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α-Glucosidase Inhibitors from Fruits of *Rosa canina* L.

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Abstract: As part of ongoing project on screening plants used in Iranian folk medicine to treatment of diabetes, α-glucosidase inhibition activities of *Rosa canina* extracts have been tested. The acetone extract of the plant exhibited significant inhibition with IC_{50} value of 0.3 µg/ml. The enzyme based assay guided fractionation of the acetone extract led to the isolation of daucosterol (**1**) and D-glucono-1,4-lactone (**2**), as highly active α-glucosidase inhibitors. Their structures were determined by ^{1}H- and ^{13}C-NMR spectroscopic evidences. The IC_{50} values of daucosterol and D-glucono-1,4-lactone on yeast α-glucosidase were 13.3 and 6.5 µM, respectively, while IC_{50} of acarbose was 16.1 µM, as a positive control. The Lineweaver-Burk plots analysis elucidated that both of the compounds inhibited the enzyme competitively. The study suggests that isolated compounds can be good candidates as α-glucosidase inhibitors and provide strong rationale for more *in vivo* studies.

Keywords: *anti-diabetic; α-glucosidase; enzyme inhibition; Rosa canina L.; daucosterol; D-glucono-1,4-lactone.*

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose level. Chronic diabetes conditions include insulin-dependent diabetes (type 1) and non-insulin-dependent diabetes (type 2). Of the diabetics, about 90 percent have type 2 diabetes [1] and finding an effective treatment for this type is not easy because of its non-insulin-dependent nature [2]. It has been demonstrated that preservation of blood glucose level in healthy range is very important for treating type 2 diabetes [3]. α-Glucosidase is a key enzyme in the digestive system, which is responsible for the final step in the hydrolysis of carbohydrates that are the major constituents of human diet [4]. Therefore, use of α-glucosidase inhibitors can delay the absorbance of carbohydrates in the gut [5]. Chemical drugs such as acarbose, miglitol and voglibose are used as α-glucosidase inhibitors, but due to their deleterious side effects such as abdominal distention, meteorism, bloating, and diarrhea, the

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search for more effective and safer inhibitors has continued [6]. Many of these efforts have involved searches for α-glucosidase inhibitors from natural sources, especially medicinal plants [5, 7-10].

Rosa canina L. (Rosaceae), also known as dog rose, is a perennial shrub that has a height of about 2-3 m [11]. Rose hips, the pseudo-fruits of *Rosa canina*, are used in food, tea and medicine form in many cultures [11, 12]. As an herbal dietary supplement, Iranian people use rose hips to prepare jam and pickle. As an herbal remedy, it has been demonstrated that the use of rose hips can be useful in prevention and treatment of various ailments such as cold, influenza, arthritis, rheumatism, gout, sciatica, gastro intestinal disorders, gastric ulcer and gall and kidney stones [13-16]. In addition, there are several reports about biological activities of *Rose canina* L., such as antioxidative [17], anti-inflammatory and anti-nociceptive [15], anti-carcinogenic [18], anti-ulcerogenic [16] and antimicrobial activities [19].

As part of ongoing project on screening of plants that are used as anti-diabetes in Iranian folk medicine [20], the inhibitory effect of *Rosa canina* fruit extracts on α-glucosidase were measured, and acetone extract possessed significant activity with IC_{50} value of 0.3 µg/ml. This high potency prompted us to investigate *Rosa canina* further.

Despite the use of *Rosa canina* in traditional medicine against diabetes, there are very few studies on the plant in relation to its anti-diabetic effect, and no study has been carried out on the characterization of principle compounds that are responsible for anti-diabetic effect. It has been shown that ethanol extract of *Rosa canina* fruits exert a remarkable hypoglycemic effect in streptozotocin (STZ) induced diabetic rats. Furthermore, the remaining H_2O fraction of the ethanol extract exhibited significant anti-diabetic activity in diabetic rats [21]. *Rosa damascena* is another member of Rosaceae family, and methanol extract of its flowers was compared to the α-glucosidase inhibitor, acarbose in normal and diabetic rats. The results showed that *Rosa damascena* extract has a strong inhibitory effect on α-glucosidase [22]. The present study was designed to evaluate the inhibitory activities of various extracts of *Rosa canina* fruits and isolation and identification of the active constituent(s) through bioassay-guided fractionation procedures.

2. Materials and Methods

2.1. Chemicals

α-Glucosidase type I from Baker Yeast (EC 3.2.1.20), *p*-nitrophenyl-α-D-glucopyranose (pNPG) and acarbose were obtained from Sigma-Aldrich (Paris, France). Silica gel (230 - 400 mesh) for column chromatography (CC), molybdatophosphoric acid hydrate and thin layer chromatography plates (TLC) were purchased from Merck. All the other solvents and reagents were obtained from local commercial sources. All solvents were distilled before use.

2.2 General

All TLC spots were visualized under UV light (λ = 254 and 365 nm) after staining with a 3 % (w/v) molybdatophosphoric acid hydrate solution in ethanol followed by heating. The ^{1}H, ^{13}C spectra were acquired on Bruker DRX 500 and Avance III 500 spectrometers, using the residual DMSO signal (δ H 2.51, δ C 39.5) as reference. The absorbance of the enzyme-assay reaction mixture was measured by a BioTek microplate reader (XS2).

2.3. Plant Material

Dog rose (*Rosa canina* L.) fruits were collected from Kandeloos village, Noshahr Mountains, Mazandaran province, Iran, in July 2010. A small amount of the raw herb sample was deposited as voucher specimen at the herbarium of Plantation Industries & Processing of Medicinal Plants, Soha Jissa Company, with voucher specimen no. 63.

2.4. Extraction and isolation

The air-dried fruits of the plant were powdered using an iron pestle and mortar. The ground samples (1.6 kg) were extracted with 4 L *n*-hexane, ethyl acetate, acetone and methanol, respectively,

by maceration at room temperature. Solvent was replenished every 24 h for 5 days to ensure that all possible compounds could be extracted. The resulting extracts were filtered and concentrated under reduced pressure at approximately 40°C to obtain *n*-hexane (10.3 g), ethyl acetate (18.6 g), acetone (47.8) and methanol (110.3 g) extracts, respectively. The extracts were kept in refrigerator before screening for their α-glucosidase inhibitory activity.

Of the above-mentioned extracts, the acetone extract showed the highest α-glucosidase inhibitory activity. Therefore, in order to isolate the active compound(s), the acetone extract (47 g) was subjected to column chromatography over silica gel (230–400 mesh) with a gradient of *n*-hexane: ethyl acetate (100:0 to 0:100) and then ethyl acetate: methanol (100:0 to 20:80) as eluent. After screening by TLC, fractions with similar compositions were pooled, to yield 15 fractions (F_1-F_{15}). All of these fractions were screened for α-glucosidase inhibitory activity. Among them, F_{11} and F_{13} showed the highest α-glucosidase inhibitory activity, and thus they were subjected for further purification. Fraction 11 contained a crude solid, which was triturated with methanol to give compound (**1**) (10 mg). Fraction 13 was triturated with methanol to separate an insoluble solid, which was recrystallized from methanol to yield compound (**2**) (53 mg).

Daucosterol (**1**), ^{1}HNMR (500 MHz, DMSO-d_6). δ: 0.67 (S, 3H, CH_3-18), 0.78-0.85 (M, 9H, CH_3-26, 27, 29), 0.92 (d, J= 6.3 Hz, 3H, CH_3-21), 0.96 (s, 3H, CH_3-19), 2.87-3.53 (M, 4H, H-2′,3′,4′,5′), 3.65 (M, 1H, H-3), 4.44 (dd, J = 11.9, 2.0 Hz, 1H, H-6′α), 4.88 (dd, J=11.8, 2.1 Hz, 1H, H-6′β), 4.91 (d, J=7.7 Hz, 1H, H-1′), 5.34 (br s, 1H, H-6).

D-glucono-1,4-lactone (**2**), ^{1}H NMR (500 MHz, DMSO-d_6). δ: 3.49 (dd, J=10.7, 5.2 Hz, 1H, H-6a), 3.60 (d, J=10.9 Hz, 1H, H-6b) ,(s, 1H, H-5), 4.07 (s, 1H, H-2), 4.18 (d, J=3.7 Hz, 1H, H-3), 4.43 (dd, J=6.7, 5.0 Hz, 1H, H-4).

2.5. α-Glucosidase inhibition assay

The yeast α-glucosidase activity was assayed using the substrate p-nitrophenyl-α-D-glucopyranoside (pNPG), which is hydrolysed by α-glucosidase to release the product p-nitrophenol, a chromogenic substance that can be monitored at 405 nm. Yeast α-glucosidase inhibitory activities were determined as per an earlier reported method with a slight modification [23]. The mixture contained 20 µl α-glucosidase (0.5 unit/ml), 120 µl of 0.1 M phosphate buffer (pH 6.9) and 10 µl of test sample at various concentrations. The mixture was preincubated in 96-well microtiter plate at 37°C for 15 min. After preincubation the enzymatic reaction was initiated by adding 20 µl of 5 mM pNPG solution in 0.1 M phosphate buffer (pH 6.9) and the reaction mixture was incubated at 37°C for another 15 min. The reaction was terminated by adding 80 µl of 0.2 M sodium carbonate solution and then the absorbance was measured at 405 nm in microplate reader. The reaction system without plant extracts was used as control and the system without α-glucosidase was used as blank to correct for background absorbance.

The inhibition of α-glucosidase by the test sample was calculated by the following formula:

$$\%\text{inhibition} = \left(\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right) \times 100$$

The mechanism of inhibition of the isolated compounds against α-glucosidase was assessed by analyzing the double reciprocal lineweaver-Burk plots data, which were calculated from the experiments, carried out with the sample solutions, in which the concentration of pNPG as a substrate was varied (0.5, 1, 2 and 4 mM) in the absence or presence of inhibitor compounds at two different concentrations (10 and 20 mM for daucoterol (**1**) as well as 5 and 10 mM for D-glucono-1,4-lactone (**2**)).

2.7. Statistical Analysis

Statistical analyses were done using GraphPad Prism version 5.00 for Windows. Differences were evaluated by the Student's t-test. Statistical significance was declared at a p value less than 0.05. All assays were performed at least in triplicate, and the results were expressed as mean ± standard deviation (SD). IC_{50} values were determined by plotting a percent inhibition versus concentration curve for all assays.

3. Results and Discussion

For the investigation of inhibitory effect of *Rosa canina* fruit extracts on α-glucosidase activity, its *n*-hexane, ethyl acetate, acetone and methanol extracts were tested. The IC_{50} values of each extract are shown in Table 1. The acetone extract exhibited the highest inhibitory activity on yeast α-glucosidase (IC_{50}: 0.3 µg/ml), followed by methanol extract, with IC_{50} equal to 0.5 µg/ml. The other two extracts, *n*-hexane and ethyl acetate, showed poor inhibition, with IC_{50} at 28.2 and 6.1 µg/ml, respectively.

Table 1. α-Glucosidase inhibitory activity of various extracts from *Rosa canina* L. fruits.

Extract of *Rosa canina* L.	IC_{50} (µg/ml)
n-Hexane extract	28.2±0.9
Ethyl acetate extract	6.1±1.2
Acetone extract	0.3±0.1
Methanol extract	0.5±0.2

Consequently, the acetone extract of *Rosa canina* fruits, as the most active extract, was subjected to silica gel column chromatography, which yielded 15 fractions. Screening of the fractions for α-glucosidase inhibitory activity demonstrated that active components in acetone extract were exclusively concentrated in the last 5 fractions. At a concentration of 2 µg/ml the last 5 fractions inhibited more than 40% of the α-glucosidase activity, while the first 10 fractions inhibited less than 30% (Figure 1). Of the last 5 fractions, F_{11} and F_{13} showed the highest α-glucosidase inhibitory activity with IC_{50} equal to 0.85 and 1.29 µg/ml.

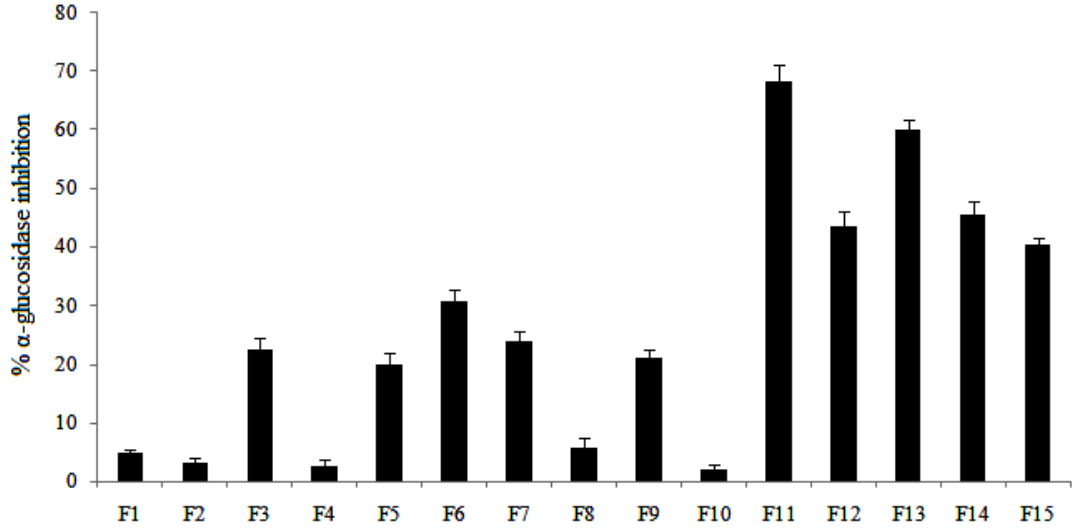

Figure 1. Inhibitory activity of *Rosa canina* L. fruit acetone extract fractions obtained by column chromatography on α-glucosidase (at 2 µg/ml). Values represent mean ± SD

Compound (**1**) was isolated from F_{11} fraction as a white powder. By comparing the ^{1}H and ^{13}C NMR data of this compound with those reported in the literatures, it was identified as β-sitosterol-3-*O*-β-D-glucopyranoside (daucosterol) [24, 25]. Compound (**2**) was also obtained as a white amorphous powder from F_{13} fraction. The spectral data of this compound were consistent with that of D-glucono-1,4-lactone [26]. Structures of the isolated compounds are shown in Figure 2. They were tested for their α-glucosidase inhibitory activity, and the results are presented as IC_{50} values in Table 2.

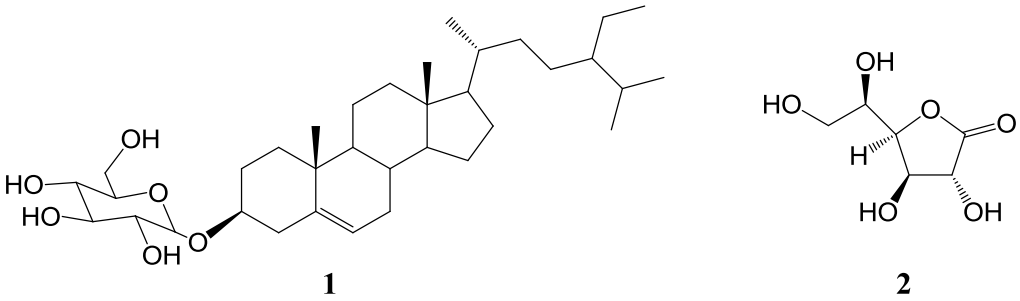

Figure 2. The structures of compounds isolated from *R. canina* fruits, daucosterol (**1**) and D-glucono-1,4-lactone (**2**)

Both daucosterol and D-glucono-1,4-lactone showed very high inhibition on yeast α-glucosidase activity, and their IC_{50} values were calculated as 13.3 and 6.5 μM, respectively, which were much lower than that of a positive control, acarbose with an IC_{50} value of 16.1 μM.

Table 2. Inhibitory effect of the compounds (**1**), (**2**) and acarbose on α-glucosidase

Compounds	IC_{50} (μM)
Daucosterol (**1**)	13.3±1.9
D-Glucono-1,4-lactone (**2**)	6.5±2.0**
Acarbose	16.1±1.1

Values represent mean±SD. Student's t-test was applied to find significant differences. ** $p<0.05$ as compared with acarbose.

Daucosterol is the 3-*O*-glucosylated form of β-sitosterol, one of the most commonly encountered phytosterols in higher plants [27]. There are two reports about the isolation of daucosterol from other members of Rosaceae family including *Rosa laevigata* Michx. [28] and *Rosa bella* Rehd. et Wils. [29]. Also, daucosterol was isolated as an efficient α-glucosidase inhibitor from the flowers of *Musa* spp.(Baxijiao). It was reported that in comparison with acarbose as a known α-glucosidase inhibitor ducosterol exerted stronger inhibition ability [30]. D-glucono-1,4-lactone is a glucose derivative, occurring naturally in various fruits and vegetables. It was reported that D-glucono-1,4-lactone is a potent inhibitor of β-glucosidase, and at 1 mM it showed complete inhibition of the enzyme [31]. D-glucono-1,4-lactone is a transition state analog of the glucose moiety of glucosides and competitively binds the glycone binding site of glucosidase active site. Furthermore, the effect of D-glucono-1,4-lactone on blood platelets under oxidative stress conditions was investigated, and the results showed that it could be helpful in reducing excessive platelet activation through antioxidant mechanisms [32].

Lineweaver-Burk plots of α-glucosidase inhibitory activities in presence of daucosterol and D-glucono-1,4-lactone were represented in Figure 3. The kinetics of inhibition was investigated by varying the concentration of pNPG as a substrate (0.5-4 mM) in the absence or presence of the isolated compounds at two different concentrations (10 and 20 mM for daucoterol as well as 5 and 10 mM for

D-glucono-1,4-lactone). According to the obtained results it is evident that α-glucosidase inhibition by compounds (**1**) and (**2**) was a competitive type of inhibition. In both of the isolated compounds K_m values increased with subsequent increase in inhibitor concentration, while V_{max} value remained unchanged.

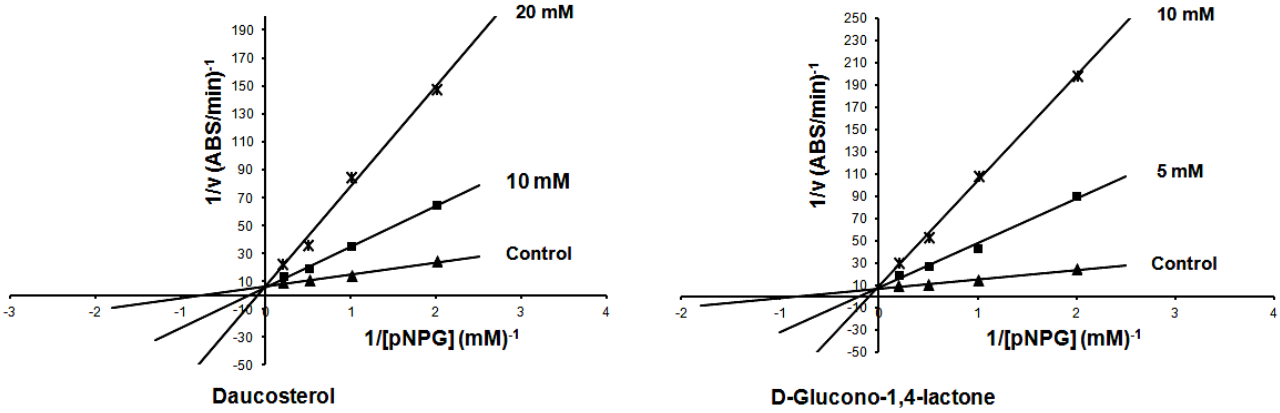

Figure 3. Lineweaver-Burk plot analysis of the inhibition kinetics of α-glucosidase by daucosterol (**1**) and D-glucono-1,4-lactone (**2**).

4. Conclusion

Inhibition of α-glucosidase is a therapeutic approach for diabetes. In this study, inhibitory activities of *Rosa canina* L. fruits extracts on α-glucosidase were investigated. Two compounds, daucosterol (**1**) and D-glucono-1,4-lactone (**2**), were identified as active compounds in fractions of the acetone extract obtained by column chromatography. To our knowledge, this is the first report on the isolation of daucosterol (**1**) and D-glucono-1,4-lactone (**2**) from *R. canina* fruits and their inhibitory effect on yeast α-glucosidase. Lineweaver-Burk plots revealed that mode of inhibition of yeast α-glucosidase by these compounds was competitive. The results of this study suggest that *R. canina* fruits can be used for the treatment of diabetes because they contain potent α-glucosidase inhibitors.

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References

[1] American Diabetes Association (2005). Diagnosis and classification of diabetes mellitus. *Diabetes Care* 28, S37–S42.

[2] E. Apostolidis and C.M. Lee (2010). *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition, *J. Food Sci*. **75**, 97-102.

[3] T. Tsujita, T. Takaku and T. Suzuki (2008). Chestnut astringent skin extract, an α-amylase inhibitor, retards carbohydrate absorption in rats and humans, *J. Nutr. Sci. Vitaminol*. **54**, 82-88.

[4] L. Lin, Y. Dong, H. Zhao, L. Wen, B. Yang and M. Zhao (2011). Comparative evaluation of rosmarinic acid, methylrosmarinate and pedalitin isolated from *Rabdosia serra* (MAXIM.) HARA as inhibitors of tyrosinase and α-glucosidase, *Food Chem*. **129**, 884-889.

[5] T. Ieyama, M. Gunawan-Puteri and J. Kawabata (2011). α-Glucosidase inhibitors from the bulb of Eleutherine Americana, *Food Chem*. **128**, 308-311.

[6] B. Kimmel and S. Inzucchi (2005). Oral agents for type 2 diabetes: An update, *Clin. Diabetes* **23**, 64-76.

[7] S. Sancheti, S. Sancheti, M. Bafna, S.H. Lee and S.Y. Seo (2011). Persimmon leaf (*Diospyros kaki*), a potent α-glucosidase inhibitor and antioxidant: Alleviation of postprandial hyperglycemia in normal and diabetic rats, *J. Med. Plants Res*. **5**, 1652-1658.

[8] K. Takahashi, Y. Yoshioka, E. Kato, S. Katsuki, O. Iida, K. Hosokawa and J. Kawabata (2010). Methyl caffeate as an α-glucosidase inhibitor from *Solanum torvum* fruits and the activity of related compounds, *Biosci. Biotechnol. Biochem*. **74**, 741-745.

[9] N. Jong-Anurakkun, M.R. Bhandari, G. Hong and J. Kawabata (2008). α-Glucosidase inhibitor from Chinese aloes, *Fitoterapia* **79**, 456-457.

[10] K. Anam, R.M. Widharna and D. Kusrini (2009). α-Glucosidase inhibitor activity of *Terminalia* species, *Int. J. Pharmacol*. **5**, 277-280.

[11] F. Demir and M. Ozcan (2001). Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey, *J. Food. Eng*. **47**, 333-336.

[12] R. Guimaraes, L. Barros, A.M. Carvalho and I. Ferreira (2010). Studies on chemical constituents and bioactivity of *Rosa micrantha*: An alternative antioxidants source for food, pharmaceutical, or cosmetic applications, *J. Agr. Food Chem*. **58**, 6277-6284.

[13] H. Tayefi-Nasrabadi, S. Sadigh-Eteghad and Z. Aghdam (2011). The Effects of the hydroalcohol extract of *Rosa canina* L. fruit on experimentally nephrolithiasic wistar rats, *Phytother. Res*. **26**, 78-85.

[14] S. Nojavan, F. Kha1i1ian, F. Momen Kiaie, A. Rahimi, A. Arabanian and S. Chalavi (2008). Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina* L. Fruit, *J. Food Compos. Anal*. **21**, 300–305.

[15] D. Deliorman Orhan, A. Harteviollu, E. Küpeli and E. Yesilada (2007). *In vivo* anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. Fruits, *J. Ethnopharmacol*. **112**, 394–400.

[16] I. Gürbüz, O. Ustün, E. Yesilada, E. Sezik and O. Kutsal (2003). Anti-ulcerogenic activity of some plants used as folk remedy in Turkey, *J. Ethnopharmacol*. **88**, 93–97.

[17] D.A. Daels-Rakotoarison, B. Gressier, F. Trotin, C. Brunet, M. Luyckx, T. Dine, F. Bailleul, M. Cazin and J.C. Cazin (2002). Effects of *Rosa canina* fruit extract on neutrophil respiratory burst, *Phytother. Res*. **16**, 157–161.

[18] A. Trovato, M.T. Monforte, A. Rossetto and A.M. Forestieri (1996). *In vitro* cytotoxic effect of some medicinal plants containing flavonoids. *Boll. Chim. Farm*. **135**, 263–266.

[19] S. Shiota, M. Shimizu, T. Mizusima, H. Ito, T. Hatano, T. Yoshida and T. Tsuchiya (2000). Restoration of effectiveness of beta-lactams on methicillin-resistant *Staphylococcus aureus* by tellimagrandin I from rose red, *FEMS Microbiol. Lett*. **185**, 135–138.

[20] P. Salehi, B. Asghari, M.A. Esmaeili, H. Dehghan and I. Ghazi (2013). α-Glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes, *J. Med. Plants Res.* **7**, 257-266.

[21] N. Orhan, M. Aslan, S. Hosbas and D. Deliorman Orhan (2009). Antidiabetic effect and antioxidant potential of *Rosa canina* fruits, *Pharmacogn. Mag*. **5**, 309-315.

[22] A. Gholamhoseinian, H. Fallah and F. Sharififar (2009). Inhibitory effect of methanol extract of *Rosa damascena* Mill. flowers on α-glucosidase activity and postprandial hyperglycemia in normal and diabetic rats, *Phytomedicine* **16**, 935–941.

[23] L. Zhang, S. Hogan, J. Li, S. Sun, C. Canning, S.J. Zheng and K. Zhou (2011). Grape skin extract inhibits mammalian intestinal α-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice, *Food Chem*. **126**, 466–471.

[24] F.M. Moghaddam, M. Moridi Farimani, S. Salahvarzi and G. Amin (2007). Chemical constituents of dichloromethane Extract of cultivated *Satureja khuzistanica*, Evid. Based Complement. *Alternat. Med*. **4**, 95-98.

[25] J.H. Lee, J.Y. Lee, J.H. Park, H.S. Jung, J.S. Kim, S.S. Kang, Y.S. Kim and Y. Han (2007). Immunoregulatory activity by daucosterol, β-sitosterol glycoside, induces protective Th1 immune response against disseminated Candidiasis in mice, *Vaccine*. **25**, 3834–3840.

[26] Z. Walaszek and D. Horton (1982). Conformational studies on aldonolactones by NMR spectroscopy. Conformations of D-glucono-, D-mannono-, D-gulono- and D-galactono-1,4-lactone in solution, *Carbohydr. Res*. **105**, 131-143.

[27] G. García-Llatas and M.T. Rodríguez-Estrada (2011). Current and new insights on phytosterol oxides in plant sterol-enriched food, *Chem. Phys. Lipids*. **164**, 607–624.

[28] Y. Gao, W.M. Cheng and G.Y. Li (1993). Chemical constituents of *Rosa laevigata* Michx., *Zhongguo Zhong Yao Za Zhi*. **18**, 426-428.

[29] F.Z. Chen, S.L. Peng, L.S. Ding, Y.H. He and M.K. Wang (2001). Chemical constituents from the fruit of *Rosa bella* Rehd. et Wils, *Zhongguo Zhong Yao Za Zhi*. **26**, 549-551.

[30] Z. Sheng, H. Dai, S. Pan, H. Wang, Y. Hu and W. Ma (2014). Isolation and characterization of an α-glucosidase inhibitor from *Musa* spp. (Baxijiao) Flowers, *Molecules* **19**, 10563-10573.

[31] P. Christakopoulos, P.W. Goodenough, D. Kekos, B.J. Macris, M. Claeyssens and M.K. Bhat (1994). Purification and characterization of an extracellular β-glucosidase with transglycosylation and exo-glucosidase activities from *Fusarium oxysporum*, *Eur. J. Biochem*. **224**, 379-385.

[32] J. Saluk-Juszczak (2010). A comparative study of antioxidative activity of calcium-D-glucarate, sodium-D-gluconate and D-glucono-1,4-lactone in a human blood platelet model, *Platelets* **21**, 632–640.