Research Journal of Pharmacognosy (RJP) 9(2), 2022: 37–44
Received: 9 Sep 2021
Accepted: 4 Jan 2022
Published online: 10 Jan 2022
DOI: 10.22127/RJP.2021.304041.1788

Original article

In Vitro Anti-Diabetic and Anti-Oxidant Activities of *Geum* Species from Iran

Avishan Farzaneh[1], Mohammad Ali Faramarzi[2], Mohammad Reza Delnavazi[1], Hamid Reza Monsef-Esfahani[1], Hamid Reza Adhami[1*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Abstract

Background and objectives: Medicinal plants have been considered as important sources of potent free radical scavengers as well as digestive enzymes inhibitors. Several plants are used in traditional and modern medicine for their biological properties such as anti-oxidant and anti-diabetic activity. The aim of this study was to investigate the anti-diabetic and anti-oxidant activities of roots and aerial parts from three of five native Iranian herbaceaous *Geum* species, including *G. iranicum*, *G. kokanicum* and *G. urbanum*. The *Geum* species and their bio-active substances are getting a lot of attention due to their various biological effects, such as anti-oxidant, anti-diabetic, anti-tumor and anti-microbial activities. **Methods:** The anti-diabetic activity of the *Geum* species was evaluated via α-glucosidase and α-amylase inhibition assays. The anti-oxidant effect was analyzed using the free radical scavenging method and the total phenolics content was determined via a colorimetric assay. **Results:** Based on our study, all the examined species revealed moderate to high anti-diabetic and anti-oxidant effects. *Geum kokanicum* roots showed the highest α-glucosidase inhibition activity (91.0%±1.7) at the concentration of 500 µg/mL and DPPH radical scavenging potential (IC_{50}: 11.6±0.5 µg/mL). **Conclusion:** The results demonstrated in-vitro anti-diabetic property of *G. kokanicum*, so detailed investigation to isolate the active compounds is suggested.

Keywords: *α*-glucosidase; *α*- amylase; antioxidant; *Geum*; phenolic content

Citation: Farzaneh A, Faramarzi MA, Delnavazi MR, Monsef-Esfahani HR, Adhami HR. In vitro anti-diabetic and anti-oxidant activities of *Geum* species from Iran. Res J Pharmacogn. 2022; 9(2): 37–44.

Introduction

Diabetes mellitus is a chronic metabolic disease, characterized by the increased blood sugar, caused due to insulin deficiency. Diabetes is divided into type 1 and type 2 and 90% of diabetic cases are accounted to be type 2. The International Diabetes Federation predicts over 435 million people worldwide will be struggling with type 2 by 2030 if no effective prevention and control programs are implemented. In addition, this disease will become the seventh cause of death by 2030 [1-3].

Current available synthetic medications such as acarbose, miglitol, and voglibose have been widely used for treatment of type 2 diabetes. Because of various gastrointestinal problems such as abdominal discomfort, bloating, flatulence and diarrhea of the mentioned drugs, researchers have been encouraged to purify natural compounds with protective effects and to design novel anti-diabetic drugs [4–6].

Intestinal α-glucosidase and pancreatic α-amylase are the key enzymes in carbohydrate digestion. The inhibition of these enzymes efficiently delays the overall carbohydrate digestion time and therefore reduces the rate of glucose absorption resulting in controlled postprandial

*Corresponding author: hr-adhami@tums.ac.ir

hyperglycemia for the type 2 diabetes patients [7]. The production of free radicals can cause several pathological disturbances such as diabetes mellitus, heart diseases and cancer in human body. Plants synthesize a wide range of non-enzymatic anti-oxidants to protect them from free radicals' reactions [8]. In addition, phytochemical components such as alkaloids, terpenoids, polysaccharides and phenolic compounds have been reported for their anti-oxidant and anti-diabetic activities [9,10].

The *Geum* genus (Rosaceae) belongs to rosoideae subfamily and consists of about 70 plant species mainly perennial herbaceous, which are distributed in temperate and arctic regions of the world [11,12]. The Iranian species are distributed in north, west north, east and central parts of the country at the altitudes of about 200 to 3400 m [13]. Since ancient time, in folk medicine, a decoction of the root and rhizome of *Geum* species has been applied for treatment of diarrhea, dysentery, dyspepsia and gastroenteritis. Also, the aerial parts infusion was used in cases of leucorrhoea, hemorrhages and fevers [14-17].

The *Geum* species and their chemicals are getting a lot of attention due to their various biological effects, including anti-oxidant, anti-tumor, anti-viral, anti-microbial, angiogenic and myogenic effects [18–22]. Tri-terpenoids and tannins have been reported as the major bioactive compounds in *Geum* species [23].

The recent studies demonstrated notable activities of the plants from Rosidae subfamily on α-glucosidase and α-amylase inhibition. *Geum urbanum* (common name: avens) has been used in some herbal anti-diabetic preparations [24–29]. In addition, the roots and aerial parts of some *Geum* species such as *G. urbanum* [30–32], *G. rivale* [30], *G. japonicum* [33], and *G. quellyon* [22] have shown potent anti-oxidant effects.

The present study investigated the anti-diabetic activities as well as anti-oxidant potentials of three *Geum* species (*Geum urbanum* L., *Geum kokanicum* Regel et Schmath & Regel and *Geum iranicum* khatamsaz) which are native to Iran. Despite some investigations of phytochemical and biological properties on *G. urbanum*, there are very limited studies available on two other species due to their dedicated places of growth [11,12].

Material and Methods

Ethical considerations

This study was approved by the Ethical Committee of Tehran University of Medical Sciences (IR. TUMS. TIPS.REC. 1397. 102; date: 09.12.2018).

Chemicals

All chemicals including α-glucosidase (*Saccharomyces cerevisiae*, EC3.2.1.20, 20 U/mg), α-amylase (*Aspergillus oryzae*), *p*-nitrophenyl glucopyranoside (PNPG), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), butylated hydroxyltoluene (BHT), 3,5-dinitrosalicylic acid (DNSA), potassium phosphate buffer (KH_2PO_4 +K_2HPO_4), Dimethyl sulfoxide (DMSO) and gallic acid were purchased from Sigma (USA). The solvents were of analytical grades.

Plant material

Geum kokanicum and *G. iranicum* were collected from northern part of Khorasan province (Sorkheh cheshmeh and Imamzadeh Zakaria, respectively) and *G. urbanum* was obtained from Masuleh, Gilan province during the flowering times in June 2018. *Geum iranicum* and *G. urbanum* were identified and deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences with the voucher specimens of 6714 THE and 6904 THE, respectively. *Geum kokanicum* was identified and deposited at the Herbarium of School of Science, University of Tehran with the voucher number of 48684 TUH. The samples were separated by root and aerial parts, dried at room temperature under the shade and ground to fine powders.

Extraction

The extraction was carried out by suspension of 1 g dried powder of each sample in 10 mL MeOH. Then it was settled in an ultrasonic water bath for 20 min at 40 °C and centrifuged for 10 min at 1008 RCF. The mixture was filtered and the residue was extracted once more with the same method. The filtrate was evaporated under reduced pressure and the dried extract was kept at 4 °C for the next step.

Fractionation

Based on bio-assays, the selected extract (*G. kokanicum* root extract), was fractionated by liquid-liquid chromatography with chloroform ($CHCl_3$) followed by ethyl acetate (EtOAc) to obtain three fractions with the different polarities. First, 2 kg of the dried root powder was macerated in methanol and stirred for 48 h by a

mechanical stirrer. The solvent was refreshed and the extraction procedure was repeated two times more. Then the filtrate was evaporated under reduced pressure at 40 °C. The methanol residue (130 g) was suspended in 2 L water and successively partitioned three times with 2 L of $CHCl_3$ followed by EtOAc with the same procedure. The extracts were filtered and subsequently evaporated using a rotary evaporator under reduced pressure at 40 °C.

In-vitro α-glucosidase inhibitory assay

The α-glucosidase inhibitory activity of the samples was evaluated according to a published study [34]. A mixture of *α*-glucosidase solution (1 U/mL, 20 µL) in potassium phosphate buffer (pH 6.8, 50 mM), different concentration of the extracts in 10% DMSO (20 µL) and 135 µL buffer were added to a 96-well plate and incubated at 37 °C. After 10 min, 25 µL PNPG (4 mM in buffer) was added to the reaction mixture and it was incubated again at 37 °C for 20 min. Finally, the absorbance was measured at 405 nm by a spectrophotometer (Gen5 Power wave xs2, BioTek, USA). DMSO (10%) and acarbose were used as the control and standard, respectively. The percentage of inhibition for each sample was assessed according the following equation:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control})] \times 100$$

$Abs_{control}$ = absorbance of control, Abs_{sample} = absorbance of the sample.

In-vitro α-amylase inhibitory assay

The α-amylase inhibitory assay was carried out based on a previous method with some modifications [35,36]. Briefly, 100 µL of different plant extracts (in 10% DMSO) were added to 50 µL starch solution containing 0.5% w/v starch in phosphate buffer (pH 7.0, 50 mM). The reaction was initiated by adding 50 µL α-amylase (2.5 U/mL) to the mixture and it was incubated at 37 °C with a shaker incubator. After 30 min, 200 µL of the reagent solution (44 mM DNSA, 106 mM potassium sodium tartrate, 40 mM NaOH) was added to the reaction mixture and heated to 100 °C for 10 min. The enzymatic hydrolysis of substrate was monitored by the released amount of 3-amino, 5-nitrosalicylic using microplate reader at 540 nm. The standard curve was obtained using different concentrations of maltose. Individual blanks were prepared for correcting the background absorbance, where the enzyme solution was replaced with 0.1 M phosphate buffer. Negative controls were conducted in an identical manner replacing the plant extracts with DMSO. Acarbose was used as standard. All experiments were carried out triplicated. The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample})/Abs_{control})] \times 100$$

$Abs_{control}$ = absorbance of control, Abs_{sample} = absorbance of the sample

Total phenolics content

The total phenolics content of the *Geum* species were defined using the method described by Velioglu et al. with slight modifications [37]. Briefly, 200 µL (0.2 mg/mL) of methanol extract from each sample was mixed with 1.5 mL of diluted Folin-Ciocalteu reagent (0.1 % V/V) in water and shaken vigorously for 5 min. After adding 1.5 mL aqueous solution of Na_2CO_3 (75 mg/mL), the solution was incubated for 2 h at room temperature and the absorbance was measured at 760 nm. Six different concentrations (6.25 to 200 µg/mL) of gallic acid as a reference standard were used to obtain the trend line.

Free radical scavenging activity

Free radical scavenging activity of the samples was determined using a published method [38]. Five mL of different concentrations (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200 µg/mL) from each extract in methanol were added to 5 mL of DPPH solution (0.08 mg/mL). The mixture was shaken properly and incubated in a dark place at room temperature for 30 min. Then, the absorbance was measured at 517 nm with a Shimadzu UV spectrophotometer (Japan). BHT was used as the standard and each experiment was conducted three times. The percentage of scavenging was calculated using the following formula:

$$(\%) \text{ scavenging activity percentage} = [(Abs_{blank} - Abs_{sample})/ (Abs_{blank})] \times 100$$

Abs_{blank}= Absorbance of blank, Abs_{sample} = Absorbance of the sample.

The IC_{50} values were determined using different concentrations of the samples.

Statistical analysis

The anti-diabetic and anti-oxidant activity tests were performed in triplicate. The results have been presented as mean±SD of three measurements. The obtained data were subjected to one-way analysis of variance (ANOVA) with IBM SPSS statistics 26.0 software (SPSS Inc., USA). Homogeneous groups were determined using Tukey's test at a significance level of ($p<0.05$).

Results and Discussion

The result revealed the root extract of *G. kokanicum* as the most potent α-glucosidase inhibitor with 91.0% of inhibition at the concentration of 500 µg/mL (Table 1). However, the aerial parts of *G. urbanum* and *G. kokanicum* as well as the root of *G. urbanum* showed moderate to good inhibitory activities by inhibition of 60.0±3.5, 72.0±2.0 and 84.0±2.6%, respectively.

Regarding the α-amylase inhibitory activity, *G. urbanum* aerial parts demonstrated the highest activity with IC_{50} value of 72.0 ± 3.1%. In addition, both aerial parts and roots of *G. kokanicum* were found to be highly active toward α-amylase by inhibitions of 61.0 ± 2.6% and 63.0 ± 2.0%, respectively (Table 1).

The total phenolic content was expressed as gallic acid equivalent (µg GAE/mg extract). The equation was obtained as $y= 0.0071x+0.0297$ ($R^2= 0.998$).

Among the three *Geum* species, the total phenolic content of *G. iranicum* root was the highest. This assay examines reduction of radical solution in the presence of a hydrogen-donating antioxidant (Table 2)

Free radical scavenging activities of the *Geum* extracts were analyzed using DPPH reagent. BHT was used as the positive control (IC_{50}= 36.3±4.3 µg/mL); the result have been presented in Table 2. The results indicated that the root extract of *G. kokanicum* and *G. iranicum* presented the highest activities, respectively. There was a significant difference between *G. urbanum* aerial parts (IC_{50}= 163.6±20.1 µg/mL) in comparison with BHT and other tested extracts ($p<0.05$).

According to the results, a detailed investigation was carried out on the *G. kokanicum* root extract. The methanol extract was fractionated stepwise by liquid-liquid extraction with chloroform and ethyl acetate for further assessment on inhibition and anti-oxidant activity. The extraction yields were 5 g for chloroform, 15 g for ethyl acetate and 110 g for methanol extracts, respectively. The results showed potent α-glucosidase inhibitory activities for all fractions. The ethyl acetate fraction showed the highest phenolics content and promising activity on DPPH reagent (Table 3).

Table 1. Inhibitory activity of methanol extracts of *Geum* species at the concentration of 500 µg/mL in α-glucosidase and α-amylase assays

Plant name	Parts used	α-Glucosidase inhibition (%)	α-Amylase inhibition (%)
Geum iranicum	Root	54.0 ± 3.6	45.0 ± 1.7
Geum iranicum	Aerial parts	56.0 ± 2.6	52.0 ± 3.6
Geum kokanicum	Root	91.0 ± 1.7	63.0 ± 2.0
Geum kokanicum	Aerial parts	72.0 ± 2.0	61.0 ± 2.6
Geum urbanum	Root	84.0 ± 2.6	18.0 ± 1.7
Geum urbanum	Aerial parts	60.0 ± 3.5	72.0 ± 3.1

Standard inhibitor (acarbose); IC_{50} values for α-glucosidase and α-amylase inhibitory activities were 484.2 ± 10.4 µg/mL and 69.7 ± 3.3 µg/mL respectively.

Table 2. Phenolics contents and DPPH radical scavenging potentials of *Geum* spp methanol extracts

Sample	Parts used	Total phenolics *	IC_{50} DPPH **
Geum iranicum	Aerial parts	121.6 ± 5.7^{b}	33.1 ± 3.7^{a}
Geum iranicum	Root	190.8 ± 6.2^{a}	18.7 ± 0.9^{a}
Geum kokanicum	Aerial parts	140.4 ± 3.2^{b}	26.5 ± 0.2^{a}
Geum kokanicum	Root	73.5 ± 0.9^{c}	11.6 ± 0.5^{a}
Geum urbanum	Aerial parts	10.2 ± 0.7^{d}	163.6 ± 20.1^{b}
Geum urbanum	Root	65.4 ± 15.1^{c}	22.8 ± 1.6^{a}
BHT	-	-	36.3 ± 4.3^{a}

Data are expressed as means±SD (n = 3) for each group. Mean values followed by different superscript lowercase letters (a, b, c, d) report significant differences between different extracts at $p<0.05$, according to Tukey's test. *µg of gallic acid equivalents/mg of dried extracts ± SD; **IC_{50} µg/mL±SD

Several studies have shown that phenolic constituents in medicinal plants greatly contribute to the anti-oxidant activity. Also, they have potential of starch digestion enzymes inhibition [30,39]. Co-administration of these compounds with synthetic inhibitors can reduce the effective dose for management of postprandial glycaemia [40].

Table 3. Phenolics contents, DPPH radical scavenging activity and α-glucosidase inhibitory activity at 500 µg/mL of different fractions of *Geum kokanicum* root

Extract	α- Glucosidase inhibition (%)	Total phenolics [a]	IC_{50} DPPH [b]
Chloroform	98.0 ± 1.0	49.2 ± 1.4	73.6 ±28.3
Ethyl acetate	94.0 ± 1.7	121.7 ± 6.1	6.4 ± 0.1
Methanol	95.0 ± 3.6	66.2 ± 0.4	13.8 ± 2.5

Data are expressed as means±SD; [a] µg of gallic acid equivalents/mg of dried extracts ± SD; [b]IC_{50} µg/mL±SD

From pharmacological standpoint, phenolic compounds have shown a strong and positive correlation in defense responses such as anti-inflammatory, anti-aging, anti-oxidant and anti-proliferative effects. They can also reduce the incidence of some chronic diseases, for instance diabetes, cardiovascular diseases and cancers due to management of oxidative stress [10].

Previous reports have demonstrated the anti-oxidant effect of various *Geum* species and some of Rosaceae medicinal plants [30]. In 2017, the ethyl acetate fraction of root and aerial parts from *G. urbanum* exhibited the promising radical scavenging potential with EC_{50} value of 0.8 and 1.5 µg/mL, respectively, which attributed to high level of phenolic compounds [31,41]. According to the reports, the hydro-methanolic extract of *G. rivale* root was found as a rich source for polyphenolic contents such as ellagic acid (2.68 g/kg) [30,42]. In 2015, the ethanol and aqueous root extracts of *G. urbanum* have shown remarkable effects against DPPH by IC_{50} values as 1.3±0.1 µg/mL and 7.8±0.5 µg/mL, respectively [32].

Some studies also described that the ellagitannins might be the active components for hypoglycemic activities [43]. The results of the present investigation suggest that the polyphenolic compounds in *Geum* extracts might be responsible for anti-oxidant and anti-diabetic effects. These polyphenol compounds have shown high activities against DPPH, O (2)(-), •OH and NO as well as good inhibition on digestive enzymes such as α-glucosidase by preventing from hydrogen binding for α- (1,4)-glucosidic hydrolysis and α-amylase by slowing the digestion and absorption of carbohydrates and viscous water-soluble dietary fibers [44–46].

Conclusion

According to our knowledge, this is the first investigation on α-glucosidase and α-amylase inhibitory activity of *Geum* species. The outcomes of this study showed the three examined species have moderate to high anti-oxidant and anti-diabetic activities which can support the indication of this genus extracts in some anti-diabetic herbal preparations [26–29].

Based on the results (Table 3), the ethyl acetate fraction from *G kokanicum* root exhibited the highest anti-oxidant and α-glucosidase inhibition activities which can present it as a suitable candidate for further studies on isolation and characterization of bio-active components with anti-diabetic activities.

Acknowledgments

The authors appreciate the deputy of research and innovation at Tehran University of Medical Sciences for grant supporting of this study with the project number of 98-01-33-41912.

Author contributions

Avishan Farzaneh performed the extraction and isolation and analyzed the experiment; Mohammad Ali Farzamarzi performed the enzymes assay; Hamidreza Monsef-Esfahani collected the plants and co-supervised the study; Mohammad-Reza Delnavazi designed; analyzed the anti-oxidant assay and Hamid-Reza Adhami supervised and managed the project. All of the contributors were involved in manuscript writing for their specific sections.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Cañizo-Gómez FJ. Update on the treatment of type 2 diabetes mellitus. *World J Diabetes*. 2016; 7(17): 354–395.

[2] Jain S, Saraf S. Type 2 diabetes mellitus, its global prevalence and therapeutic strategies. *Diabetes Metab Syndr*. 2010; 4(1): 48–56.

[3] Word Health Organization: Diabetes overview [Accessed 2020]. Available from: https://www.who.int/health-topics/diabetes.
[4] Toeller M. α-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. *Eur J Clin Investig.* 1994; 24(S3): 31–35.
[5] Ross SA, Gulve EA, Wang M. Chemistry and biochemistry of type 2 diabetes. *Chem Rev*. 2004; 104(3): 1255–1282.
[6] Hollander P. Safety profile of acarbose, an α-glucosidase inhibitor. *Drugs*. 1992; 44(3): 47–53.
[7] Bischoff H. The mechanism of alpha-glucosidase inhibition in the management of diabetes. *Clin Investig Med.* 1995; 18(4): 303–311.
[8] Asgarpanah J, Motamed SM, Farzaneh A, Ghanizadeh B, Tomraee S. Antioxidant activity and total phenolic and flavonoid content of *Astragalus squarrosus* Bunge. *Afr J Biotechnol*. 2011; 10(82): 19176–19180.
[9] Carocho M, Ferreira IC. A review on antioxidants, pro oxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol*. 2013; 51: 15–25.
[10] Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, Kong M, Li L, Zhang Q, Liu Y, Chen H. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*. 2016; 21(10): 1–19.
[11] Mozaffarian V. A dictionary of Iranian plant names. Tehran: Farhang-e- Moaser, 2003.
[12] Khatamsaz K. Flora of Iran. No. 6: Rosaceae. Karaj: Research Institute of Forest and Rangelands, 1992.
[13] Faghir MB, Armudian MM, Shahi SR. Micro-macro morphology of the genus *Geum* L. (Rosaceae) in Iran and their taxonomic significance. *Iran J Bot*. 2015; 21(2): 103–117.
[14] Menković N, Šavikin K, Tasić S, Zdunić G, Stešević D, Milosavljević S, Vincek D. Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). *J Ethnopharmacol.* 2011; 133(1): 97–107
[15] Granica S, Kłębowska A, Kosiński M, Piwowarski JP, Dudek MK, Kaźmierski S, Kiss AK. Effects of *Geum urbanum* L. root extracts and its constituents on polymorphonuclear leucocytes functions. significance in periodontal diseases. *J Ethnopharmacol*. 2016; 188: 1–12.
[16] Abotorabi H. The ethnobotany and phytochemistry of plant distributed in the northern part of Iran in the Roeen and Esfarayen regions. Pharm.D. thesis, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, 2002.
[17] Vogl S, Picker P, Mihaly-Bison J, Fakhrudin N, Atanasov AG, Heiss EH, Wawrosch C, Reznicek G, Dirsch VM, Saukel J, Kopp B. Ethnopharmacological in vitro studies on Austria's folk medicine-an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *J Ethnopharmacol*. 2013; 149(3): 750–771.
[18] Li M, Yu CM, Cheng L, Wang M, Gu X, Lee KH, Wang T, Sung YT, Sanderson JE. Repair of infarcted myocardium by an extract of *Geum japonicum* with dual effects on angiogenesis and myogenesis. *Clin Chem.* 2006; 52(8): 1460–1468.
[19] Panizzi L, Catalano S, Miarelli C, Cioni P, Campeol E. In vitro antimicrobial activity of extracts and isolated constituents of *Geum rivale*. *Phytother Res.* 2000; 14(7): 561–563.
[20] Kurokawa M, Hozumi T, Basnet P, Nakano M, Kadota S, Namba T, Kawana T, Shiraki K. Purification and characterization of eugeniin as an anti-herpesvirus compound from *Geum japonicum* and *Syzygium aromaticum*. *J Pharmacol Exp Ther*. 1998; 284(2): 728–735.
[21] Khorramizadeh MR, Shahverdi AR, Saadat F, Monsef-Esfahani HR. Inhibitory effect of *Geum kokanicum* roots on matrix metalloproteinases expression. *Pharm Biol*. 2006; 44(4): 266–270.
[22] Russo A, Cardile V, Lombardo L, Vanella L, Vanella A, Garbarino JA. Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* sweet roots in human tumor cell lines. *J Ethnopharmacol*. 2005; 100(3): 323–332.
[23] Cheng XR, Jin HZ, Qin JJ, Fu JJ, Zhang WD. Chemical constituents of plants from the genus *Geum*. *Chem Biodivers*. 2011; 8(2): 203–222.
[24] da Silva Pinto M, de Carvalho JE, Lajolo FM, Genovese MI, Shetty K. Evaluation of antiproliferative, anti-type 2 diabetes, and antihypertension potentials of ellagitannins from strawberries (*Fragaria× ananassa*

Duch.) using in vitro models. *J Med Food.* 2010; 13(5): 1027–1035.

[25] Tomczyk M, Latté KP. *Potentilla*- a review of its phytochemical and pharmacological profile. *J Ethnopharmacol.* 2009; 122(2): 184–204.

[26] Wichtl M. Herbal drugs and phytopharmaceuticals. 3rd ed. Stuttgart: Medpharm, 2004.

[27] Kuenzle J. Herbs and weeds. A practical booklet on Medicinal herbs. Ticino: Krauterpfarrer Kunzle AG, 1967.

[28] Madic V, Jovanovic J, Stojilkovic A, Vasiljevic P. Evaluation of cytotoxicity of 'anti-diabetic' herbal preparation and five medicinal plants: an *Allium cepa* assay. *Biol Nyssana*. 2017; 8(2): 151–158.

[29] Duke J. Handbook of medicinal herbs. 2nd ed. Florida: CRC Press LLC, 2002.

[30] Owczarek AL, Gudej J, Olszewska MA. Antioxidant activity of *Geum rivale* L. and *Geum urbanum* L. *Acta Pol Pharm*. 2015; 72(6): 1239–1244.

[31] Dimitrova L, Zaharieva MM, Popova M, Kostadinova N, Tsvetkova I, Bankova V, Najdenski H. Antimicrobial and antioxidant potential of different solvent extracts of the medicinal plant *Geum urbanum* L. *Chem Cent J*. 2017; 11(1): 1–11.

[32] Paun G, Neagu E, Albu C, Radu GL. Inhibitory potential of some Romanian medicinal plants against enzymes linked to neurodegenerative diseases and their antioxidant activity. *Pharmacogn Mag.* 2015; 11(1): 110–116.

[33] Kim JB, Kim JB, Cho KJ, König GM, Wright AD. Antioxidant activity of 3, 4, 5-trihydroxybenzaldehyde isolated from *Geum japonicum. J Food Drug Anal*. 2006; 14(2): 190–193.

[34] Saeedi M, Raeisi-Nafchi M, Sobhani S, Mirfazli SS, Zardkanlou M, Mojtabavi S, Faramarzi MA, Akbarzadeh T. Synthesis of 4-alkylaminoimidazo [1,2-a] pyridines linked to carbamate moiety as potent α-glucosidase inhibitors. *Mol Divers*. 2020; 25(4): 2399–2409.

[35] Ardeshirlarijani E, Namazi N, Jalili RB, Saeedi M, Imanparast S, Adhami HR, Faramarzi MA, Ayati MH, Mahdavi M, Larijani B. Potential anti-obesity effects of some medicinal herb: in vitro α-amylase, α-glucosidase and lipase inhibitory activity. *Int Biol Biomed J*. 2019; 5(2): 1–8

[36] Wickramaratne MN, Punchihewa JC, Wickramaratne DB. In-vitro alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. *BMC Complement Altern Med*. 2016; 16(1): 1–5.

[37] Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agric Food Chem*. 1998; 46(10): 4113–4117.

[38] Sarikurkcu C. Antioxidant activities of solvent extracts from endemic *Cyclamen mirabile* Hildebr tubers and leaves. *Afr J Biotechnol*. 2011; 10(13): 831–839.

[39] Agatonovic-Kustrin S, Kustrin E, Gegechkori V, Morton DW. Bioassay-guided identification of α-amylase inhibitors in herbal extracts. *J Chromatogr A.* 2020; Article ID 460970.

[40] Ali Asgar MD. Anti-diabetic potential of phenolic compounds: a review. *Int J Food Prop*. 2013; 16(1): 91–103.

[41] Schmitt M, Magid AA, Nuzillard JM, Hubert J, Etique N, Duca L, Voutquenne-Nazabadioko L. Investigation of antioxidant and elastase inhibitory activities of *Geum urbanum* aerial parts, chemical characterization of extracts guided by chemical and biological assays. *Nat Prod Commun*. 2020; 15(3): 1–9.

[42] Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K. Antioxidant tannins from Rosaceae plant roots. *Food Chem*. 2007; 100(2): 579–583.

[43] Yuan T, Ding Y, Wan C, Li L, Xu J, Liu K, Slitt A, Ferreira D, Khan IA, Seeram NP. Antidiabetic ellagitannins from pomegranate flowers: inhibition of α-glucosidase and lipogenic gene expression. *Org Lett.* 2012; 14(20): 5358–5361.

[44] Nakagawa T, Yokozawa T. Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem Toxicol.* 2002; 40(12): 1745–1750.

[45] de Melo EB, da Silveira Gomes A, Carvalho I. α-and β-Glucosidase inhibitors: chemical structure and biological activity. *Tetrahedron.* 2006; 62(44): 10277–10302.

[46] Oyedemi SO, Oyedemi BO, Ijeh II, Ohanyerem PE, Coopoosamy RM, Aiyegoro OA. Alpha-amylase inhibition and antioxidative capacity of some antidiabetic plants used by the traditional healers in

Southeastern Nigeria. *Sci World J*. 2017; Article ID 3592491.

Abbreviations

CN: common name; $CHCl_3$: chloroform; MeOH: methanol; EtOAc: ethyl acetate; PNPG: p-nitro phenyl glucopyranoside; DNSA: 3, 5-dinitrosalicylic acid; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; BHT: butylated hydroxytoluene; Abs: absorbance; DMSO: dimethyl sulfoxide; NaOH: sodium hydroxide; GAE: gallic acid equivalent; Na_2CO_3: sodium carbonate; NO: nitric oxide