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Biochemical Composition of *Lathyrus* L. Seeds: Antioxidant Activities, Phenolic Profiles, β-ODAP and Protein Contents

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ABSTRACT

Lathyrus taxa are used in different areas including nutritive, agricultural areas, and they are seen as the source of both protein and phenolics. *Lathyrus* taxa seeds contain a neurotoxic substance called β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) in different amounts. *Lathyrus* species grown in Turkey is reported to have low β-ODAP and high protein content. In this study, antioxidant activities, phenolic profiles, β-ODAP and protein contents of six *Lathyrus* taxa were investigated. Total phenolic content (TPC) in *Lathyrus* taxa used was ranging from 0.17±0.05 to 5.10±0.02. The antioxidant activities were observed in a wide interval that IC_{50} values were between 7.05±0.11 to 1.15±0.08 mg/mL. The highest TPC and antioxidant activity were recorded for *L. clymenum*. In HPLC analysis, gallic, *p*-hydroxybenzoic, caffeic, chlorogenic acid and epicatechin in all of the extracts were determined. Also, β-ODAP and protein contents in seeds of the *Lathyrus* taxa were found between 0.20-1.18 mg/g and 22.66-29.74%, respectively. β-ODAP contents of investigated *Layhyrus* taxa were within the safe consumption range in terms of health (<2.00 mg/g). The investigated *Lathyrus* taxa were found to be excellent protein sources with low β-ODAP content and contain natural antioxidants. As a result, this study has provided important data for wild *Lathyrus* taxa to be used as a cheap protein source and functional food for promoting health.

Key words: β-ODAP, *Lathyrus* L., Phenolic profile, Protein content.

INTRODUCTION

Legumes are important dietary components in human nutrition. They are sources of inexpensive proteins, fat, carbohydrates, dietary fiber and micronutrients like polyphenols. Consumption of legumes is known to have favorable effects on health associated with the amount of dietary fiber and polyphenols (Fratianni *et al.*, 2014; Zhao *et al.*, 2014). Grain products, vegetables and fruits are natural sources of antioxidants and other phytochemicals. Legume seeds include the main phenolic compounds such as flavonoids, phenolic acids and procyanidins (Zhao *et al.*, 2014).

Genus *Lathyrus* L. contains 79 taxa at the level of species, subspecies and variety in Turkey and 25 of these taxa are endemic (Davis *et al.*, 1988; Guner *et al.*, 2000; Genc and Sahin, 2011; Gunes 2018). *Lathyrus* L. has many species with high protein content, while *Lathyrus sativus* L. (grass pea) is the most cultivated species (Başaran *et al.*, 2016). It can provide significant yield in areas prone to drought and excessive precipitation. In the past, *Lathyrus* L. species was widely cultivated as feed and grain crops. Today, long-term consumption of *Lathyrus* and β-ODAP is suggested to cause neurological disorder called as 'Lathyrism'. Studies show that nutritional deficiencies in methionine and cysteine may increase β-ODAP-induced neurotoxicity. Now, β-ODAP content is considered to pose no risk to health if *L. sativus* is consumed as part of a balanced diet combined with cereals. Moreover, β-ODAP content can be partially removed from seed by easy processing methods like cooking, fermentation, soaking in water (Vaz Patto and Rubiales, 2014; Xu *et al*., 2017).

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Lathyrus L. species is seen as the source of both protein and phenolics which are antioxidant, but studies on the composition of polyphenols in *Lathyrus* spp. are limited (Chavan *et al.*, 1999; Chavan *et al.*, 2003; Pastor-Cavada *et al.*, 2009). To our knowledge, there is no report on antioxidant potentials and phenolics compositions of *Lathyrus* species used in the study. The objective of the study was to define phenolic profiles, antioxidant capacities,

β-ODAP contents, and protein contents of *L. tuberosus* L., *L. annuus* L., *L. hierosolymitanus* Boiss., *L. gorgoni* Parl. var. *gorgoni*, *L. stenophyllus* Boiss. & Heldr. and *L. clymenum* L. seeds.

MATERIALS AND METHODS

Materials

Folin-Ciocalteau reagent, Gallic acid, butylated hydroxyanisole (BHA), DPPH·, fluoro-2, 4 dinitrobenzene, β-ODAP were purchased from Sigma-Adrich. Also, all standard for HPLC were purchased from Sigma-Aldrich (Steinheim, Germany).

Plant Materials

The seeds of *Lathyrus* L. taxa, used for the study, were collected from their natural habitats in 2014. Taxonomic identification of these plant materials was confirmed by Genç and Yıldırım. Taxa and their localities are given below:

L. tuberosus	Isparta, Aksu-Sütçüler road, around Pazarköy.
L. annuus	Muğla, Yatağan, around Stratonikeia ancient city.
L. hierosolymitanus	Muğla, Dalyan, around Eskiköy.
L. gorgoni var. *gorgoni*	Antalya, Serik, around Burmahancı village.
L. stenophyllus	Isparta, Aksu-Yenişarbademli road, 3.5 km.
L. clymenum	Muğla, Datça, around Yazıköy.

Extraction

For the extraction, the dried seeds were pulverized and were firstly defatted with hexane for 24 h. To obtain ethanol extracts, the seed materials (10 g) were macerated with 100 mL of 70% ethanol at room temperature for 24 h. The extracts were concentrated using a rotary evaporator and stored at + 4°C in dark.

Total Phenolic Content (TPC)

The TPC in extracts was quantified using Folin-Ciocalteu method with slight modifications (Singleton *et al.*, 1999). Gallic acid was used as a standard for comparison and the results are expressed in milligrams of gallic acid equivalent (GAE) per gram dry weight *Lathyrus* seed (mg GAE/g DW) based on the calibration curve.

Radical scavenging assay

Determination of free radical scavenging capacity (2,2-diphenyl-1-picryl-hydrazyl-DPPH) of the extract was performed based on the method of Sánchez-Moreno *et al.* (1998). Antioxidant activities of extracts were expressed as IC_{50}, defined as the concentration of the extract required to cause a 50% decrease in initial DPPH· concentration. The same test was performed for the butylated BHA solutions which were used as standard.

HPLC-DAD analyses of phenolic compounds

The analytical HPLC system 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), a SIL 20ACHT automatic sampler, a CTO10ASVp column oven and a 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. The mobile phase consisted of water with 3% glacial acetic acid (A) versus (B) methanol (Table 1) (Kose *et al.*, 2011). The quantification was performed by using the standards and the levels were expressed as µg phenolic compounds/g dry seed weight (dw).

Protein content determination

The Dumas method was employed to identify the protein content of the samples (Dumas, 1831).

ODAP content determination by HPLC

β-ODAP content of the seed was analyzed based on the methods of Wang *et al.* (2000). β-ODAP was used as a standard for quantitative analysis. The β-ODAP content was expressed as mg/g dry weight (dw) and percent (%) in dry weight of the seed sample.

RESULTS AND DISCUSSION

In the study, the extract yield percent of the seeds ranged from 7.1 to 15.2%. The maximum extract yield was obtained from *L. annuus* (15.2%) followed by *L. clymenum* (13.5%). Similar yield results were reported for *L. czeczottianus* Bässler and *L. nissolia* L. collected from Turkey (Llorent-

Table 1: HPLC-DAD gradient solvent system for phenolic compounds.

Time (min)	A %	B %
1	80	20
25	50	50
40	20	80

Table 2: Extraction yields, TPCs and DPPH radical scavenging activities of the extracts.

Extracts/Standart	Extract yield (%)	TPC(mg/g)	DPPH-IC_{50} (mg/ml)
L. tuberosus	7.1	0.17±0.05	7.05±0.11
L. annuus	15.2	1.07±0.12	2.64±0.03
L. hierosolymitanus	7.5	1.78±0.06	1.48±0.04
L. gorgoni var. *gorgoni*	8.9	1.63±0.05	1.99±0.01
L. stenophyllus	10.6	1.41±0.01	1.59±0.09
L. clymenum	13.5	5.10±0.02	1.15±0.08
BHA			0.032±0.01

Martínez *et al.*, 2017a). In addition, TPC in *Lathyrus* taxa was ranged from 0.17 ± 0.05 to 5.10 ± 0.02 (Table 2). The highest total phenolic content was recorded for *L. clymenum* seed extract (5.10±0.02 mg/g). The differences in the TPCs of *Lathyrus* taxa may be explained by genetic factors and habitat properties.

The phenolic content of commonly consumed legumes varies in the range of 0.325-6.378 mg GAE/g (Marathe *et al.*, 2011). It has been suggested that *Lathyrus* species can be a new source of natural phenolic antioxidants as well as high quality proteins for human and animal nutrition (Pastor-Cavada *et al.*, 2009). Our results are in the TPC range of the legumes. There are not many studies on phenolic content and compounds in *Lathyrus* species. Fratianni *et al.* (2014) found the total polyphenolic content of the two ecotypes of *L. sativus* from Southern Italy to be low like *L. tuberosus* it is in the present work. In another study conducted on *Lathyrus* species from Turkey (Llorent-Martínez *et al.*, 2017b), the amount of total phenolic in extracts of *L. digitatus* (Bieb.) Fiori and *L. cicera* L. are close to *L. clymenum* and *L. gorgoni* var. *gorgoni* in the study. As seen in the literature, phenolic content of *Lathyrus* species is varied. The variation in phenolic content may be attributed to regions where species grow.

In the present study, the antioxidant activities were observed in a wide interval which was IC_{50} values ranged from 7.05±0.11 to 1.15±0.08 mg/mL (Table 2). The DPPH scavenging activity of *L. clymenum* was higher in comparison to the other *Lathyrus* taxa and its high total phenolic content directly corresponded with its antioxidant activity. In previous study, IC_{50} values of extracts from *L. czeczottianus* and *L. nissolia* were found as 1.42 mg/mL and 3.19 ± 0.11 mg/mL, respectively, in DPPH assay (Llorent-Martínez *et al.*, 2017a). The results are generally consistent with the findings obtained in our study.

In this study, phenolic composition of the *Lathyrus* taxa studied was reported for the first time, and HPLC was used to quantify 10 phenolic compounds (protocatechuic, *p*-hydroxybenzoic, gallic, vanillic, chlorogenic, and caffeic acid, epicatechin, naringin, rutin and quercetin). The retention times and wavelengths for detection for analyses of phenolic compounds in *Lathyrus* taxa are given in Table 3. Phenolic compositions of the seed extracts are shown in Table 4. Gallic acid, *p*-hydroxybenzoic acid, epicatechin, caffeic acid, chlorogenic acid in all of the extracts were determined in different amounts. The content of gallic, p-hydroxybenzoic, chlorogenic acid and epicatechin was the highest in *L. clymenum* among all the extracts, as well as the highest TPC.

Table 3: Correlation coefficients (R^2), limits of detection (LOD), limits of quantification (LOQ) of HPLC system employed for phenolic profile determination.

Phenolic Compounds	(nm)	RT (min)	Standard curve (regression equation)	R^2	LOD (µg/ml)	LOQ (µg/ml)
Gallic acid	280	6.8	y=320183x+20047	0.999	0.01	0.033
p-hydroxybenzoic acid	280	15.7	y=419586x+38675	0.999	0.01	0.033
Chlorogenic acid	320	18.2	y=325955x+67549	0.999	0.01	0.033
Caffeic acid	280	22.7	y=6181,4x-4294.8	0.999	0.01	0.03
Vanilic acid	320	19.2	y=78672x+7413.6	0.999	0.11	0.36
Protocatechuic acid	280	10.7	y=433017x+ 87123	0.999	0.03	0.102
Epicatechin	260	21.3	y=51980x+6328.6	0.999	0.43	1.41
Naringin	280	49.7	y=109126x+51750	0.999	0.40	1.32
Quercetin	360	70.4	y=663587x+7091.1	0.999	0.57	1.88
Rutin	360	45,6	y=299028x+25250	0.999	0.57	188

Table 4: Determination of phenolic compounds in the extracts by HPLC (µg/g).

Compounds	L. *tuberosus*	L. *annuus*	L. *hierosolymitanus*	L. *gorgoni*var. *gorgoni*	L. *stenophyllus*	L. *clymenum*
Gallic acid	0,81	2.4	60.06	29.76	10.6	63.18
p-hydroxybenzoic acid	0,25	0.45	0.21	0.16	0.15	0.52
Chlorogenic acid	0,35	0.9	0.49	0.96	0.71	1.04
Caffeic acid	21.01	114.15	29.47	16.8	23.13	40.43
Vanilic acid	nd	nd	nd	0.88	nd	nd
Protocatechuic acid	nd	nd	nd	nd	1.07	nd
Epicatechin	2,05	1.8	36.19	18.72	2.99	198.25
Naringin	nd	nd	nd	Nd	nd	nd
Quercetin	nd	nd	nd	Nd	nd	nd
Rutin	nd	nd	nd	Nd	nd	nd

nd: not detected.

Table 5: Seed protein and β-ODAP contents of the *Lathyrus* taxa.

Taxon	Protein %	β-ODAP	
		mg/g	(%)
L. tuberosus	22.66	1.18	(0.118)
L. annuus	24.45	0.94	(0.094)
L. hierosolymitanus	24.60	1.02	(0.102)
L. gorgoni var. *gorgoni*	29.74	1.13	(0.113)
L. stenophyllus	25.79	0.20	(0.020)
L. clymenum	29.65	1.07	(0.107)

Food legumes comprise phenolic acids, flavonoids and condensed tannins. Studies on the phenolic composition of *Lathyrus* taxa seeds are scarce. Llorent-Martínez *et al.* (2017b) found that both *Lathyrus* species contained 3-caffeoylquinic acid, coumaric acid, coutaric acid, ferulic acid, verbascoside, rosmarinic acid and derivatives of kaempferol and quercetin. Fratianni *et al.* (2014) detected phenolic acids such as gallic, chlorogenic, caffeic, coumaric and ferulic acids in *L. sativus* seeds. On the other hand, it was reported that *L. maritimus* and *L. cicera* seeds contained flavonoids as main phenolic compounds and no phenolic acid derivatives were detected (Chavan *et al.*, 1999; Ferreres *et al.*, 2017). In our study, 4 flavonoids were investigated and the epicatechin detected in all seed extracts was sole flavonoid. Similarly, the two ecotypes of *L. sativus* also contained different amounts of epicatechin while rutin was available in one of the ecotypes of *L. sativus* (Fratianni *et al.*, 2014). A correlation was suggested between antiradical activity and phenolic compounds such as gallic acid, epicatechin, protocatechuic acid, chlorogenic acid, quercetin and catechin (Kaliora *et al.*, 2014). In addition, our study showed that the extracts contained phenolic compounds with antioxidant activities.

Legumes are considered to be an essential source of nutrients. The protein content of legume seeds fall within the range of 21-25%. Legume seeds are rich in lysine and poorer in sulfur-containing amino acids when in comparison to cereals (Aniszewski *et al*., 2013; Arslan 2017). In the study, the mean protein content of *Lathyrus* taxa was 26.15% (Table 5). It was reported that some cultivars of *L. cicera* grown in Turkey are as high in nutritional value as soybeans (Karadeniz *et al*., 2010). In previous study, Pastor-Cavada *et al.* (2011) determined that protein contents of 15 *Lathyrus* species fall within the range of 17.7 and 25.6%. In agreement with our findings, local cultivars including *L. sativus* in Turkey was found to range from 26.77% and 30.20% regarding their protein content (Basaran *et al.*, 2016). In conclusion, the high protein content of *L.* taxa studied is similar to those of other legumes and the most cultivated *Lathyrus* species.

β-ODAP is suggested to cause Lathyrism which is a neurological disorder. Therefore, β-ODAP content is the main reason that limits the consumption of *Lathyrus* species. In the *Lathyrus* species breeding, low β-ODAP and high protein are major interest (Basaran *et al*., 2016). However, β-ODAP has been determined not only in *Lathyrus* species, but also in other leguminous plants (Karadeniz *et al*., 2010). The presence of β-ODAP was shown in 13 *Crotalaria* species and 17 *Acacia* Willd. species, besides in 21 *Lathyrus* species. β-ODAP content in plant is related to environment and genotype. Also, β-ODAP was considered to play a role in drought tolerance and in resistance to oxidative stress (Xu *et al*., 2017). *Lathyrus species* that grows in India and Indonesia were found to contain high β-ODAP levels; while Syria, Cyprus and Turkey were reported to be at low levels (Abd El Moneim *et al*., 2001). β-ODAP amounts of *L. sativus* vary from 0.02 to 2.59% while from 0.09 to 0.49% in *L. cicera* seeds (Vaz patto and Rubies 2014). In the present study, β-ODAP contents of the samples were between 0.20 and 1.18 mg/g. Karadeniz *et al.* (2010) found that there was no β-ODAP in the seeds of *L. aphaca* L., *L. sphaericus* Retz. and *L. aureus* (Stev.) Brandza and β-ODAP levels of *L. cicera* L. and *L. clymenum* L. seeds were 0.053 and 0.171%, respectively. Basaran *et al.* (2016) reported the β-ODAP contents in 52 *L. sativus* seeds varied from 2.62 mg/g up to 5.59 mg/g. Abd El Moneim *et al.* (2001) have suggested that the β-ODAP content may help to determine its toxic effect and less than 2 mg/g is assumed safe for consumption as food. In the study, all *Lathyrus* taxa had lower β-ODAP content than 2 mg/g. The results imply that the *Lathyrus* taxa with low β-ODAP contents are promising as food.

CONCLUSION

In the study, the results showed that the *Lathyrus* taxa had as high protein content as other legume and were rich in phenolic compounds with important biological activities. At the same time, all *Lathyrus* taxa had low β-ODAP content in the safe consumption range for health. The implication is that *Lathyrus* taxa with excellent nutritional value, both with high protein content and important phenolic compounds, can be a healthy alternative as food and feed all over the world. Moreover, the study provides important data on the nutritional characteristics of wild *Lathyrus* species that much information doesn't exist.

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