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Radical Scavenging Activity of Some *Lathyrus* Taxa Distributed in Burdur-Isparta Regio

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Abstract: Radical scavenging activity of the extracts of *Lathyrus aphaca* L. var. *pseudoaphaca* (Boiss.) Davis, *L. aureus* (Stev.) Brandza, *L. cicera* L., *L. sphaericus* Retz., *L. digitatus* (Bieb.) Fiori in Fiori & Paol. and *L. setifolius* L. taxa distributed in Burdur-Isparta regio was determined. Methanol extracts of air dried aerial parts and the seeds of the plants were dissolved in water and chlorophylls and lipophilic compounds were removed from the aqueous extracts. 2,2-diphenyl-1-picryl hydrazyl, superoxide and nitric oxide radical scavenging activity of aqueous and methanol extracts of the plants were tested. Gallic acid equivalent total phenolic contents of the plant extracts were also detected using Folin-Ciocalteu reagent. According to the 2,2-diphenyl-1-picryl hydrazyl radical scavenging activity test, the highest antioxidant activity was found in aerial parts of *L. aphaca* var. *pseudoaphaca* and highest total phenolic content was found as gallic acid equivalent in *L. sphaericus* seed extracts.

Burdur-Isparta Yöresinde Yayılış Gösteren Bazı *Lathyrus* Taksonlarının Radikal Süpürücü Aktivitesi

Anahtar Kelimeler
DPPH,
Lathyrus,,
NO,
Radikal süpürücü aktivite,
SO,
Toplam fenolik içeriği

Özet: Burdur-Isparta yöresinde yayılış gösteren *Lathyrus aphaca* L. var. *pseudoaphaca* (Boiss.) Davis, *L. aureus* (Stev.) Brandza, *L. cicera* L., *L. sphaericus* Retz., *L. digitatus* (Bieb.) Fiori in Fiori & Paol. and *L. setifolius* L. taksonlarına ait ekstraktların radikal süpürücü aktivitesi belirlenmiştir. Kurutulmuş topraküstü kısımları ve tohumlarının metanol ekstraktlarından klorofil ve lipofilik bileşikler uzaklaştırılmıştır. Sulu ve metanol ekstraktlarının 2,2-difenil-1-pikril hidrazil, süperoksit ve nitrik oksit radikali süpürücü aktiviteleri araştırılmıştır. Ayrıca gallik aside eşdeğer toplam fenolik madde miktarı Folin-Ciocalteu reaktifi kullanılarak belirlenmiştir. En yüksek 2,2-difenil-1-pikril hidrazil radikali süpürücü aktivite *L. aphaca* var. *pseudoaphaca*'nın topraküstü kısımlarında, en yüksek toplam fenolik madde içeriği gallik aside eşdeğer olarak *L. sphaericus*'un tohumlarında bulunmuştur.

1. Introduction

The Fabaceae family is in the third position in largeness among flowering plants in the world [1]. The genus *Lathyrus*, belonged to the tribe Fabeae, is one of the largest genera with about 200 species worldwide, mainly in the Mediterranean [2]. Legumes bare great economic importance for the world and are used for human food, animal feed and other products. Legumes contain different bioactive compounds. When regularly consumed, they might have beneficial effects against metabolic diseases like diabetes mellitus, coronary heart disease, colon cancer and neurodegenerative disturbances including Alzheimer's and Parkinson's diseases. Besides phenolic compounds which have antioxidant effect, Lathyrus species also include neurotoxic substances such as ODAP (-β-N-oxalyl-L-α, β-diamino propionic acid), [3].

In spite of the antioxidant activity and phenolic content of Lathyrus species [3-9], poor attention has been given to them. Since *Lathyrus* species are important for their nutritional values and bioactivities as potential functional foods, we investigated the radical scavenging activity (RSA) of *Lathyrus aphaca* L. var. *pseudoaphaca* (Boiss.) Davis, *L. aureus* (Stev.) Brandza, *L. cicera* L., *L. sphaericus* Retz., *L. digitatus* (Bieb.) Fiori in Fiori & Paol. and *L. setifolius* L., distributed in Burdur-Isparta regio which we determined ODAP content of in our previous study [10].

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2. Material and Method

2.1. Plant materials and extraction

Plants were collected from different locations distributed to Isparta-Burdur regio. *L. aphaca, L. cicera, L. sphaericus* and *L. setifolius* are annual while *L. aureus* and *L. digitatus* are perennial herbs, and they generally distribute in forests and scrublands [11] (Table 1). Air-dried and powdered aerial parts and seeds of the plant materials were extracted for three times with methanol by using magnetic stirrer at 40°C for three days. Afterwards, the obtained methanolic extracts were filtered and evaporated in a rotator evaporator to gain crude extracts (Table 2). Subsequently, crude methanolic extracts were dissolved in distilled water followed by partition with equal volume of petroleum ether to remove chlorophyll and other lipophylic compounds. Finally, the remaining aqueous extracts were lyophilized [12].

Table 1. *Lathyrus* taxa and the collection stations

Taxa	Station	Altitude (m)
L. aphaca var. *pseudoaphaca*	Isparta-Eğirdir, pine forest	1190
L. aureus	Isparta-Eğirdir, Kasnak oak forest	1530
L. cicera	Burdur, urban area	850
L. sphaericus	Isparta-Eğirdir, pine forest	1190
L. digitatus	Isparta-Eğirdir, lake shore	875
L. setifolius	Isparta-Eğirdir, lake shore	875

2.2. 2,2-diphenyl-1-picril hydrazyl (DPPH) radical scavenging activity

DPPH RSA potentials of the methanolic extracts of the plants were detected [13]. Each methanol sample or control of 200 µL was added DPPH, a stable free radical, (50 µL, 1 mM) at certain concentrations and then mixed well. The remaining DPPH had an absorbance value of 517 nm after a 30-minute period. The RSA of a negative control well which contained only DPPH and solvent was taken as comparison to the RSA of the extract. Positive control was ascorbic acid. All analyses were done as three replicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$IP\ (\%) = (ABS_{control} - ABS_{sample} / ABS_{control}) * 100$$

2.3. Superoxide radical scavenging activity test by alkaline DMSO method

Superoxide (SO) RSA was detected according to Kunchandy and Rao [14]. In sum, a non-enzymatic system was established to generate a superoxide radical. The mixture which include 10 µL of nitroblue tetrazolium (NBT) (1 mg/mL solution in dimethyl sulphoxide, (DMSO) and 30 µL of each sample (in DMSO). 100 µL of alkaline DMSO (1 mL of which containing 5 mM NaOH in 0.1 mL of water) was put in order to make a final volume of 140 µL and the absorbance was read at 560 nm. Positive control was ascorbic acid. All analyses were done as three replicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$IP\ (\%) = (ABS_{sample} - ABS_{control} / ABS_{sample}) * 100$$

2.4. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of the extracts was also determined [15]. Briefly, 60 µL of 10 mM sodium nitro prussiate, in phosphate buffered saline (PBS), were added to 60 µL of the extract or control wells and the plate was incubated at room temperature under light for 150 min. Finally, 60 µL of Griess reagent (1 g sulphanilamide + 0.1 g N-(1-naphthyl) ethylene diamine dihydrochloride + 2.5 mL phosphoric acid, completed 100 mL distilled water) was added into each well in order to measure the nitrite content at 577 nm. Ascorbic acid was used as positive control. All analyses were done as three replicates. Radical scavenging activity was represented as the inhibition percentage and was calculated using the formula below:

$$IP\ (\%) = (ABS_{control} - ABS_{sample} / ABS_{control}) * 100$$

2.5. Total phenolic content

The total phenolic content (TPC) was detected via the method of Singleton and Rossi [16] with minimal changes. 10 µL of sample or standard (10-500 µg/mL of gallic acid) and a diluted 150 µL of Folin-Ciocalteu reagent (1:4 reagent/water) were put in the wells of the plate and later taken into incubation at room temperature during three minutes. After the admixture of 50 µL of saturated sodium carbonate (7.5%) and an additional session of incubation for two hours at room temperature, absorbance was at 725 nm. TPC was figured as gallic acid equivalent (GAE).

2.6. Statistical analysis

Statistical significance was assessed using Analysis of Variance, ANOVA (one way), followed by Tukey test for multiple comparisons, at $p< 0.05$ level. Graphics for linear regression curves, inhibition percentage, and correlation coefficients were drawn and used to determine calculations. Correlation coefficients between were defined via Pearson correlation test. All the tests were done in triplicates.

3. Results

Extract yields of *Lathyrus* taxa were given in Table 2. Aerial parts (IC_{50} = 222.27±5.61 µg/mL) and seeds (IC_{50} = 43.42±0.39 µg/mL) of *L. aphaca* have the highest DPPH RSA values. The lowest IC_{50} value indicates the highest RSA, meaning the extract has

effect even in low concentrations (half-maximal inhibitory concentration). Seeds (IC_{50} = 102.52±6.31 µg/mL) of *L. aphaca* have the highest SO RSA. Seeds and aerial parts of L. cicera and aerial part of *L. digitatus* showed negligible SO RSA. NO RSA of taxa were found negligible (IC_{50} > 1000 µg/mL). The highest total phenolic contents were found in both seed (288.89±0.05 mg/g GAE) and aerial (273.16±0.35 mg/g GAE) parts of *L. sphaericus*. In addition, there were no or weak correlation between both the total phenolic content and DPPH RSA ($p<0.05$) (r_{seed} -0.34; $r_{aerial\ parts}$ 0.55) (Table 3).

Table 2. Extract yields of *Lathyrus* taxa.

	Extract Yield (%)	
Taxa	Seed	Aerial Part
L. aphaca var. *pseudoaphaca*	6.69	14.00
L. aureus	6.66	12.58
L. cicera	6.25	20.00
L. sphaericus	5.69	5.00
L. digitatus	nd	11.16
L. setifolius	nd	24.87

nd: not determined

4. Discussion and Conclusion

Our results indicate that *L. aphaca* and *L. sphaericus* are significant in antioxidant capacity. *Lathyrus* extracts had negligible NO RSA (IC_{50} >1000 µg/mL) and our extracts can scavenge DPPH and SO radicals more than the NO radical. Flavonoids must contain a catechol group to scavenge NO radical in the plant extracts. Catechins are one of the compounds responsible for the NO RSA [17]. *Lathyrus* extracts did not show a significant NO RSA which can be explained due to lack of NO active substances in the plant contents.

Maximum phenolic contents were found both in seed (288.89±0.05 mg/g GAE) and aerial parts (273.16±0.35 mg/g GAE) of *L. sphaericus* while the lowest were determined in the seed (5.38±0.01 mg/g GAE) and aerial parts (13.85±0.16± mg/g GAE) of *L. cicera*. These results were nearly the same as of the records of Pastor-Cavada et al. [3]. In that study, the highest total phenolic content (catechin equivalent) was determined in *L. sphaericus* (29.2 mg/g), while the lowest was found in *L. cicera* (3.8 mg/g).

DPPH RSA and total phenolic content of *Lathyrus* extracts didn't show any correlation (r_{seed} -0.34; $r_{aerial\ parts}$ 0.55). Moreover, the highest total phenolic content was found in *L. sphaericus* extract, but this taxon had weak or negligible inhibition values in all the RSA tests when compared to most of the other taxa (Table 3). However; *L. aphaca* extract, exhibiting a relatively high RSA, possessed a low total of phenolic content (GAE). In this context, the high RSA of *L. aphaca* extract could be thought due to another compound(s). Antioxidant activity is generally related to total phenolic content and radical scavenging activity deals with numbers and positions of the hydroxyl groups of the phenolic compounds [18]. Furthermore, antioxidant activity is not fully contributed by phenolic compounds alone [19]. Essential oils, vitamins, carotenoids and other secondary compounds are also responsible for antioxidant activities. In addition, a wide range of secondary metabolites can show antagonistic or synergistic effects.

In the past decades nutritional and antinutritional factors of *L. sativus* and other *Lathyrus* species, ODAP and related neurotoxins, their metabolism [20], neurotoxicity [21], physiological [22] and genetic [23] studies to decrease neurotoxin content [24] besides their bioactivities [25, 26] such as antioxidant [3] and antimicrobial [25] properties have been investigated. Therefore some researchers found that neurotoxins such as ODAP which leguminous seeds could contain might be radical scavengers [27]. *Lathyrus* neurotoxins especially ODAP can result in irreversible neurodegeneration causing spastic paraparesis of the lower limbs. This disease called Lathyrism usually happens when seeds are consumed in large amounts during famine episodes triggered by droughts in countries like Ethiopia, India and Bangladesh [28].

There are some studies on bioactive components and their bioactivity of *Lathyrus* taxa. For example, *L. binatus*, *L. cicera* [5], L. digitatus [6], *L. czeczottoianus* [2] contain phenolics, *L. japonicus* triterpene saponins [7] and flavonoids [4], *L. cicera* [5] and *L. davidii* flavonoids and saponins [8] and *L. odoratus* contains phytoalexins [9]. *Lathyrus* neurotoxins especially ODAP and its metabolites have been broadly studied in terms of their metabolism [29]. Also neurotoxin

Table 3: Radical scavenging activities (IC_{50} values) and total phenolic contents of *Lathyrus* taxa

Taxa	DPPH (µg/mL)		NO (µg/mL)		SO (µg/mL)		TPC (mg GAE/g dw)	
	Seed	Aerial part	Seed	Aerial part	Seed	Aerial part	Seed	Aerial part
L. aphaca* var. *pseudoaphaca	43.42±0.39*a	222.27±5.61b	>1000	>1000	102.52±6.31a	171.39±6.66b	75.33±0.03b	22,36±0.02a
L. aureus	480.11±0.66de	441.61±11.79de	>1000	>1000	142.36±5.34ab	562.36±22.38d	29.90±0.02a	67.60±0.03b
L. cicera	768.95±23.21f	354.49±10.82bcd	>1000	>1000	>1000	>1000	5.8±0.01a	13,85±0.16a
L. sphaericus	410.58±3.72cde	496.52±1.12e	>1000	>1000	920.78±30.98f	667.67±25.32e	288.89±0.05c	273,16±0.35c
L. digitatus	-	275.62±3.48bc	-	>1000	-	>1000	-	32.84±0.03a
L. setifolius	-	531.73±39.07de	-	>1000	-	377.30±9.32c	-	13.18±0.01a

*: means of three replicates±sd, different letters in the same column indicates significant differences, DPPH: 2,2-diphenyl-1-picryl hydrazyl, NO: nitric oxide, SO: superoxide, TPC: total phenolic content, GAE: gallic acid equivalent, dw: dry weight

content of *Lathyrus* species and its changing in different ecological and physiological conditions was studied. Researches for plant breeding to eliminate the non-protein amino acid β-ODAP from the seed have been conducted for four decades. A large number of "low-toxin" varieties of grass pea emerged but it didn't result in the development of "toxin-free" varieties, yet [30].

There are some data on interactions with neurotoxin content, stress and antioxidant activity especially in legume seeds and *L. sativus*. β-ODAP carries out a protective role in *L. sativus* as it significantly increases when *L. sativus* is under drought stress. β-ODAP pile up in high amounts in order to manage osmotic adjustments in plants exposed to drought stress [31] as well as oxalic acid, a precursor for ODAP biosynthesis [32].

We investigated RSAs of some *Lathyrus* taxa which we determined ODAP contents of. In our previous study, some *Lathyrus* species such as *L. cicera* which has relatively larger and lighter coloured seeds contain ODAP while *L. sphaericus* and *L. aphaca* having antioxidant activity, did not contain ODAP [10]. More studies are needed on *Lathyrus*' neurotoxins and secondary metabolism and genetic and environmental effects on them.

ODAP content increases in stress conditions and it could have radical scavenging ability. Similarly some secondary metabolites like phenolics increase when plants face stress. Phenolics have antioxidant activity beside having some other ecological properties such as allelopathy [33]. *Lathyrus* species are rich in protein and some other nutritional factors, thus scientists have concentrated on their breeding to decrease neurotoxin content and to use for animal and human food in recent. Overall these studies, two substantial outcomes could be obtained:

1. Interactions between ODAP, its metabolites, other secondary metabolites and stress could be enlightening in terms of ecology, physiology and biochemical studies.
2. Breeding strategies of *Lathyrus* species concerning both high nutritional values and lowest neurotoxin content, contribute to agricultural economy

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Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

References

[1] Lewis, G. P. 2005. Tribe Acacieae. In: Legumes of the World. Lewis G, Schrire B, Mackinder B, Lock M, eds. Royal Botanic Gardens, Kew: UK, pp: 187–191.

[2] Ceylan, R., Zengin, G., Guler, G. O., Aktumsek, A. 2020. Bioactive constituents of *Lathyrus czeczottianus* and ethyl acetate and water extracts and their biological activities: An endemic plant to Turkey. South African Journal of Botany, 1-6.

[3] Pastor-Cavada, E., Juan, R., Pastor, J. E., Alaiz, M., Vioque, J. 2009. Antioxidant activity of seed polyphenols in fifteen wild *Lathyrus* species from South Spain. LWT - Food Science and Technology, 42, 705–709.

[4] Ohtsuki, T., Murai, Y., Iwashina, T., Setoguchi, H. 2013. Geographical differentiation inferred from flavonoid content between coastal and freshwater populations of the coastal plant *Lathyrus japonicus* (Fabaceae). Biochemical Systematics and Ecology, 51, 243-250.

[5] Ferreres, F., Magalhães, S. C. Q., Gil-Izquierdo, A., Valentão, P., Cabrita, A. R., Fonseca, A. J., Andrade, P. B. 2017. HPLC-DAD-ESI/MSn profiling of phenolic compounds from *Lathyrus cicera* L. seeds. Food chemistry, 214, 678-685.

[6] Llorent-Martínez, E. J., Ortega-Barrales, P., Zengin, G., Mocan, A., Simirgiotis, M. J., Ceylan, R., Uysal, S., Aktumsek, A. 2017. Evaluation of antioxidant potential, enzyme inhibition activity and phenolic profile of *Lathyrus cicera* and *Lathyrus digitatus*: Potential sources of bioactive compounds for the food industry. Food and Chemical Toxicology, 107, 609-619.

[7] Kang, S. S., Ahn, B. T., Kim, J. S., Bae, K. H. 1998. *Lathyrus* saponin, a new trisaccharide glycoside from *Lathyrus japonicus*. Journal of Natural Products, 61(2), 299-300.

[8] Park, S. Y., Kim, J. S., Li, S. Y., Bae, K. H., Kang, S. S. 2008. Chemical constituents of *Lathyrus davidii*. Natural Product Sciences, 14(4), 281-288.

[9] Robeson, D. J., Ingham, J. L., Harborne, B. 1980. Identification of two chromone phytoalexins in

the sweet pea, *Lathyrus odoratus*. Phytochemistry, 19, 2171–2173.

[10] Karadeniz. A., Erdoğan, N., Genç, H., Emre, İ. 2010. ODAP levels in some Lathyrus species distributed on Burdur-Isparta provinces in Turkey. Genetic Resources and Crop Evolution, 57, 1121-1126.

[11] Tubives, 2021. Türkiye Bitkileri Veri Servisi (Turkish Plants Data Services). http://www.tubives.com (accessed 1 March 2021, in Turkish).

[12] Harput, U. S., Genc, Y., Saracoglu, I. 2012. Cytotoxic and antioxidative activities of *Plantago lagopus* L. and characterization of its bioactive compounds. Food and Chemical Toxicology, 50, 1554–1559.

[13] Blois, M. S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199-1200.

[14] Kunchandy, E. Rao, M. N. A. 1990. Oxygen radical scavenging activity of curcumin, International Journal of Pharmaceutics, 58, 237–240.

[15] Tsai, P. Y., Tsai, T. H., Yu, C. H., Ho, S. C. 2007. Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea, Food Chemistry, 103, 181-187.

[16] Singleton, V. L., Rossi, J. A. Jr. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

[17] Paquay, J. B. G , Haenen, G. R. M. M., Stender, G., Wiseman, S. A., Tijburg, L. B. M., Bast, A. 2000. Protection against nitric oxide toxicity by tea. Journal of Agriculture and Food Chemistry, 48, 5768-5772.

[18] Awah, F. M., Uzoegwu, P. N., Ifeonu, P., Oyugi, J. O., Rutherford, J., Yao, X., Fehrmann, F., Fowke, K. R., Eze, M. O. 2012. Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. Food Chemistry, 131(4), 1279-1286.

[19] Babbar, N., Oberoi, H. S., Uppal, D. S., Patil, R. T. 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Research International, 44(1), 391-396.

[20] Kusama Eguchi, K., Kusama, T., Suda, A., Masuko, T., Yamamoto, M., Ikegami, F., Igarashi, K., Kuo, Y. H., Lambein, F., Watanabe, K. 2004. Partial involvement of group I metabotropic glutamat receptors in the neurotoxicity of 3-N-oxalyl-L-2, 3- diaminopropionic acid (L-ODAP). Biological and Pharmaceutical Bulletin, 27(7), 1052-1058.

[21] Kawaguchi, K., Lambein, F., Kusama-Eguchi, K. 2012. Vascular insult accompanied by overexpressed heme oxygenase-1 as a pathophysiological mechanism in experimental neurolathyrism with hind-leg paraparesis. Biochemical and Biophysical Research Communications, 428, 160-166.

[22] Xiong, Y. C., Xing, G. M., Li, F. M., Wang, S. M., Fan, X. W., Li, Z. X., Wang, Y. F. 2006. Abscisic acid promotes accumulation of toxin ODAP in relation to free spermin level in grass pea seedlings (*Lathyrus sativus* L.). Plant Physiology and Chemistry, 44, 161-169.

[23] Arslan, M. 2018. Genetic diversity analysis of low β-ODAP population of grass pea wtih SSR markers. Journal of Biotechnolology 280, Supplement, p:554.

[24] Kumar, S., Bejiga, G., Ahmed, S., Nakkoul, H., Sarker, A. 2011. Genetic improvement of grass pea for low neurotoxin β-ODAP content. Food and Chemical Toxicology, 49, 589-600.

[25] Khan, N. A., Kuereshi, S., Pandey, A., Srivastava, A. 2009. Antibacterial activity of seed extracts of commercial and wild Lathyrus species. Turkish Journal of Biology, 33, 165-169.

[26] Sharma, D., Singh, P., Singh, S. S. 2018. β-N-oxalyl-l-α, β- diaminopropionic acid induces, wound healing by stabilizing HIF-1α and modulating associated protein expression. Phytomedicine, 44, 9-19.

[27] Gongke, Z., Yingzhen, K., Kairong, C., Zhixiao, L., Yafıu, W. 2001. Hydroxyl radical scavenging activity of β-N-oxalyl-α, β- diaminopropionic acid. Phytochemistry, 58, 759-762.

[28] Getahun, H., Mekonnen, A., TekleHaimanot, R., Lambein, F. 1999. Epidemic of neurolathyrism in Ethiopia. The Lancet, 354, 306.

[29] Polignano, G. B., Bisignano, V., Tomasell, V., Uggenti, P., Alba, V., Della Gatta, C. 2009. Genotype X environment interaction in grass pea (*Lathyrus sativus* L) lines. International Journal of Argonomy, Article ID: 898396, 7 pages.

[30] Lambein, F., Kuo, Y. H., Kusama-Eguchi, K., Ikegami, F. 2007. 3-N-oxalyl-L-2,3-diaminopropanoic acid, a multifunctional plant metabolite of toxic reputation. ARKIVOC, 9, 45-52.

[31] Xiong, J., Bai, X., Batool, A., Kong, H. Y., Tan, R., Wang, Y. F., Jiao, C. J., Xiong, Y. 2014. Ecological Function and and application of toxin β-ODAP in grass pea (*Lathyrus sativus*). Chinese Journal of Applied Ecology 25(4), 1-10.

[32] Dawei, Z., Gengmei, X., Hui, X., Zeyi, Y., Chongying, W., Yafu, W., Zhixiao, L. 2005. Relationship between oxalic acid and the metabolism of β-N-oxalyl- α,β-diaminopropionic acid (ODAP) in grass pea (*Lathyrus sativus* L.). Israel Journal of Plant Science, 53, 89-96.

[33] Djurdjevic, L., Gajic, G., Kostic, O., Jaric, S., Pavlovic, M., Mitrovic, M., Pavlovic, P. 2012. Seasonal dynamics of allelopathically significant phenolic compounds in globally successful invader *Conyza canadensis* L. plants and associated sandy soil. Flora, 207, 812-820.