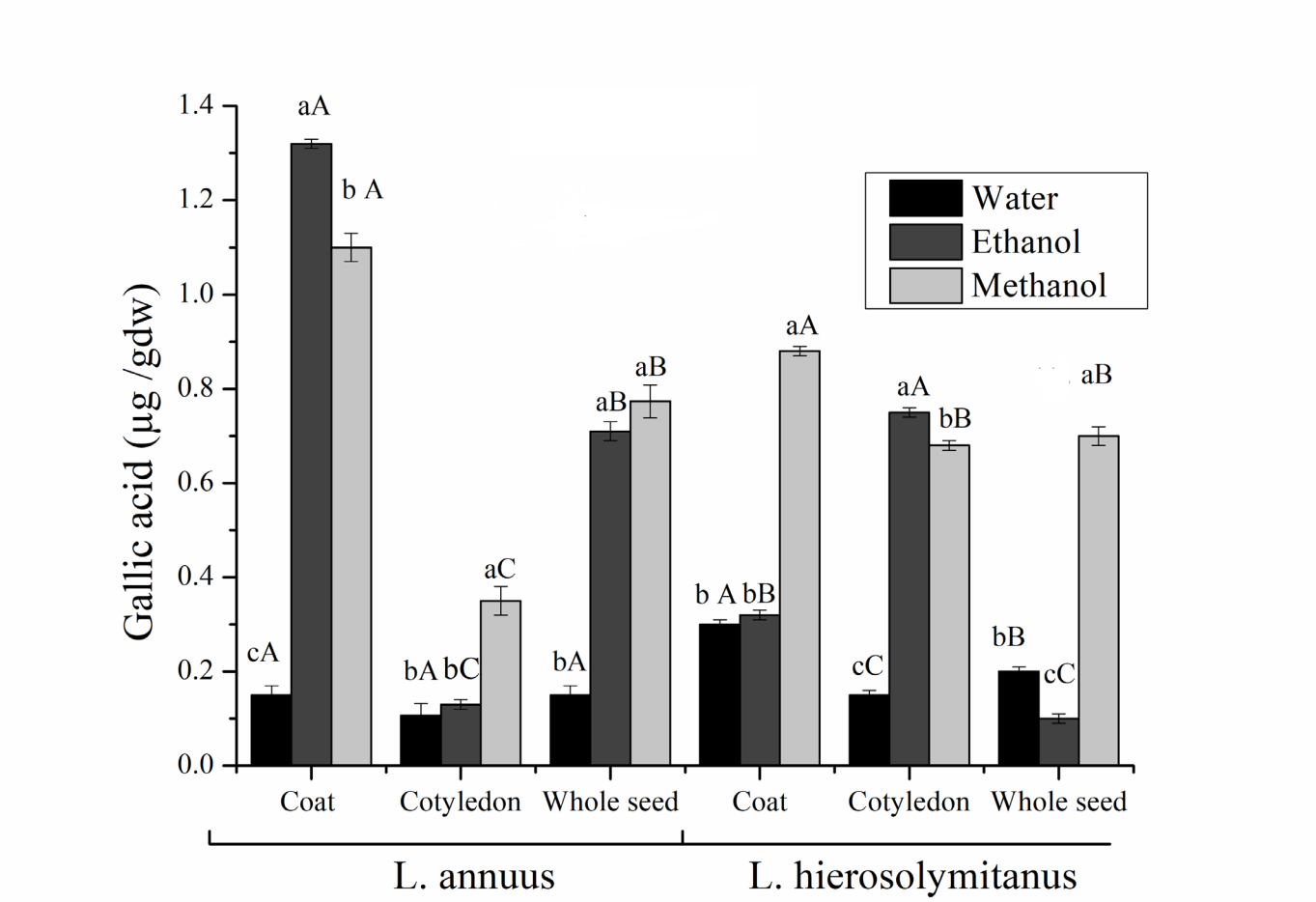

FIGURE 1 - Distribution of gallic acid in *Lathyrus* seed extracts. Different letter show differences of means among different solvents for each part of each plant species ($p < 0.05$). Different caps letter shows differences between extracts from different part of the plant in the same solvent for *Lathyrus* species ($p < 0.05$).

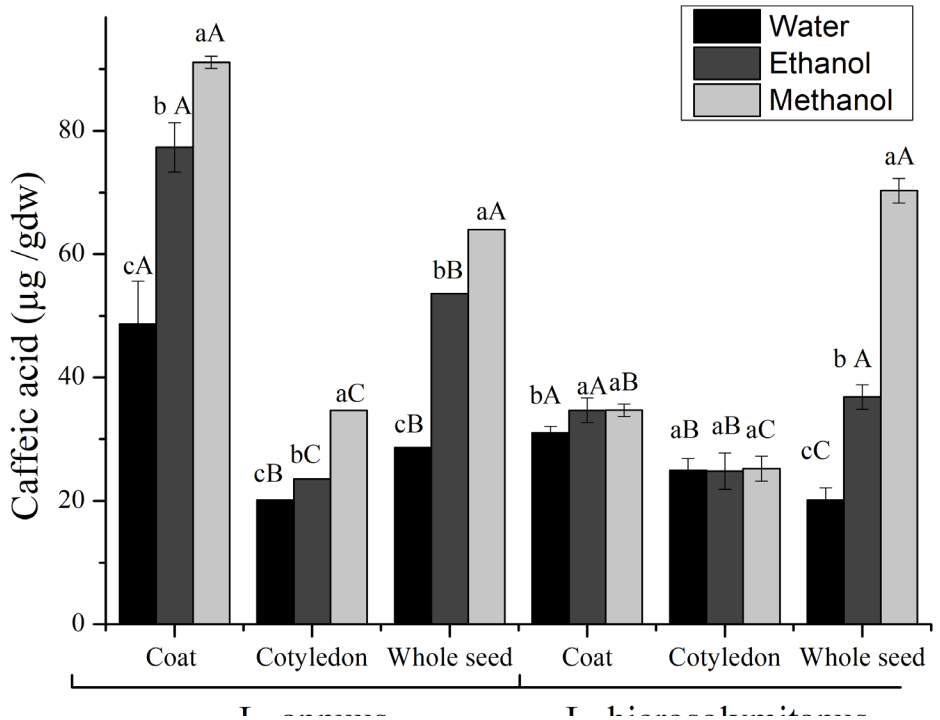

FIGURE 2 - Distribution of caffeic acid in *Lathyrus* seed extracts. Different letter show differences of means among different solvents for each part of each plant species ($p < 0.05$). Different caps letter shows differences between extracts from different part of the plant in the same solvent for *Lathyrus* species ($p < 0.05$).

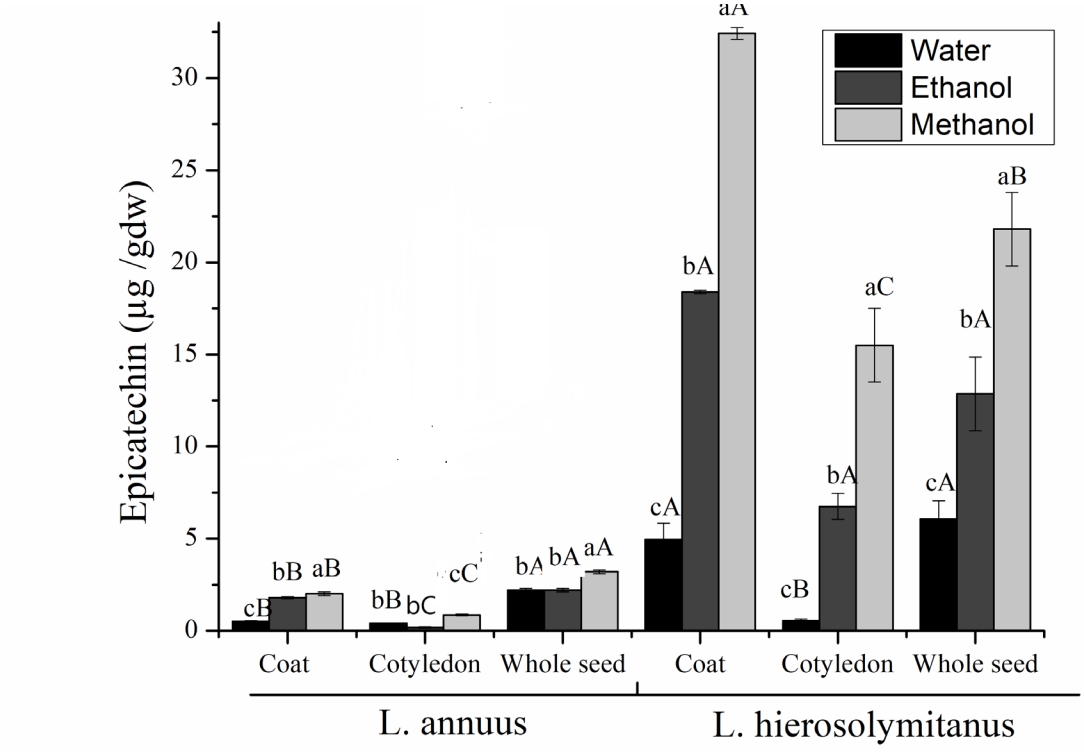

FIGURE 3 - Distribution of epicatechin in *Lathyrus* seed extracts. Different letter show differences of means among different solvents for each part of each plant species ($p < 0.05$). Different caps letter shows differences between extracts from different part of the plant in the same solvent for *Lathyrus* species ($p < 0.05$).

In the present study, the higher total phenolic contents were mostly obtained from methanol extracts compared with ethanol and water extracts, but this is not the case for all the individual phenols. Similar effects of the solvents were observed in a previous study (Jouki**,** Khazaei, 2010). The highest concentration of gallic acid (1.32 µg/gdw) was extracted from seed coat of *L. annuus* with ethanol while the lowest from whole seed of *L. hierosolymitanus* with ethanol (0.1 µg/gdw) as seen in Figure 1. Significant differences were observed when the gallic acid concentrations in the same seed parts were evaluated with respect to solvents. This difference was particularly marked in water extract. The highest gallic acid concentrations were obtained from the bark extracts of plants and showed a similar trend to their total phenolic contents.

Caffeic acid has gained an increasing attention related to its antioxidant, anticancer, and anti-inflammatory properties. Strong antioxidant property of caffeic acid was attributed to the dihydroxylation of the 3,4 position on the phenolic ring (Jafri *et al.,* 2017). In the current study, more caffeic acid was observed in all extracts compared to gallic acid and epicatechin concentrations (Figure 2). The highest concentration of caffeic acid was detected in methanol extract from seed coat of *L. annuus* (91.11 µg/gdw), whereas the least in water extract from cotyledon of *L. annuus* (20.11µg/gdw) as seen in the HPLC chromatogram (Figure 4-5). The epicatechin showed a wide range of variation according to Figure 3. The range for epicatechin varied between 0.17 and 32.55 µg/gdw. The highest amount of epicatechin was detected in methanol extract from the seed coat of *L. hierosolymitanus* while the least in ethanol extract from cotyledon of *L. annuus* (Figure 3).

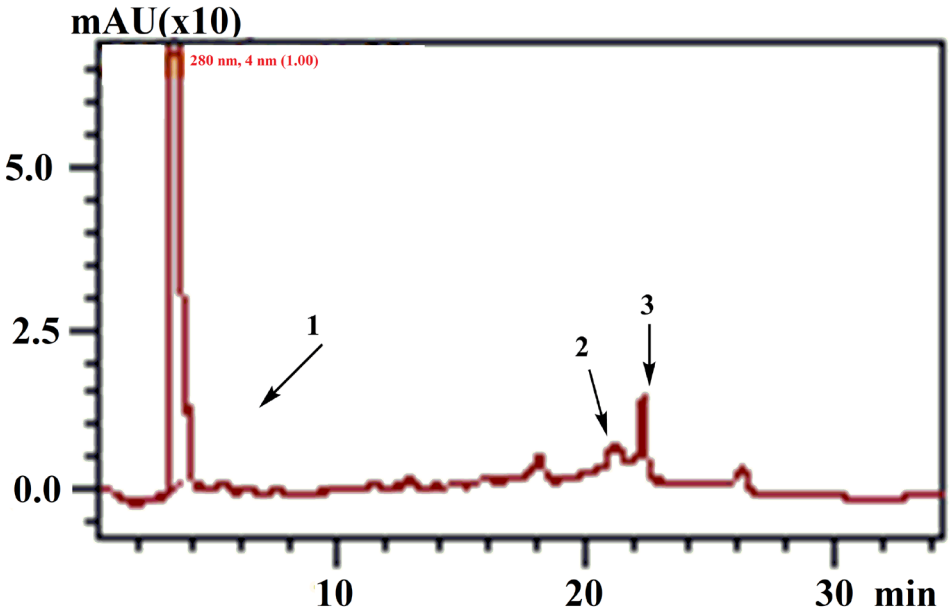

FIGURE 4 - HPLC chromatogram of *L. annuus* seed coat methanol extract. The peaks correspond to: 1 gallic acid (280 nm wavelength); 2 epicatechin (260 nm wavelength); 3 caffeic acid (280 nm wavelength).

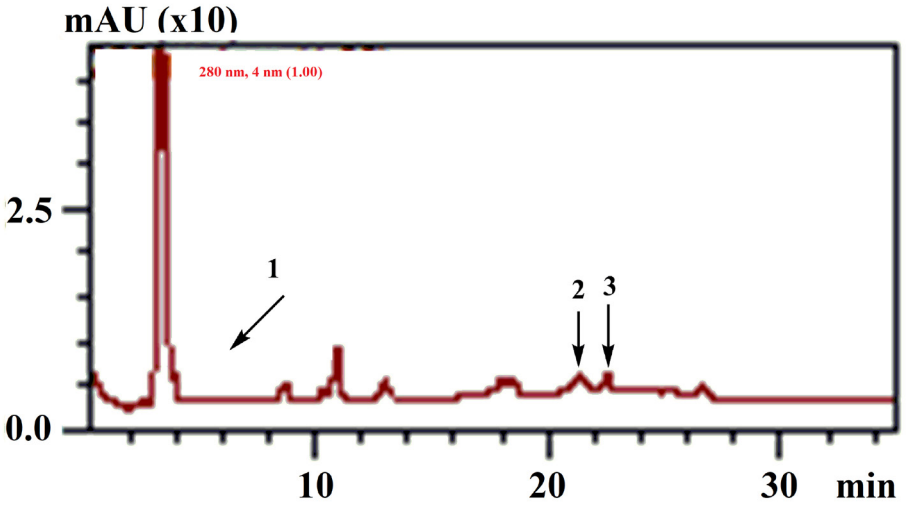

FIGURE 5 - HPLC chromatogram of *L. annuus* cotyledon water extract. The peaks correspond to: 1 gallic acid (280 nm wavelength); 2 epicatechin (260 nm wavelength); 3 caffeic acid (280 nm wavelength).

At the same time, when phenolic concentration obtained with different solvents were compared statistically, the concentrations of epicatechin and caffeic acid from the methanol extracts was the highest and significantly different from those of the water extracts ($p < 0.05$), except for cotyledon extracts from L. *hierosolymitanus* for caffeic acid (Figure 2). Similarly, the gallic acid concentrations from methanol extracts were different from those of the water extracts, but there was no difference among the water extracts from different parts of *L. annuus*. In addition, the difference between the epicatechin and caffeic acid from extracts was found to be significant in terms of *Lathyrus* species ($p < 0.05$). It was observed that caffeic acid was higher in *L. annuus*, while L. *hierosolymitanus* was richer in epicatechin. HPLC analysis results of the extracts imply that methanol and ethanol are the appropriate ones as the solvents to be preferred for phenolic extraction from *L. annuus* and *L. hierosolymitanus*.

As a result, the amount of individual phenolic components in the extracts significantly varied in relation to different solvents as well as *Lathyrus* species and their seed parts. The variations in phenolic compounds might attribute to the polarities of solvents and the chemical nature of components extracted from the plant as well as the plant matrix. This emphasizes that different phenolic compounds can individually be extracted by using specific solvent extraction. Therefore, a comparison of different solvents provides valuable information for efficient phenolic extraction from *Lathyrus* species.

Antimicrobial activity of the extracts from *Lathyrus* seeds

Antimicrobial properties of plants are being increasingly reported from different parts of the world. The search for new plants with potential antimicrobial properties has intensified owing to the side effects of drugs (Khan *et al*., 2009). Considering the dominant resistance of *B. cereus, E. coli,* and *P. aeruginosa* to a wide range of antibiotics, antibacterial effects of *Lathyrus* species extracts were investigated to find a suitable alternative to antibiotics. This study is the first to report the extracts from *L. annuus* and *L. hierosolymitanus* to be against microorganisms. In Table II, the extracts for antibacterial activity were tested based on minimal inhibition concentration (MIC). According to the results, methanol extracts from all parts of *Lathyrus* species showed mostly higher antibacterial activity for bacteria tested. The methanol extract from the seed coat of *L. hierosolymitanus* also showed the highest antibacterial activity against *E. coli*, while the highest antibacterial activity against *B. cereus* was obtained with methanol extract from whole seed of *L. annuus* and methanol extract

from the seed coat of *L. hierosolymitanus* ($p < 0.05$). The methanol extract from whole seed of *L. hierosolymitanus* was also observed to have the highest antibacterial activity against *P. aeruginosa*. When the parts were compared, extracts obtained from cotyledons showed significantly lower antibacterial activity than seed coat and whole seed extracts as seen in Table II ($p < 0.05$). In particular, the water and ethanol extracts from the cotyledon parts showed no activity at all against the tested microorganisms. However, no statistically significant difference was found among the extracts in terms of *Lathyrus* species ($p > 0.05$). In terms of solvents, similarly, methanol extracts showed better antibacterial activity than other solvents ($p < 0.05$). These differences can be attributed to the high phenolic content. The phenolic content is suggested to increase the antibacterial activity. Phenolic compounds are the most important antimicrobial agents and bioactive constituents in the plant (Mabrouki *et al.,* 2018).

TABLE II - Minimum inhibitory concentrations of extracts from *Lathyrus* seeds on tested bacteria

Species	Parts	Solvents	*E. coli* mg/mL	*B. cereus* mg/mL	*P. aeruginosa* mg/mL
L. annuus	Seed coat	Water	1.25 ± 0.25^{aB}	1.25 ± 0.25^{aB}	1.25 ± 0.2^{aC}
		Ethanol	0.5 ± 0.1^{bB}	0.5 ± 0.1^{bB}	0.75 ± 0.05^{bC}
		Methanol	0.5 ± 0.1^{bB}	0.5 ± 0.1^{bB}	0.5 ± 0.1^{bC}
	Cotyledon	Water	nd	nd	nd
		Ethanol	nd	nd	nd
		Methanol	1.5 ± 0.1^{A}	1.5 ± 0.2^{A}	1.5 ± 0.1^{A}
	Whole seed	Water	1.5 ± 0.1^{aB}	1.5 ± 0.05^{aB}	1.5 ± 0.15^{aB}
		Ethanol	1.0 ± 0.1^{bB}	0.75 ± 0.05^{bB}	1.0 ± 0.1^{bB}
		Methanol	0.5 ± 0.1^{cB}	0.25 ± 0.05^{cB}	0.75 ± 0.05^{bB}
L. hierosolymitanus	Seed coat	Water	1.25 ± 0.25^{aC}	1.25 ± 0.15^{aB}	1.25 ± 0.05^{aB}
		Ethanol	0.5 ± 0.1^{bC}	0.5 ± 0.25^{bB}	0.5 ± 0.15^{bB}
		Methanol	0.25 ± 0.05^{bC}	0.25 ± 0.05^{bB}	0.5 ± 0.1^{bB}
	Cotyledon	Water	nd	nd	nd
		Ethanol	nd	nd	nd
		Methanol	1.25 ± 0.25^{A}	1.5 ± 0.25^{A}	1.5 ± 0.15^{A}
	Whole seed	Water	1.25 ± 0.1^{aB}	1.25 ± 0.05^{aB}	1.25 ± 0.15^{aB}
		Ethanol	0.5 ± 0.1^{bB}	0.75 ± 0.1^{bB}	0.5 ± 0.05^{bB}
		Methanol	0.75 ± 0.15^{bB}	0.5 ± 0.1^{cB}	0.25 ± 0.02^{cB}

(nd): not determined

Values are mean ± SD (n =3). Different letter within the same column show differences of means among different solvents for each part of each plant species ($p < 0.05$). Different caps letter shows differences between extracts from different part of the plant in the same solvent for *Lathyrus* species ($p < 0.05$).

There are not many reports of research on the antibacterial screening of *Lathyrus* species. In another study, MIC values of butanolic extracts from *L. aphaca* and *L. ratan* seeds were found to be below 100 µg/ml against selected bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* species and *Bacillus subtilis* (Khan *et al*., 2009). The methanol and ethanol extracts of the leaf and body of *L. karsianus* P. H. Davis showed antibacterial activity against *K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis, B. cereus, Salmonella enteritidis, Proteus mirabilis, E. coli,* and *Enterococcus faecalis* (Özkan *et al*., 2014). Heydari *et al*. (2019) found that ethyl acetate fraction from *L. armenus* (Boiss. & Huet) Širj., *L. aureus* (Stev.) Brandza, *L. cilicicus* Hayek & Siehe, *L. laxiflorus* (Desf.) O. Kuntze, and *L. pratensis* L. were more effective than the methanol extracts, and n-hexane, chloroform, and water fractions against *S. aureus, B. subtilis, E. coli, P. aeruginosa,* and *Candida albicans.*

The antimicrobial effect of the plant extracts against the microorganisms may be due to the secondary metabolites like phenolic compounds. Krishnappa *et al*. (2017) found that the antibacterial activity of green gram seeds, sprouts, husks, and exudate was correlated with TPC, as the extracts showing higher phenolics showed lower MIC values. In the present study, it was found that methanol extracts and extracts from the seed coat and whole seed with higher TPC showed lower MIC values, but there was no correlation between antibacterial activity and TPC in the extracts. Beside the phenolic compounds, the synergistic effects of the variety of major and minor components in the extract constitute another factor to be considered for the antibacterial activity of the extracts. It can be concluded that these extracts, especially ethanol and methanol extracts, have a potential inhibitory effect against pathogenic microorganisms.

CONCLUSIONS

This study provides comprehensive information on the biological activities of *Lathyrus* species. The results indicated that absolute methanol was the most effective extraction agent on the recovery of phenolic compounds among the tested solvents. Besides, the seed coat extracts showed higher antioxidant activities than cotyledon and the whole seed extracts. The seed coat of *L. hierosolymitanus* generally contained the maximum phenolic compounds. The correlations between phenolic content and antioxidant activities of the extracts were also found. Therefore, the results are critical to optimize the extraction procedures for the analysis of different classes of phenolic compounds of *Lathyrus* species which are a rich source of antioxidants. *Lathyrus* species with biological activities shown in the study can be considered as potential candidates for their use in industrial areas such as pharmaceutical and cosmetic fields.

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