IRSTI 61.45.36 https://doi.org/10.26577/ijbch.2021.v14.i1.020

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The isolation of lignan containing fractions from flaxseed *Linum usitatissimum L.*

Abstract. This article is dedicated to the isolation of lignin-containing fractions from the flaxseeds *Linum usitatissimum L.* The main component of these fractions is lignan-secoisolariciresinol diglucoside (SDG). The authors have developed an effective extraction method for obtaining lignan-containing fractions from flaxseed with a satisfactory yield of SDG-containing fraction and its isomers. This method was implemented by microwave irradiation pretreatment of the seeds and by using environmentally friendly extraction solvents. A 50/50 mixture of water and ethanol was chosen as extraction agents. Microwave irradiation pretreatment of ground flaxseeds was carried out at 450W and 800W for 5 seconds, 4 times with an interval of 5 seconds. SDG-identification was performed on an Agilent 1200 series high performance liquid chromatograph and a Shimadzu UV-1900 automatic dual-beam UV spectrophotometer. The results of the conducted studies showed that the microwave irradiation pretreatment of flaxseeds at 800W increases the yield of SDG relative to the dry residue by 34.76% in comparison with the classical alkaline method, and the content of the amount of lignans in recalculation on chicory acid was 43.53%. Thus, the extraction method of lignans-containing extract including the stage of pretreatment by microwave irradiation with power of 800W is the most effective method.

Key words: flaxseeds, extraction, hydrolysis, lignan, secoisolariciresinol diglucoside, extract, HPLC, optimal conditions.

Introduction

One of the raw materials for the production of phytoestrogens is flaxseeds, one of the key industrial crops grown in Kazakhstan. Flaxseeds accumulate in their seedings a macromolecular complex consisting of lignans. The main lignan in flaxseeds is secoisolariciresinol diglucoside (SDG) (its specific content is more than 2%). Along with SDG, other substances similar in physicochemical characteristics are also contained: p-coumaric acid, ferulic acid, sinapic acid, caffeic acid and their glucosides [1; 2], and other minor lignans (matairesinol, isolariciresinol, pinoresinol, lariciresinol).

SDG has powerful antioxidant activity and also exhibits bactericidal and antiviral properties. This substance in flaxseed extract has been found to have anti-cancer activity by blocking the effect of CDK4 protein. When exposed to cancerous forms in diabetic diseases, this substance shows sufficient activity to inhibit the growth of malignant cells in the gastrointestinal tract [3; 4]. Researchers have shown a positive effect of SDG on menopausal syndrome and sexual dysfunction in women during non-hormonal therapy [5].

All these properties make the isolation of these substances, both in pure form and in the form of lignan-enriched compositions, currently topical.

The most effective way to isolate biologically active compounds from plant material is extraction, which consists in the transference of substances of plant material into a solution with an appropriate solvent. The general scheme of lignans extraction from flaxseeds consists of the following stages: seeds grinding, their degreasing, extraction and hydrolysis of the degreased mass, neutralization and concentration of the obtained extract [6-8]. Degreasing with hexane is necessary to remove lipids and to facilitate the extraction process of phenolic components of flaxseed. The polyphenol fraction containing secoisolariciresinol diglucoside is a polymeric chain bound by a polysaccharide

complex. Therefore, acidic, enzymatic and alkaline hydrolysis methods are used to release SDG from the bound state. Hydrolysis and extraction are the most important stages as they determine the yield of the extracts that include SDG. The most effective method of hydrolysis is the alkaline method of hydrolysis because the fermentative method is expensive and has a low yield [9], and the acidic method has a partial release of SDG as its major part is converted into secoisolariciresinol [10].

Methods with the highest yield of SDG in the extract using a mixture of dioxane and ethanol, methanol and water are known, but due to the high toxicity of the solvents, these methods are unsafe [5; 6]. To increase the efficiency of extraction and to increase the yield of the target product from plant raw materials, microwave irradiation treatment methods are increasingly used [11-13], which make it possible to reduce solvent consumption and to reduce the duration of extraction. It was found that with prolonged microwave action, the yield of SDH is significantly decreasesdue to catalytic destruction in plant raw materials, therefore, short-term treatment with microwave radiation is optimal [14].

Based on the above, the process of obtaining extracts dominated by SDG remains a priority. In this regard, this work was carried out to increase the mass yield of the SDG-containing fraction and to study its properties.

Work objective: Optimization of method of obtaining lignan-containing extract by microwave irradiation pretreatment of flaxseeds using environmentally friendly solvents.

Materials and methods

Flaxseeds *Linum usitatissimum L.* were taken as an initial raw material for isolation of lignans fraction. Manufacturing country is Kazakhstan (KazBAD). Water-ethanol mixture (50/50) was chosen as extract agents.

The physicochemical parameters of the initial raw materials were determined according to GOST [15].

Extraction of lignans fraction was carried out according to the following methods:

1) According to the method (1) (control) of extraction with parallel hydrolysis, the raw material was crushed, a quantity of substance weighing 5 g was taken, de-greasing of raw material was carried out by hexane boiling for 1 hour in water bath with a reflux condenser at raw material-hexane ratio equal to 1:6 [16]. After degreasing hexane was removed, the raw material was dried and subjected to extraction in water-alcoholic solvent (50/50) for 1.5 hours [17]. The resulting seed cake was separated from the extract by filtration through a fine-mesh gauze filter; the suspensoid was precipitated by centrifugation at 3000 rpm. The resulting extract as a true color solution was subjected to NaOH hydrolysis at pH=10 [18] and a temperature of 80℃ for one hour. Neutralization of the obtained extract was carried out to pH=6 using 0.1H HCl solution [19], the residual suspension was precipitated by centrifugation at 3000 rpm. The resulting extract was evaporated to dry weight.

The method (2) included pretreatment of ground flaxseeds with microwave irradiation at 450W for 5s, 4 times with an interval of 5 seconds. Before treatment the seeds were moistened with water in an amount (1 part raw material to 3 parts water). The treated raw material was further extracted according to method 1.

The method (3) included microwave irradiation pretreatment of the ground flaxseeds at 800W for 5s, 4 times with an interval of 5 seconds. Before treatment the seeds were moistened with water in an amount (1 part raw material to 3 parts water). The treated raw material was further extracted according to method 1.

Preparation of solutions of extracts for UV spectral analysis: From dry extracts an accurate quantity of substance weighing 0.010 g was taken and dissolved in 10 ml of solvent (50% ethanol solution in water), from the obtained solution with a concentration of 1 mg/ml an aliquot of 1 ml was taken, which was diluted 10 times to obtain a solution with a concentration of 0.1 mg/ml, this solution was studied by UV spectroscopy. UV spectral analysis was performed on a Shimadzu UV-1900 (Shimadzu, Japan) automatic dual-beam UV spectrophotometer. The working spectral range was 200-500 nm. Spectral slit width: 1 nm. The wavelength setting error is within ±0.5 nm.

The confirmation of the presence and determination of SDG was performed on an Agilent 1200 series high performance liquid chromatograph (HPLC) (Agilent Technologies, Waldbronn, Germany) equipped with a four-channel gradient pump, degasifier, autosampler, column thermostat and diode matrix detector. Separation was performed on an Agilent Zorbax SB-C18 column of 4.6x150 mm with a particle diameter of 5 μm at an eluent flow rate of 1 ml/min. Time of analysis was 60 min. The eluent used was a mixture of acetonitrile and 0.5% (vol.) aqueous solution of ortho-phosphoric acid. The elution was conducted in gradient mode, varying the concentration of acetonitrile from 15 to 85%. The column temperature was kept constant at 300C. The

applied sample volume was 20 µl. Detection was performed at 4 wavelengths: 280±8 nm, 330±10 nm, 350±8 nm, 370±8 nm.

Results and discussion

The physicochemical characteristics of the initial raw materials are shown in Table 1, which are within acceptable limits in accordance with literature data.

Table 1 – Physicochemical characteristics of flax seed *Linum usitatissimum L.*

Index	Values
Moisture, %	7.4
Total ash, %	2.34
Ash insoluble in 10% hydrochloric acid, %	0.3

It was empirically found that the exposure time in a microwave oven at 800 W should not exceed more than 5 seconds for samples weighing 5 g, since the destruction of substances begins to occur, and the extraction efficiency decreases. A direct relationship between microwave power and extraction efficiency was also established.

As shown in Table 2, treatment of flaxseeds with microwave irradiation increases the yield of extraction by dry residue. The highest yield of extraction by dry residue is achieved when treating flaxseeds with microwave irradiation at a power of 800 W.

Table 2 – The yield of extraction by dry residue

Extraction method	Yield of dry residue, %
Method 1 (control)	9.82
Method 2	10.76
Method 3	12.35

To compare the quality of the extracts obtained, their UV spectral analysis was carried out at a concentration of 0.1 mg/ml and at different wavelengths.

Figure 1 shows the dependence of UV spectra of flaxseed extract depending on the methods of their preparation. As can be seen from the Figure there is an increase in optical density in extracts obtained by the microwave irradiation treatment of seeds compared to the control method, therefore, this extract is more saturated with substances that absorb light at these wavelengths.

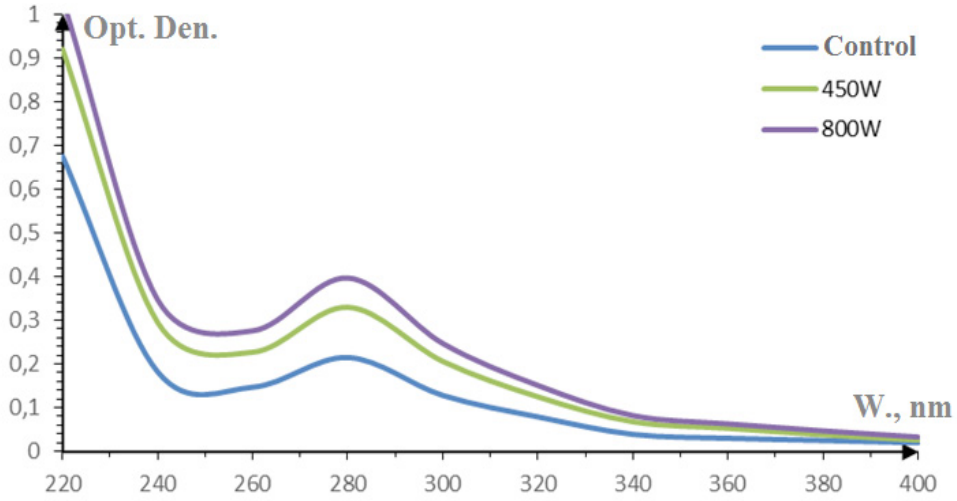

Figure 1 – Dependence of optical density on wavelength of flaxseed extracts at a concentration of 0.1 mg/ml

The UV spectra of the flaxseed extract show two intense peaks at 220 and 280 nm (Figure 1). The maximum at 280 nm is of analytical importance as it corresponds with the literature data of the secoisolariciresinol fraction [20]. The results of quantitative determination of the amount of lignans converted to chicoric acid at 280 nm and a concentration of 0.1 mg/mg are shown in Table 3. These results indicate the increase of lignan fraction yield in the extract with pretreatment of flaxseeds by microwave irradiation at 800W.

Using HPLC, the percentages of individual compounds were measured depending on the resulting fractions when the extracts were checked (Figure 2).

Percentage results are in Table 4. According to the obtained spectra, at 2.7 min and 3.6 min the compounds whose spectra coincide with those of the SDG were detected.

Table 3 –Quantitative analysis of the amount of lignans converted to chicoric acid for the extracts.

Concentration of the solution to be tested	Alkaline extract (Control), % of the weight of quantity of substance of dry residue	Microwave extract (450W), % of the weight of quantity of substance of the dry residue	Microwave extract (800W), % of the weight of quantity of substance of the dry residue
0.1 µg / ml	8.77	36.18	43.53

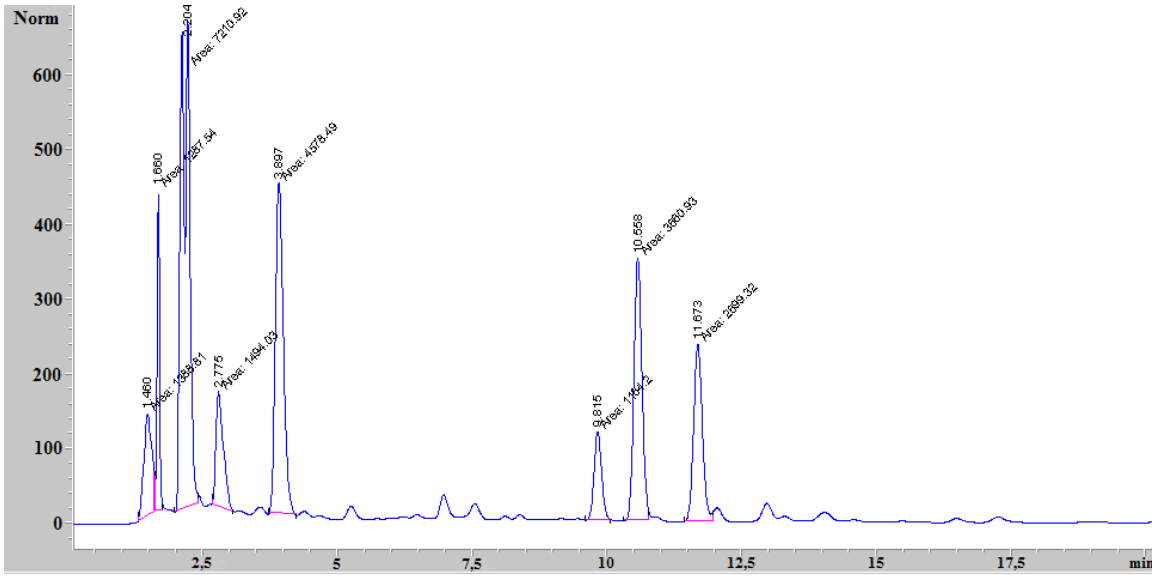

Figure 2 – HPLC spectrum of the extract obtained containing SDG

Table 4 – Percentage fractions as a function of retention time

No.	Time	Area	Height	Width	Area%
1	1.46	1388.8	137.1	0.1688	5.914
2	1.66	1287.5	428.2	0.0501	5.483
3	2.204	7210.9	653.2	0.184	30.705
4	2.775	1494	154.3	0.1614	6.362
5	3.897	4578.5	442.2	0.1726	19.496
6	9.815	1164.2	118.6	0.1636	4.957
7	10.558	3660.9	351	0.1739	15.589
8	11.673	2699.3	236.7	0.1901	11.494

According to the Table, the UV spectra of the peaks, which correspond to the literature data of the SDG peaks, fall on peaks 4-8. The total yield of the SDG containing fraction and its isomers is 58% of the obtained peak.

The above calibration graph was built on the basis of a SDG analytical sample (Merk, USA). The sensitivity of the method was 1 μg/ml. The average error of the method does not exceed 7% with repeated measurements. According to this graph (Figure 3), the total yield of peaks 4-8 was 45.4% of the dry matter weight. The obtained quantitative data on the content of SDG of similar substances are consistent with the above-obtained data.

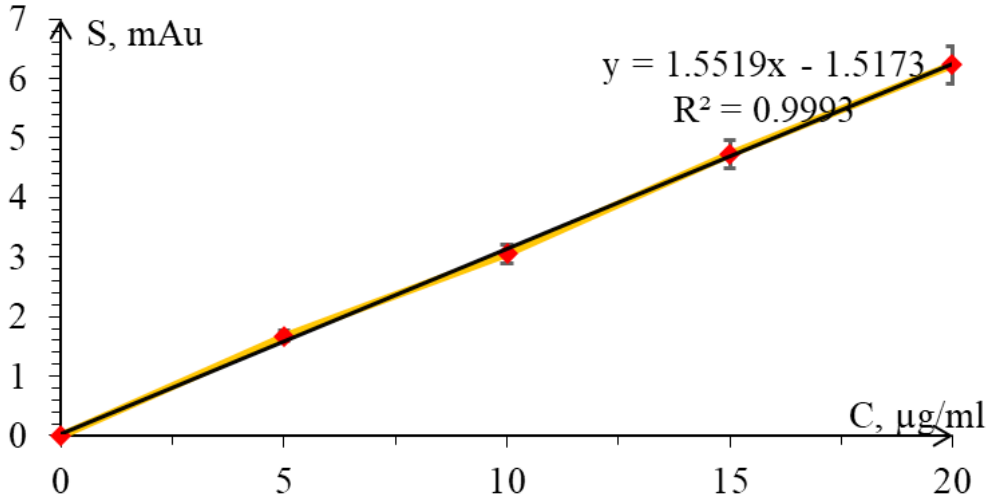

Figure 3 – Dependence of the area under the chromatographic peak, on the concentration of the SDG substance.

Conclusion

SDG is one of the main lignans contained in flaxseeds. Lignans belong to a group of natural phenolic compounds that have a wide range of biological activity. These include reduction in the risk of osteoporosis, as well as anti-cancer, antioxidant, cardioprotective, hepatoprotective and neuroprotective activity [21]. Concomitant polyphenolic substances may, in some cases, exhibit synergy with the target substances. Therefore, lignans are of great interest in the fields of chemistry and medicine for the creation of preventive and medicinal products.

Due to the above factors, method for obtaining a lignan-containing extract from flaxseeds *Linum usitatissimum L* has been developed, which includes pre-treatment with microwave radiation using environmentally friendly solvents for extraction. Obtaining these extracts is of great practical importance, due to their multiple useful properties.

The results of the conducted studies have shown that the most optimal method for obtaining a lignan-containing extract is the method involving pretreatment of flaxseeds with microwave radiation at 800W. It was found that the yield of SDG relative to the dry residue increased by 34.76% with microwave treatment relative to the classical alkaline method. The obtained extracts contain a sufficient amount of active target substances that have been confirmed by HPLC.

Acknowledgments

Research is funded by the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan, Grant No. AP08956875.

References

1 Liggins J., Grimwood R., Bingham S.A. (2000). Extraction and quantification of lignan phytoestrogens in food and human samples. *Analytical Biochemistry.* vol. 287, pp. 102-109. https://doi.org/10.1006/abio.2000.4811

2 Struijs, K. (2008) The lignan macromolecule from flaxseed. Structure and bioconversion of

lignans. Ph.D. thesis Wageningen Universiteit, The Netherlands, 180 p. ISBN: 978-90-8585-247-6

3 Niyati R.S., Bhoomika M.P. (2016). Secoisolariciresinol diglucoside rich extract of L. usitatissimum prevents diabetic colon cancer through inhibition of CDK4. *Biomedicine & Pharmacotherapy.* vol. 83, pp. 733-739. https://doi.org/10.1016/j.biopha.2016.07.041

4 Colli M.C., Bracht, A., Soares A.A., de Oliveira A.L., Boer C.G., de Souza C.G.M., Peralta R.M. (2012) Evaluation of the Efficacy of Flaxseed Meal and Flaxseed Extract in Reducing Menopausal Symptoms. *J. Of Med. Food.* vol. 15, no. 9, pp. 840-845. https://doi.org/10.1089/jmf.2011.0228

5 Rickard S. E., Thompson L.U. (1997) Health effects of flaxseed mucilage, lignans. *Inform*, vol. 8, pp. 860-865.

6 Johnsson P., Kamal-Eldin A., Lundgren L.N., Aman P. (2000). HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. *J. Agric. Food Chem.*, vol. 48, pp. 5216-5219. https://doi.org/10.1021/jf0005871

7 Madhusudhan B., Wiesenborn D., Schwarz J., Tostenson K., Gillespie J. (2000). A dry mechanical method for concentrating the lignan secoisolariciresinol diglucoside in flaxseed. *Lebensm.-Wiss. U.-Technol.,* vol. 33, pp. 268-275. https://doi.org/10.1006/fstl.2000.0652

8 Eliasson C., Kamal – Eldin A., Andersson R., Aman P. (2003). High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucoside in flaxseed by alkaline extraction. *J. Chromatogr. A*, vol. 1012, no. 2, pp. 151-159. https://doi.org/10.1016/S0021-9673(03)01136-1

9 Obermeyer W.R., Musser S.M., Betz J.M., Casey R.E., Pohland A.E., Page S.W. (1995). Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral. *Proc. Soc. Exp. Biol. Med.,* vol. 208, no. 1, pp. 6-12. https://doi.org/10.3181/00379727-208-43824

10 Charlet S., Bensaddek L., Raynaud S., Gillet F., Mesnard F., Fliniaux M-A. (2002). An HPLC procedure for the quantification of an hydrosecoisolariciresinol. Application to the evaluation of flax lignan content. *Plant Physiol. Biochem.,* vol. 40, pp. 225–229. https://doi.org/10.1016/S0981-9428(02)01368-2

11 Egizabal A., Zuloaga O., Etxebarria N., Fernández L.A., Madariaga J.M. (1998). Comparison of microwave-assisted extraction and Soxhlet extraction for phenols in soil samples using experimental designs. *The Analyst,* vol. 123, no. 8, pp. 1679-1684. https://doi.org/10.1039/a802117a.

12 Stasevich O.V., Mihalenok S.G., Kurchenko V.P. (2009) Antioksidantnye svojstva sekoizolaricirezinoladigljukozida i ego proizvodnyh [Effective method for obtaining lignan-containing extract from flaxseeds] *Trudy BGU*, vol 4, no. 1. pp. 154-160. https://elib.belstu.by/handle/123456789/32475

13 Fliniaux O., Grand E., Lamblin F., Bensaddek L., Christen Ph., Kovensky J., Fliniaux M-A., and Mesnard F. (2007) Microwave-assisted extraction of the main phenolic compounds in flaxseed. *Phytochem. Analysis*, vol. 18, no. 4, pp. 275-282. http://dx.doi.org/10.1002/pca.973

14 Zang W., Xu S. (2007). Microwave-assisted extraction of secoisolariciresinol diglucoside from flaxseed hull. *J. Sci. Food Agric.*, vol. 87, pp. 1455–1462. https://doi.org/10.1002/jsfa.2793

15 RMG 24027.2 – 1980. Medicinal plant raw materials. Methods for determining humidity, ash content, extractive and tannic substances, and essential oil. [GSI. Syre lekarstvennoe rastitelnoe. Metody opredeleniia vlazhnosti soderzhaniia zoly ekstraktivnykh i dubilnykh veshchestv efirnogo masla.]. Moscow, Russia, 1981.

16 Yuk H.J., Lee J.H., Curtis-Long M.J., Lee J.W., Kim Y.S., Ryu H.W., Park Ch.G., Jeong T.S., Park K.H. (2011) The most abundant polyphenol of soy leaves, coumestrol, displays potent α–glucosidase inhibitory activity. *Food Chem.*, vol. 126, pp. 1057-1063. https://doi.org/10.1016/j.foodchem.2010.11.125

17 Deepika K., Kumar M-V.S-M., Radhika, T-S.D., Rajagopal S.V. (2016) Studies on isolation, purification and structure elucidation of chemical constituents from methanolic flower extract of blepharismolluginifolia pers. And their biological activities. *Int. J. Of Pharm. Sci. And Res.*, vol. 7, pp. 4893-4904. https://doi.org/10.13040/IJPSR.0975-8232.7(12).4893-04

18 Dey B., Bhunia S.K., Maity K.K., Patra S., Mandal S., Maiti S., Maiti T.K., Sikdar S.R., Islam S.S. (2012) Glucans of Pleurotusflorida blue variant: Isolation, purification, characterization and immunological studies. *Int. J. Biol. Macromol.,* vol. 50, no. 3, pp. 591-597. https://doi.org/10.1016/j.ijbiomac.2012.01.031

19 Konar N., Poyrazoglu E.N., Demir K., Artik N. (2012) Effect of different sample preparation methods on isoflavone, lignin, coumestan and

flavonoid contents of various vegetables determined by triple quadrupole LC-MS/MS. *J Food Compost Anal.,* vol. 26, pp. 26-35. https://doi.org/10.1016/j.jfca.2012.01.002

20 Kurkin V.A., Klimova L.D., Velmiaikina E.I., Pervushkin S.V., ZHelonkin N.N., Ber O.L., Sokhina A.A. (2014) Issledovanie po sozdaniiu immunomoduliruiushchego lekarstvennogo sredstva tabletok s ekstraktom ekhinatsei [Research on the creation of an immunomodulatory drug tablets with echinacea extract]. *Izvestiia Samarskogo nauchnogo tsentra Rossiiskoi akademii nauk. – Bulletin of the Samara Scientific Center of the Russian Academy of Sciences*, vol. 16, no. 5(2), pp. 990-994.

Islam A., Islam M.S., Uddin M.N., Hasan M.M.I., Akanda M.R. (2020) The potential health benefits of the isoflavone glycoside genistin. *Arch. of Pharm. Res.,* vol. 43, no. 4, pp. 395-408. https://doi.org/10.1007/s12272-020-01233-2