

RESEARCH CONCERNING METABOLIC AND ANTIOXIDANT EFFECTS OF THE ETHANOLIC EXTRACT FROM *SYRINGAE VULGARIS FLOS F. ALBA* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Since it was proved that diabetes mellitus complications, especially nephropathy, retinopathy, arteriopathy or neuropathy, are closely related to increased oxidative stress, researches were directed to find natural antioxidants which can be safely administered to patients on long-term safety. Hence, many medicinal plants have been recommended for the management of diabetes due to their significant metabolic and antioxidant effects. The aim of our research was to evaluate the influence of an ethanolic extract of *Syringae vulgaris flos f. alba* on the metabolic and antioxidant status of streptozotocin-induced diabetic rats in comparison with an ethanolic extract of *Phaseoli sine seminibus fructus*, recognized worldwide for its antidiabetic and antioxidant virtues. The effects of oral administration of plant extracts as a once daily dose for six-weeks to diabetic rats were investigated on blood glucose and cholesterol concentration, the degree of lipid peroxidation and the activity of several antioxidant enzymes (glutathione peroxidase, glutathione reductase, superoxide dismutase).

Rezumat

Deoarece complicațiile diabetului zaharat, precum nefropatia, retinopatia, arteriopatia sau neuropatia, s-au dovedit a fi în strânsă legătură cu intensificarea stresului oxidativ, cercetările în domeniu au fost direcționate spre găsirea unor antioxidanți naturali care să fie administrați pacienților în condiții de siguranță pe termen lung. Prin urmare, multe dintre plantele medicinale au fost recomandate în managementul diabetului zaharat datorită activității lor metabolice și antioxidante semnificative. Scopul cercetării noastre a fost de a evalua influența extractului etanolic de *Syringae vulgaris flos f. alba* asupra statusului metabolic și antioxidant la șobolani cu diabet experimental indus cu streptozotocină, în comparație cu un extract de *Phaseoli sine seminibus fructus*, plantă recunoscută în întreaga lume pentru virtuțile sale antidiabetice și antioxidante. Efectele administrării orale a unei singure doze de extract zilnic, timp de șase săptămâni, au fost investigate prin determinarea concentrației sanguine a glucozei și colesterolului, a gradului de peroxidare lipidică și activității unor enzime antioxidante (glutathione peroxidază, glutathione reductază, superoxid dismutază).

Keywords: *Syringa vulgaris flos f. alba*, ethanolic extract, streptozotocin experimental diabetes, antioxidant activity

Introduction

Diabetes mellitus is associated with abnormalities in the metabolism of carbohydrates, proteins and lipids. Therefore it is characterized by hyperglycaemia due to an insufficient secretion of insulin or a peripheral tissue resistance to its action and hyperlipidaemia responsible for the acceleration of

atherosclerotic disease. The metabolic abnormalities of diabetes induce the oxidative stress either due to mitochondrial overproduction of reactive oxygen species (ROS) or the reduction of the scavenging capacity of free radicals [15].

In the presence of excessive amounts of very unstable ROS (superoxide anion, hydroxyl radical),

which could transfer their free unpaired electron, occurs the damage of biological compounds after the interactions between ROS and proteins, fatty acids, nucleotides or disulphide bridges, with membrane damages and mutagenic conditions, oxidative stress playing a pivotal role in the appearance of late diabetes complications [6, 17, 22]. The antioxidant enzymatic systems defence (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and the non-enzymatic free radical scavenging systems (glutathione, vitamins A, E, C, P, melatonin) have a major role in counteracting these unwanted effects of ROS [4, 5, 7, 10, 17, 19]. Apart from these endogenous systems, the human body can use natural or synthetic inhibitors of the oxidation of basic cell constituents. Natural antioxidants which neutralize such reactive species preserving the body homeostasis can be obtained much more easily from diet and medicinal herbs intake.

In the recent years it has been a rising concern for discovering strategies to improve the metabolic parameters and to inhibit the overproduction of free radical species or exogenous supplements of potent scavenger to support the antioxidant defence systems in a preventive intervention for disorders caused by oxidative stress. Therefore, it was an opportunity of exploring various plant products or extracts, already recognized as valuable medicinal plants and others waiting to be validated by scientific studies based on the presence of several phytochemical constituents important for the reduction of oxidative stress, such as polyphenolic acids, flavonoids, anthocyanins or tannins [8, 9, 11]. These various phytochemicals found in all plant parts (leaves, flowers, fruits, roots or bark) are a potential source of natural antioxidant supplements better tolerated, with fewer side effects and conferring a protective and disturbance-preventing established role of herbal medicine [2, 4, 5, 10, 14, 16, 17].

Syringa vulgaris L. (*Oleaceae*) commonly known as lilac is recorded in ethno-pharmacology with anti-inflammatory and immunomodulatory actions; thus it can be used in traditional medicine to treat rheumatic disease or joint manifestations from gout [12], but it also showed results in other animal models of experimental inflammation such as periodontitis or spinal cord trauma [14, 20]. Our previous study with the tincture of violet lilac flowers on diabetic rats revealed its antioxidant capacity [7]. The present study was designed to evaluate the influence of an ethanolic extract of *Syringae vulgaris* flos f. *alba*, a variant of lilac with white flowers less investigated for its therapeutic properties, on the metabolic and antioxidant status of streptozotocin-induced diabetic rats, compared to the ethanolic extract of *Phaseoli pericarpium*.

Phaseolus vulgaris (common bean) belongs to the *Fabaceae* family, worldwide spread and utilized for its edible dry seeds (known as beans) or unripe fruit (known as green beans). Several studies recommend it not only as a good source of proteins, vitamins, carbohydrates, or minerals [8, 9, 18], but also with potential bioactivities of its natural compounds enough to be used as adjuvants to restore near normal several biological parameters disturbed in metabolic disorders such as blood glucose, lipid profile and antioxidant status [21, 23, 26].

Phaseoli sine seminibus fructus (pericarpium) known as bean pods is the plant part used in traditional medicine for diuretic and antidiabetic activities [8, 9]. The hypoglycaemic action and antioxidant properties of bean pods extracts were demonstrated in several *in vitro* and *in vivo* studies and are assigned to phenolic compounds which have cytoprotective effects on pancreatic β -cells, organosulphur compounds able to neutralize factors that inactivate insulin and also to phaseolamine, a proteinaceous inhibitor of α -amylase [8, 13, 18, 23].

Materials and Methods

Reagents. Streptozotocin (STZ) for induction of experimental diabetes and thiobarbituric acid (TBA), used for the assay of lipid peroxidation, were obtained from Sigma-Aldrich GmbH. For testing the antioxidant enzymes activities there were used kits (Ransod, Ransel and Glut Red) obtained from Randox Labs., UK. The absorbance was measured on a Beckman UV/VIS DU-65 spectrophotometer with a temperature control module from Beckman Instruments, Fullerton, USA. Accu-Chek® InstantPlus Meter, a device that tests both blood glucose and cholesterol, was purchased from Roche Diagnostics.

Plant materials. *Syringae vulgaris* flos f. *alba* (white lilac flowers) and *Phaseoli sine seminibus fructus* or *Phaseoli pericarpium* (bean pods) were collected from the Botanical Garden of the University of Craiova, Romania and identified at the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania. Voucher specimens were deposited in the department herbarium. Collected plant materials were shade air dried at room temperature and stored. The dried flowers and respectively pods were pulverized with an electric grinder. Ethanolic extracts were obtained by simple percolation in a ratio vegetable/solvent (ethanol 70°) of 1:5 (according the Xth edition of the Romanian Pharmacopoeia) and then were stored into tightly closed brown glass bottles, at room temperature.

Animals. Experiments were performed on adult male Wistar rats, weighing 176-260g, maintained

on standard food and water *ad libitum*. All experiments were conducted according to the ethical guidelines of scientific research approved by our institution regarding the maintenance and treatment of animals.

Toxicity study. Normal healthy rats orally treated with different high concentrations from the ethanolic extract of *Syringae vulgaris flos f. alba* revealed, after a careful examination, a normal behaviour. No toxic or lethal effects were reported for 48 h after a single dose (acute toxicity), respectively for two-weeks of daily administration, so we ascertained the extract as non-toxic.

Induction of diabetes and experimental design. For the experimental STZ-induced hyperglycaemia it was administrated a single intraperitoneal injection (in a dose of 70 mg/kg body weight) to overnight fasted rats. On the third day after STZ injection, the rats with fasting glucose levels higher than 150 mg/dL were considered diabetics, according to experimental methods previously described by other authors [1, 3]. In the control group, the healthy rats were injected with physiologic saline solution. Rats were divided into four groups comprising five animals each: group 1 (G1) – untreated healthy rats serving as normal control group; group 2 (G2) – untreated STZ-induced diabetic rats serving as diabetic control group; group 3 (G3) – STZ-induced diabetic rats treated daily, for six-weeks, with 150 mg/kg b.w. from the 20% ethanolic extract of *Syringae vulgaris flos f. alba* (SA) given orally; group 4 (G4) – STZ-induced diabetic rats treated daily, with 150 mg/kg b.w. from the 20% ethanolic extract of *Phaseoli pericarpium* (PP) given orally for six-weeks.

Blood glucose level and body weight were monitored every morning at the same hour. Cholesterol level was measured at the onset and the end of the experiment. After six weeks, the animals were slaughtered and blood was collected and processed for plasma and erythrocytes separation in order to assess oxidative stress biomarkers: lipid peroxides, glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) activities.

Biochemical assays. Fasting blood glucose and cholesterol levels were measured with the Accu-Chek® Instant Plus Meter. We estimated lipid peroxidation by measuring the thiobarbituric acid reactive substances (TBARS) according to the method of Buege and Aust (1978) that uses malondialdehyde (MDA) as a standard. The activities of the antioxidant enzymes (GPx, GR and SOD) were measured with commercially available kits from Randox according to the manufacturer's protocols. The method for GPx activity is based on that of Paglia and Valentine (1967). GR activity was estimated according to the method of Goldberg and

Spooner (1983). Determination of SOD activity involved measuring the degree of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium (INT) transformation into a red formazan dye by the superoxide generated from the oxidation of xanthine with xanthine oxidase.

Statistical analysis. Values are presented as mean $\pm$ SEM. The results were compared with the *t*-test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Table I and Figure 1 display the variation of blood glucose and cholesterol levels and one can observe that these were significantly increased in STZ-diabetic control rats compared to normal control and STZ-diabetic groups treated with ethanolic extracts. The administration of SA ethanolic extract on the metabolic parameters had a similar influence with that of PP ethanolic extract.

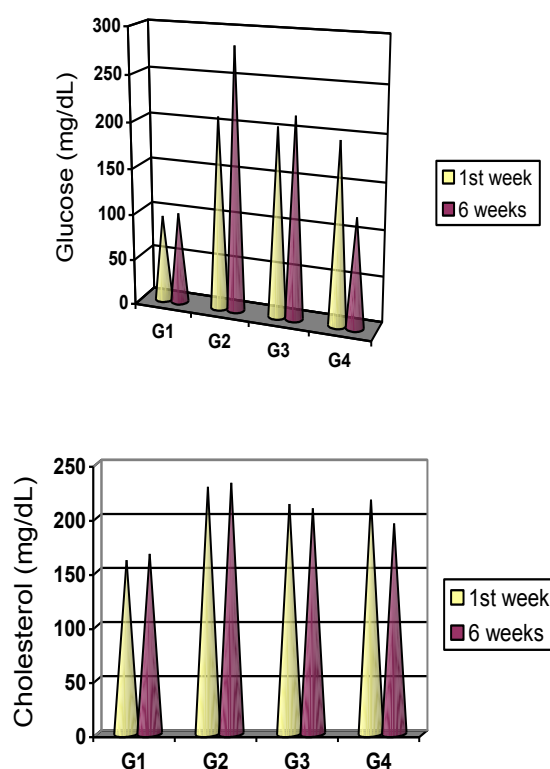


Figure 1.

Variation of the metabolic parameters during the six weeks experiment

(G1 – normal control; G2 – STZ-diabetic control; G3 – STZ-diabetic treated with SA; G4 – STZ-diabetic treated with PP)

Table II shows the variation of TBARS level and antioxidant enzymes activities in the different groups of study after six-weeks of treatment with the ethanolic extracts of *Syringae vulgaris flos f. alba* and *Phaseoli pericarpium*.

Table I

Effect of six weeks treatment with the extracts on blood glucose and cholesterol levels

		Group 1 Normal Control	Group 2 STZ-diabetic Control	Group 3 STZ-diabetic treated SA	Group 4 STZ-diabetic treated PP
Glucose (mg/dL)	1st week	94.3 ± 8.2	208.4 ± 32.5*	203.8 ± 26.8*	195.9 ± 32.6*
	After 6 weeks	99.2 ± 6.8	282.8 ± 52.6*	216.4 ± 20.2* [#]	118.4 ± 20.4 [#]
Cholesterol (mg/dL)	1st week	160.5 ± 15.3	228.4 ± 18.7*	212.4 ± 12.8* ^{##}	216.4 ± 16.4*
	After 6 weeks	166.3 ± 14.6	232.1 ± 21.2*	208.4 ± 15.3* ^{##}	194.3 ± 20.1* ^{##}

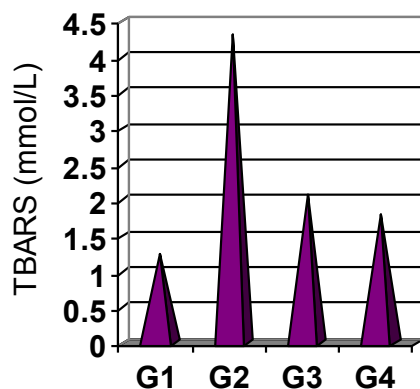
*p < 0.001; **p < 0.005 compared with the normal control group; [#]p < 0.001; ^{##}p < 0.05 compared with STZ-diabetic control.**Table II**

Effect of six weeks treatment with the ethanolic extracts on lipid peroxides and antioxidant enzymes

	Group 1 Normal Control	Group 2 STZ-diabetic Control	Group 3 STZ-diabetic treated SA	Group 4 STZ-diabetic treated PP
TBARS (mmol/L)	1.25 ± 0.25	4.30 ± 0.52*	2.07 ± 0.12* [#]	1.80 ± 0.15* [#]
GPx (U/g Hb)	38.6 ± 3.62	12.2 ± 4.25*	22.5 ± 3.02* [#]	24.5 ± 2.12* [#]
GR (U/g Hb)	13.5 ± 1.14	10.4 ± 0.2*	8.9 ± 0.35*	11.2 ± 1.14* ^{##}
SOD (U/mL)	154.4 ± 9.8	116.8 ± 16.4*	125.2 ± 8.2*	136.2 ± 10.7* ^Δ

*p < 0.001; **p < 0.005 compared with the normal control group; [#]p < 0.001; ^{##}p < 0.05; ^Δp < 0.005 compared with STZ-diabetic control.

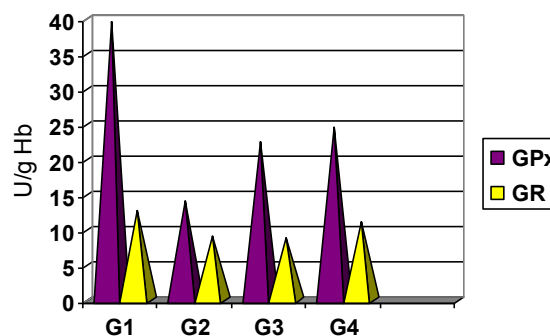
We noted that TBARS levels presented a very high significant decrease in both groups of STZ-diabetic rats treated with SA and PP ethanolic extracts compared to the animals from the STZ-diabetic control group ($p < 0.001$) (Figure 2).

**Figure 2.**

Variation of lipid peroxides (measured as TBARS) after six weeks of treatment

(G1 – normal control; G2 – STZ-diabetic control; G3 – STZ-diabetic treated with SA; G4 – STZ-diabetic treated with PP)

Treatment of STZ-diabetic rats with both SA and PP ethanolic extracts had a very high significant influence on GPx activity levels ($p < 0.001$) compared with untreated STZ-diabetic animals (Figure 3). Concerning GR activity, we observed that only PP ethanolic extract determined its' significantly increase in diabetic rats treated ($p < 0.05$) compared with the animals from the STZ-diabetic control.

**Figure 3.**

Variation of GPx and GR activities after six weeks of treatment

(G1 – normal control; G2 – STZ-diabetic control; G3 – STZ-diabetic treated with SA; G4 – STZ-diabetic treated with PP)

Regarding blood SOD activity we observed a high significant increase only in STZ-diabetic rats treated with PP ethanolic extract ($p < 0.005$) compared to the diabetic animals untreated.

In conclusion, daily treatment for six weeks with the SA ethanolic extract had a very significant effect only on GPx activity and lipid peroxides level.

Studies carried out on STZ-induced diabetes in rats demonstrated that treatment with an aqueous extract of *Phaseolus pericarpium* had not only hypoglycaemic and hypolipidemic effects, but also caused a significant decrease in plasma TBARS, hydroperoxides and restore to near normal levels HDLC, plasma insulin, vitamin C and glutathione [25]. Regarding our study, after six-weeks of daily administration of the ethanolic extracts of SA and PP to diabetic rats we noted that both extracts reduced blood glucose and cholesterol levels compared to untreated STZ-diabetic animals.

Similar researches on plants revealed that the antidiabetic effect of various vegetal products might be based on the improvement of insulin, C-peptide or amylin levels by stimulating their release from the pancreatic islets of Langerhans or by the augmentation of synthesis due to pancreatic β -cells regeneration [9, 11, 26].

The antidiabetic effect of *Phaseolus vulgaris* pods ethanolic extract might be based on various peptides from common bean proteins with potential antioxidant activity, dipeptidyl peptidase IV inhibitor or angiotensin converting enzyme inhibitor recommending such a product in managing to prevent or control type-2 diabetes and hypertension [18].

Persistent hyperglycaemia leads to glucose oxidation, lipid peroxidation or accumulation of advanced glycation end products (AGEs), processes which are considered the main sources of ROS [17, 19]. Several *in vitro* and *in vivo* studies revealed that the imbalance between the production of ROS and scavenger activity of antioxidant systems impairs the function and structure of many biomolecules, and the oxidative stress that follows causes damage of pancreatic β -cells, peripheral insulin resistance and endothelial dysfunction in blood vessels, being associated with the progression of diabetes mellitus complications [10, 15].

Diabetes mellitus induces alteration in the structure and function of antioxidant enzymes [17] and affects free radicals detoxification, showed by decreased levels of GPx, GR and SOD activities. Moreover, the increase of lipid peroxidation after STZ-induced diabetes sustains either an intensive production of ROS or a decrease in the activity of antioxidant defence systems.

In the performed study, the activities of GPx, GR and SOD decreased significantly in diabetic rats compared to untreated controls, the explanation being the presence of an excess of ROS. Our findings indicate that daily oral administration of SA and PP ethanolic extracts in diabetic rats had a significant antioxidant effect with diminished lipid peroxidation. The research also revealed increased GPx, GR and SOD activities, especially for PP tincture, while the SA ethanolic extract determined only an enhancement of GPx activity. This suggests the protective role of the ethanolic extracts which might be closely related to the content in natural antioxidant plant constituents such as flavonoids and polyphenols that act by detoxifying the oxygen free radicals [4, 12, 14, 20, 23, 24].

In traditional medicine, lilac remedies have been used especially due to its antirheumatic and anti-inflammatory properties; subsequently, phyto-pharmaceutical researches have revealed increased amounts of constituents with antioxidant capacity

such as flavonoids, polyphenols and verbascosides [12, 14, 20, 24].

Our results demonstrated that the capacity of SA ethanolic extract to reduce hyperglycaemia and hypercholesterolemia, to enhance GPx activity and diminish lipid peroxidation is approaching to PP ethanolic extract and indicates a protective role of *Syringae vulgaris flos f. alba* extracts in preventing pathological events due to the enhancement of oxidative burden.

Conclusions

The present research reveals that both extracts, one recognized as a natural remedy for diabetes, *Phaseoli pericarpium* extract, and the other one, a candidate for recognizing its therapeutic adjuvant potential, showed hypoglycaemic, hypocholesterolemic effects and free radicals scavenging capacity. These results confer a scientific evidence for the possible use of *Syringae vulgaris flos f. alba* extract as an adjuvant in order to improve biological parameters of patients whom inherit a predisposition to diabetes mellitus. Further, the research will continue with the isolation of phytochemical constituents with pharmacological properties in order to achieve some optimal combinations more effective in reducing cell damage and avoiding occurrence of diabetic complications.

References

1. Abu Abeeleh M., Ismail Z.B., Alzaben K., Abu-Halaweh S., Al-Essa M., Abu Abeeleh J., Alsmady M., Induction of Diabetes Mellitus in Rats Using Intraperitoneal Streptozotocin: A Comparison between 2 Strains of Rats. *Eur. J. Sci. Res.*, 2009; 32(3): 398-402.
2. Adisakwattana S., Jiphimai P., Prutanopajai P., Chanathong B., Sapwarobol S., Ariyapitipan T., Evaluation of alpha-glucosidase, alpha-amylase and protein glycation inhibitory activities of edible plants. *Int. J. Food Sci. Nutr.*, 2010; 61(3): 295-305.
3. Akbarzadeh A., Norouzian D., Mehrabi M.R., Jamshidi Sh., Farhangi A., Verdi A.A., Mofidian S.M., Rad B.L., Induction of diabetes by Streptozotocin in rats. *Indian J. Clin. Biochem.*, 2007; 22(2): 60-64.
4. Akinmoladun A.C., Ibukun E.O., Afor E., Oboutor E.M., Farombi E.O., Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci. Res. Essays*, 2007; 2(5): 163-166.
5. Arsene A.L., Rodino S., Butu A., Petrache P., Iordache O., Butu M., Study on antimicrobial and antioxidant activity and phenolic content of ethanolic extract of *Humulus lupulus*. *Farmacia*, 2015; 63(6): 851-857.
6. Baynes J.W., Thorpe S.R., Role of oxidative stress in diabetic complications: A new perspective of an old paradigm. *Diabetes*, 1999; 48(4): 1-9.
7. Berbecaru-Iovan A., Stănculescu E.C., Berbecaru-Iovan S., Andrei A.M., Ceașu I., Pisoschi C.G.,

- Study regarding the antioxidant activity of *Syringae vulgaris* flos f. *violacea* tincture in experimental diabetes. *Farmacia*, 2014; 62(6): 1072-1081.
8. Bnouham M., Ziyat A., Mekhfi H., Tahri A., Legssyer A., Medicinal plants with potential anti-diabetic activity – A review of ten years of herbal medicine research (1990-2000). *Int. J. Diabetes and Metabolism*, 2006; 14(1): 1-25.
 9. Bojor O., Popescu O., Fitoterapie tradițională și modernă, ediția a IV-a, revizuită și adăugită, Ed. Fiat Lux, București, 2005; 48-56, 251-258.
 10. Drăgoi C.M., Nicolae A.C., Grigore C., Dinu-Pîrvu C.E., Arsene A.L., Characteristics of glucose homeostasis and lipidic profile in a hamster metabolic syndrome model, after the co-administration of melatonin and irbesartan in a multiparticulate pharmaceutical formulation. *The 2nd International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, INTERDIAB 2016*, Diabetes Mellitus as Cardiovascular Disease, Ed. Niculescu Bucharest, 2016; 221-229.
 11. Eddouks M., Khalidi A., Zeggwagh N.A., Lemhadri A., Michel J.B., Burcelin R., An understanding mechanistic approach of hypoglycemic plants. *Advances in phytotherapy research*, Research Signpost Publishing House, 2009; 109-128.
 12. Fedoseeva A.A., Lebedkova O.S., Kanibolotskaya L.V., Shendrik A.N., Dudzinskaya V.V., Tkachenko L.N., Shineva N.V., Composition and antiradical activity of lilac extracts. *Pharm. Chem. J.*, 2011; 45(2): 91-92.
 13. Frassinetti S., Gabriele M., Caltavuturo L., Longo V., Pucci L., Antimutagenic and antioxidant activity of a selected lectin-free common bean (*Phaseolus vulgaris* L.) in two cell-based models. *Plant Foods Hum. Nutr.*, 2015; 70(1): 35-41.
 14. Genovese T., Paterniti I., Mazzon E., Esposito E., Di Paola R., Galuppo M., Bramanti P., Cuzzocrea S., Efficacy of treatment with verbascoside, biotechnologically produced by *Syringa vulgaris* plant cell cultures in an experimental mice model of spinal cord trauma. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 2010; 382(4): 331-345.
 15. Giacco F., Brownlee M., Oxidative stress and diabetic complications. *Circ. Res.*, 2010; 107(9): 1058-1070.
 16. Jurca T., Vicas L., Marian E., Vicas S., Muresan M., A new natural antioxidant supplement – design and development. *Farmacia*, 2016; 64(1): 135-142.
 17. Maritim A.C., Sanders R.A., Watkins III J.B., Diabetes, Oxidative Stress, and Antioxidants: A Review. *J. Biochem. Mol. Toxicol.*, 2003; 17(1): 24-37.
 18. Mojica L., de Mejía E.G., Characterization and Comparison of Protein and Peptide Profiles and their Biological Activities of Improved Common Bean Cultivars (*Phaseolus vulgaris* L.) from Mexico and Brazil. *Plant Foods Hum. Nutr.*, 2015; 70(2): 105-112.
 19. Nicolae A.C., Drăgoi C.M., Ceașu I., Poalelungi C., Iliescu D., Arsene A.L., Clinical implications of the indolergic system and oxidative stress in physiological gestational homeostasis. *Farmacia*, 2015; 63(1): 46-51.
 20. Paola R.D., Oteri G., Mazzon E., Crisafulli C., Galuppo M., Toso R.D., Pressi G., Cordasco G., Cuzzocrea S., Effects of verbascoside, biotechnologically purified by *Syringa vulgaris* plant cell cultures, in a rodent model of periodontitis. *J. Pharm. Pharmacol.*, 2011; 63(5): 707-717.
 21. Pari L., Venkateswaran S., Protective role of *Phaseolus vulgaris* on changes in the fatty acid composition in experimental diabetes. *J. Med. Food*, 2004; 7(2): 204-209.
 22. Petlevski R., Hadzija M., Slijepcevic M., Juretic D., Effect of 'antidiabetic' herbal preparation on serum glucose and fructosamine in NOD mice. *J. Ethnopharmacol.*, 2001; 75(2-3): 181-184.
 23. Reverri E.J., Randolph J.M., Steinberg F.M., Kappagoda C.T., Edirisinghe I., Burton-Freeman B.M., Black Beans, Fiber, and Antioxidant Capacity Pilot Study: Examination of Whole Foods vs. Functional Components on Postprandial Metabolic, Oxidative Stress, and Inflammation in Adults with Metabolic Syndrome. *Nutrients*, 2015; 7(8): 6139-6154.
 24. Tóth G., Barabás C., Tóth A., Kéry Á., Béni S., Boldizsár I., Varga E., Noszá B., Characterization of antioxidant phenolics in *Syringa vulgaris* L. flowers and fruits by HPLC-DAD-ESI-MS. *Biomed Chromatogr.*, 2016; 30(6): 923-932.
 25. Venkateswaran S., Pari L., Saravanan G., Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in rats with streptozotocin-induced diabetes. *J. Med Food*, 2002; 5(2): 97-103.
 26. Venkateswaran S., Pari L., Antioxidant effect of *Phaseolus vulgaris* in streptozotocin-induced diabetic rats. *Asia Pac. J. Clin. Nutr.*, 2002; 11(3): 206-209.