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Original Article

Chemical constituents of *Swertia longifolia* Boiss. with α-amylase inhibitory activity

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Abstract

α-Amylase inhibitors play a critical role in the control of diabetes and many of medicinal plants have been found to act as α-amylase inhibitors. *Swertia* genus, belonging to the family Gentianaceae, comprises different species most of which have been used in traditional medicine of several cultures as antidiabetic, anti-pyretic, analgesic, liver and gastrointestinal tonic. *Swertia longifolia* Boiss. is the only species of *Swertia* growing in Iran. In the present investigation, phytochemical study of *S. longifolia* was performed and α-amylase inhibitory effects of the plant fractions and purified compounds were determined. Aerial parts of the plant were extracted with hexane, chloroform, methanol and water, respectively. The components of the hexane and chloroform fractions were isolated by different chromatographic methods and their structures were determined by ^{1}H NMR and ^{13}C NMR data. α-Amylase inhibitory activity was determined by a colorimetric assay using 3,5-dinitro salysilic acid. During phytochemical examination, α-amyrin, β-amyrin and β-sitosterol were purified from the hexane fraction,while ursolic acid, daucosterol and swertiamarin were isolated from chloroform fraction. The results of the biochemical assay revealed α-amylase inhibitory activity of hexane, chloroform, methanol and water fractions, of which the chloroform and methanol fractions were more potent (IC_{50} 16.8 and 18.1 mg/ml, respectively). Among examined compounds, daucosterol was found to be the most potent α-amylase inhibitor (57.5% in concentration 10 mg/ml). With regard to α-amylase inhibitory effects of the plant extracts, purified constituents, and antidiabetic application of the species of *Swertia* genus in traditional medicine of different countries, *S. longifolia* seems more appropriate species for further mechanistic antidiabetic evaluations.

Keywords: *Swertia longifolia*; Daucosterol; Amyrin; Swertiamarin; Ursolic acid; α-Amylase inhibition

INTRODUCTION

Diabetes mellitus, a well-known major health risk in the world, is a chronic endocrine disease characterized by persistent hyperglycemia along with abnormalities in metabolism of carbohydrates, proteins and lipids. It is induced by a failure of insulin secretion and/or increased cellular resistance. In this condition, microvascular and macrovascular problems including failure of organs especially eyes, kidneys, nerves, heart and blood vessels occur (1). Different types of drugs are used in diabetes management influencing via different mechanisms of action such as improving insulin action, increasing pancreatic insulin secretion and inhibition of α-glucosidase and α-amylase enzymes (2). The α-amylase enzyme has a strategic role in digestion of carbohydrates; therefore, its inhibitors (AAI) are important in the treatment of diabetes. It has been established that some medicinal plants are capable of controling diabetes by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzyme, α–amylase, in digestive tract (3). The genus *Swertia* L. belongs to the family Gentianaceae, and comprises 170 known species worldwide (4). Ninety *Swertia* species are cosmopolitan. They have

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distributed in the mountainous parts of tropical Asia, Europe, America and Africa just like most species of the Gentianaceae, although areas around Himalayas are considered as the main origin. About 40 species of this genus are known to grow in India. Moreover, 97 *Swertia* species have been reported from China (5). Different plants of *Swertia* have been employed in traditional medicine of diverse countries (6).

A variety of *Swertia* species are used as crude drugs in the Indian pharmacopoeia, of which *S. chirayita* is commonly available in India, Nepal and China, and used as the most important and valuable one (7). This species is abundantly used in traditional medicine for treatment of type 2 diabetes mellitus due to lowering the blood glucose level (8). Surprisingly, some other *Swertia* species are used as substitutes and adulterant of *S. chirayita*. For instance, in Pakistan, *S. purpurascens* is known as a substitute of *S. chirayita*, while *S. japonica* is an important species in Japan (4). Furthermore, in Chinese traditional medicine, about twenty species of *Swertia* genus are used for treatment of choleric, hepatic and inflammatory disorders (9). Aerial parts of *S. corymbosa* are used as a major ingredient for the preparation of Ayurvedic herbal medicines against diabetes (10).

According to the importance of *Swertia* in traditional medicine, many phytochemical investigations have been performed on this genus to find its active constituents. About 200 components with different structural patterns have been reported from the genus including xanthones, iridoids, secoiridoids, triterpenoids, steroids, flavonoids and alkaloids (6). The only *Swertia* species that grows in Iran is *S. longifolia* Boiss. called "*Maryam Koohi*" in Persian.

The plant is found in northern regions of Iran in mountainous areas (11). Some phytochemical (12-14) and biological (15,16) studies have been carried out on the mentioned species demonstrating that the plant mainly contains xanthone diglycosides, iridoid and secoiridoids (12-14).

To the best of our knowledge, there is no report on the α-amylase inhibitory activity of this plant. Thus, in the present work, α-amylase inhibitory effects of hexane, chloroform, methanol and water extracts of *S. longifolia* as well as some constituents isolated and identified from these fractions were investigated.

MATERIALS AND METHODS

Materials

Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker Avance 400 & 500 NMR spectrometers (Germany) with tetramethylsilane as an internal standard. Chemical shifts are given in σ (ppm) in $CDCl_3$, pyridine or CD_3OD as solvent. Column chromatography was performed using silica gel (kieselgel 60, 0.2-0.5 mm, 0.063-0.2 mm, Merck, Germany) and Sephadex LH-20 (0.025-0.1 mm, Sigma, Germany). Separation by solid phase extraction (SPE) was carried out using silica gel (0.04-0.063 mm, Merck, Germany). Silica gel $60F_{254}$ pre-coated plates (Merck) were used for TLC. The spots on the plates were detected by spraying a methanol-H_2SO_4 10% reagent followed by heating the plates at 120 ºC using a hot plate for 5 min. PTLC was performed on silica-gel plates 20 × 20 cm. High-performance liquid chromatography (HPLC) was performed on HPLC analytical and semi-preparative instruments from Shimadzu company.

Palnt material

The aerial parts of *S. longifolia* Boiss. were collected in July 2010 from the northern parts of Iran, Mazandaran province, Lavashm mountains and identified by botanists Dr. A. Pirani and Dr. H. Moazzeni. A voucher specimen was deposited at the Herbarium of Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (No. 3058 TMRC).

Extraction and isolation

The aerial parts of the plant (1 kg) were powdered, and then extracted with maceration method using hexane, chloroform, methanol

and water. The extracts were evaporated under reduced pressure using a rotary evaporator (16).

Purification of compounds from hexane fraction

A portion (12.0 g) of the hexane fraction was dissolved in 200 ml of chloroform. The chloroform solution was washed with 5% aquous NaOH (4 × 200 ml) to remove phenolics (12). These were set aside and the organic solution further washed with water, dried, and evaporated to dryness. The resulting fraction (2.5 g) was chromatographed over silica gel column (0.063-0.200 mm, 3 × 55 cm) using a petroleum ether:CH_2Cl_2:EtOAc gradient (30:70:0, 20:80:0, 10:90:0, 5:95:0, 0:100:0, 0:95:5, 0:90:10, and 0:80:20, consequently). The volume was 1000 ml for each solvent system to give five fractions. The fractions from petroleum ether:CH_2Cl_2:EtOAc 20:80:0 (A_1, 200 mg) and 0:95:5 (A_2, 30 mg) contained the major constituents.

Isolation of the main components of fraction A_1 and A_2

PTLC was used with a mixture of petroleum ether:CH_2Cl_2 (1:9) as a mobile phase. Finally, two compounds (**1**, 8 mg and **2**, 4 mg) were purified from fraction A_1. In addition, compound **3** (22 mg) was obtained by recrystallization of the fraction A_2 using petroleum ether.

Isolation of compounds from chloroform fraction

Chloroform fraction (12 g) was subjected to silica gel column (0.2-0.5mm, 5 × 90 cm). The mobile phase was CH_2Cl_2:EtOAc:MeOH (100:0:0), (90:10:0), (80:20:0), (70:30:0), (60:40:0), (50:50:0), (40:50:10), (30:50:20), (20:50:30), (10:50:40), (0:50:50), (0:40:60), (0:30:70), (0:20:80), (0:10:90), and (0:0:100), consequently. The volume was 2000 ml for each solvent system to give nine fractions. The fractions obtained from CH_2Cl_2:EtOAc:MeOH 80:20:0 (fraction B_1, 1 g) and CH_2Cl_2:EtOAc:MeOH 50:50:0 (fraction B_2, 3.3 g) were chosen for further purifications. The fraction B_1 (1 g) was eluted using silica gel column (0.063-0.2 mm, 3 × 65 cm) with CH_2Cl_2:EtOAc (95:5, 92.5:7.5, 90:10, 87.5:12.5, 85:15, 80:20, 75:25, 60:40, 50:50, 40:60, 30:70, and 20:80, consequently). The volume was 500 ml for each solvent system to give six fractions. The fractions obtained from CH_2Cl_2:EtOAc 75:25 (fraction B_{1a}, 71 mg) and CH_2Cl_2:EtOAc 50:50 (fraction B_{1b}, 300 mg) were selected for further purifications. Fraction B_{1a} was chromatographed over a silicagel column (0.043-0.063 mm, 1 × 3cm) using SPE method and eluted with CH_2Cl_2:EtOAc (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, and 0:100, consequently). The volume was 15 ml for each solvent system. The fraction obtained from CH_2Cl_2:EtOAc 80:20 contained a pure substance (compound **4**, 9 mg).

In order to separate the components of B_{1b}, the fraction was added to silica gel column (0.04-0.063 mm, 2 × 13 cm) with CH_2Cl_2:EtOAc (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, and 50:50, consequently), and volume was 120 ml for each one. Fraction obtained from CH_2Cl_2:EtOAc (80:20) contained compound **5** (20 mg). The fraction B_2 (3.3 g) was purified by a silica gel column (0.063-0.2 mm, 2.5 × 90 cm) with EtOAc:MeOH (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 0:100, consequently), and the volume for each solvent system was 800 ml. Six fractions were obtained. The fractions obtained from EtOAc:MeOH (70:30) (B_{2a}, 600 mg) was chosen for further purifications. For separating the main components of the fraction B_{2a}, sephadex LH-20 column (2 × 30 cm) was used with MeOH as the mobile phase. The obtained fractions were compared by TLC, and finally fraction B_{2a1} purified by HPLC method. At first, an analytical HPLC equipped with VP-ODS column (4.6 × 250 mm, 5 µm) and diode array detector was used. The mobile phase was methanol:water (50:50) with isocratic mode. The flow rate and run time were 0.8 ml/min and 30 min, respectively. After setting up the method of separation, a semi-preparative HPLC was used with following chromatographic conditions: RP-18 column (20 × 250 mm, 15 µm), UV detector set at 245 nm, methanol:water (50:50) as mobile phase (isocratic), 5 ml/min flow rate and run time of 30 min. Compound **6** was eluted at retention time of 13.11 min (4.5 mg).

α-Amylase inhibition assay

The α-amylase inhibition assay was performed according to the method proposed by Giancarlo and colleagues by some modifications (17,18). The starch solution (1% w/v) was obtained by boiling and stirring 1 g of potato starch in 100 ml of sodium phosphate buffer for 30 min. The enzyme (EC 3.2.1.1) solution (50 unit/1 ml) was prepared by mixing 0.01 g of α-amylase in 10 ml of sodium phosphate buffer (pH 6.9) containing 0.0006 mM sodium chloride. The extracts were dissolved in dimethyl sulfoxide (DMSO) to give concentrations from 10 to 50 mg/ml, while the concentration of 10 mg/ml was made from componds **3**, **5** and mixture of compounds **1** and **2** in DMSO. Compounds **4** and **6** were purified in small quantities and not enough for enzyme assay. The color reagent was a solution containing 0.1 g of 3,5-dinitrosalicylic acid plus 2.99 g sodium potassium tartrate in 0.16 g sodium hydroxide and phosphate buffer (10 ml).

An aliquot of each sample (50 µl) and portions of each starch solution (150 µl) and the enzyme solution (10 µl) were mixed in a 96-well plate and incubated at 37 °C for 30 min. Then, 20 µl of sodium hydroxide and 20 µl of color reagent were added and the closed plate was placed into a water bath set at 100 °C. After 20 min, the reaction mixture was removed from the water bath and cooled, thereafter, α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm in Elisa stat fax 2100 (Awarness Technology Inc., USA). Blank samples were used to correct the absorbances of the mixture in which the enzyme was replaced with buffer solution.

A control reaction in which the sample had been replaced with 50 µl of DMSO was also used, and the maximum enzyme activity was determined. Acarbose solution at the concentrations 1-5 mg/ml was used as a positive control. The inhibition percentage of α-amylase was determined by the following equation:

$$\alpha\text{-Amylase inhibition } \% = 100 \times (\Delta A_{control} - \Delta A_{sample}) / \Delta A_{control}$$

$$\Delta A_{control} = A_{test} - A_{Blank}$$

$$\Delta A_{sample} = A_{test} - A_{Blank}$$

Statistical analysis

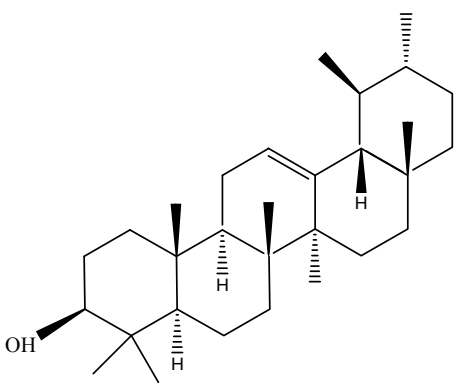
Statistical analysis was performed using the SPSS version 21.0. The IC_{50} values were estimated by non-linear curve and presented as their respective 95% confidence limits. Probit analysis of variance was used to assess the presence of significant differences ($P<0.05$) between the samples.

RESULTS

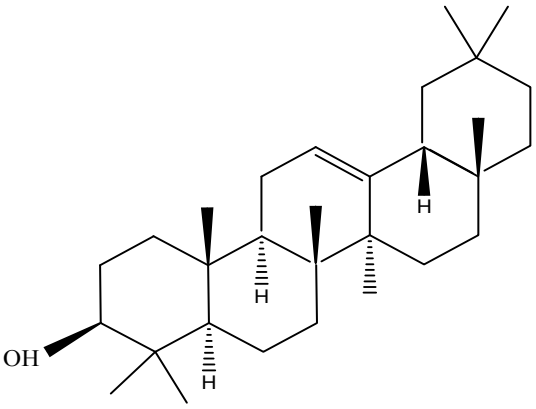
The present study led to the isolation of six compounds from aerial parts of *S. longifolia,* three of which were triterpenoid, two were steroid and one was a secoiridoid (Fig. 1). The structures of these compounds were elucidated by comparison of their NMR data with those reported in the literature (19-23). The data of ^{1}H-NMR and ^{13}C-NMR of the compounds are given as follows:

Compound **1**: **α-amyrin**, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.75 (1H, *d*, J=11.6 Hz, H-5), 0.82 (3H, *s*, H-25), 0.82 (3H, *s*, H-24), 0.97 (3H, *s*, H-26), 1.02 (3H, *s*, H-23), 1.03 (3H, *s*, H-28), 1.09 (3H, *s*, H-27), 1.85 (2H, *td*, J=4.8, 13.6 Hz, H-16), 1.92 (2H, *dt*, H-22), 1.99 (2H, *td*, J=4.4, 13.2 Hz, H-15), 3.23 (1H, *dd*, J=5.2, 11.2 Hz, H-3), 5.14 (1H, *t*, J=3.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 400 NMR): 15.60 (C-24), 15.69 (C-25), 16.87 (C-26), 17.48 (C-29), 18.36 (C-6), 21.41 (C-30), 23.28 (C-27), 23.37 (C-11), 26.62 (C-16), 27.28 (C-15), 28.11 (C-28), 28.11 (C-23), 28.76 (C-2), 31.26 (C-21), 32.36 (C-7), 33.34 (C-17), 36.66 (C-10), 38.79 (C-1), 38.79 (C-4), 39.62 (C-19), 39.67 (C-20), 41.54 (C-22), 42.09 (C-14), 47.72 (C-9), 55.19 (C-5), 59.07 (C-18), 79.06 (C-3), 124.43 (C-12), 139.59 (C-13).

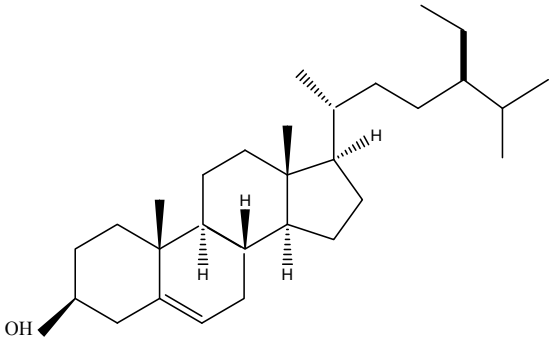
Compound **2: β-amyrin**, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.72 (1H, *d*, J=10.4 Hz, H-5), 0.81 (3H, *s*, H-25), 0.85 (3H, *s*, H-23), 1.01 (3H, *s*, H-24), 1.81 (2H, *m*, H-16), 1.98 (2H, *td*, J=4.4, 13.2 Hz, H-15), 1.15 (3H, *s*, H-28), 1.27 (3H, *s*, H-27), 3.24 (1H, *dd*, J=4.8, 10.8 Hz, H-3), 5.19 (1H, *t*, J=3.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): 15.50 (C-24), 15.63 (C-25), 16.81 (C-26), 18.44 (C-6), 23.54 (C-11), 23.70 (C-30), 25.75 (C-27), 26.16 (C-16), 26.51 (C-15), 27.24 (C-2), 27.98 (C-23), 28.4 (C-28), 31.09 (C-20), 32.66 (C-7), 32.83 (C-17), 33.76 (C-29), 34.74 (C-21), 36.90 (C-10), 37.15 (C-22), 38.59 (C-4), 38.67 (C-1), 40.02 (C-8), 41.54 (C-14), 46.83 (C-19), 47.23 (C-18), 47.64 (C-9), 55.35 (C-5), 79.06 (C-3), 121.73 (C-12), 145.3 (C-13).



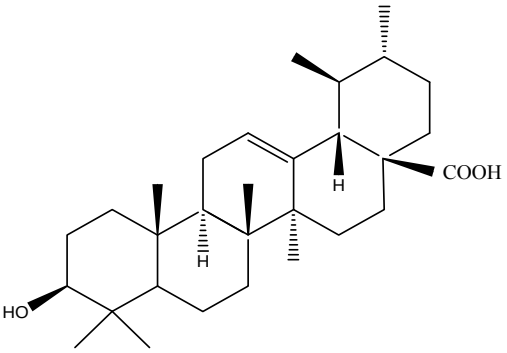
α-amyrin (**1**)



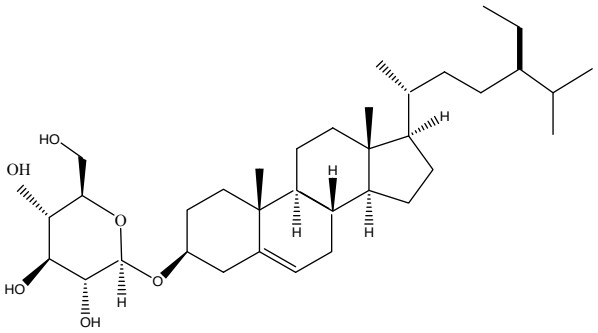
β-amyrin (**2**)



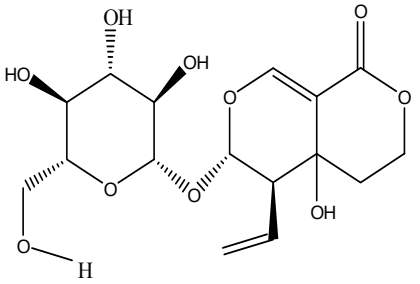
β-sitosterol (**3**)



ursolic acid (**4**)



daucosterol (**5**)



swertiamarin (**6**)

Fig. 1. Structures of the isolated compounds from *Swertia longifolia*.

Compound **3**: **β-sitosterol**, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.69 (3H, *s*, H-18), 0.82 (3H, H-26), 0.82 (3H, H-27), 0.86 (3H, H-29), 0.93 (3H, *d*, J=5.8 Hz, H-21), 1.02 (3H, *s*, H-19), 1.08 (2H, *m*, H-1b), 1.08 (2H, *m*, H-15b), 1.08 (1H, *m*, H-17), 1.08 (2H, *m*, H-22b), 1.16 (2H, *m*, H-12b), 1.16 (2H, *m*, H-23), 1.25 (2H, *m*, H-16b), 1.25 (2H, *m*, H-28), 1.48 (2H, *m*, H-2b), 1.48 (2H, *m*, H-7), 1.48 (2H, *m*, H-11), 1.58 (2H, H-15a), 1.67 (1H, *m*, H-25), 1.85 (2H, *m*, H-1a), 1.85 (2H, *m*, H-2a), 1.85 (2H, *m*, H-16a), 1.97 (1H, H-8), 2.02 (2H, m, H-12a), 2.27 (2H, m, H-4), 3.54 (1H, s, H-3), 5.37 (1H, s, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): 11.83 (C-18), 11.95 (C-29), 18.75 (C-21), 19 (C-27), 19.37 (C-19), 19.79 (C-26), 21.05 (C-11), 23.03 (C-28), 24.27 (C-15), 26.02 (C-23), 28.22 (C-16), 29.10 (C-25), 31.60 (C-2), 31.87 (C-7), 31.87 (C-8), 33.90 (C-22), 36.12 (C-20), 36.47 (C-10), 37.22 (C-1), 39.74 (C-12), 42.25 (C-4), 42.25 (C-13), 45.78 (C-24), 56.02 (C-17), 50.09 (C-9), 56.73 (C-14), 71.75 (C-3), 121.68 (C-6), 140.72 (C-5).

Compound **4**: **Ursolic acid**, ^{1}H NMR (pyridine-d5, 500 MHz): δ 0.88 (3H, *s*, H-25), 0.94 (3H, *d*, J=6.2 Hz, H-30), 1.00 (3H, *d*, J=6.4 Hz, H-29), 1.02 (3H, *s*, H-24), 1.05 (3H, *s*, H-26), 1.22 (3H, *s*, H-27), 1.24 (3H, *s*, H-23), 2.12 (1H, *dt*, J=4.2, 13.3Hz, H-16a), 2.32 (1H, *dt*, J=4.3, 13.1 Hz, H-16b), 2.64 (1H, *d*, J=11.1 Hz, H-18), 3.45 (1H, *dd*, J = 5.4, 10.2 Hz, H-3), 5.49 (1H, *t*, J=3.6 Hz, H-12); ^{13}C - NMR (pyridine-d5, 125 MHz): 15.68 (C-25), 16.58 (C-24), 17.46 (C-26), 17.52 (C-29), 18.78 (C-6), 21.41 (C-30), 23.63 (C-11), 23.91 (C-27), 24.91 (C-16), 28.13 (C-2), 28.69 (C-15), 28.81 (C-23), 31.07 (C-21), 33.57 (C-7), 37.28 (C-10), 37.45 (C-22), 39.07 (C-4), 39.39 (C-1), 39.39 (C-20), 39.49 (C-19), 39.97 (C-8), 42.50 (C-14), 48.05 (C-9), 48.05 (C-17), 53.55 (C-18), 55.82 (C-5), 78.12 (C-3), 125.65 (C-12), 135.80 (C-13), 179.90 (C-28).

Compound **5**: **daucosterol**, ^{1}H NMR (pyridine-d5, 500 MHz): δ 0.64 (1H, *s*, H-18), 0.84 (1H, *d*, J=6.5 Hz, H-27), 0.88 (1H, *d*, J=3.1 Hz, H-29), 0.89 (1H, *d*, J=5.6 Hz, H-26), 0.92 (1H, *s*, H-19), 0.97 (1H, *d*, J=6.5 Hz, H-21), 1.00 (1H, H-9), 1.05 (1H, *m*, H-11b), 1.05 (1H, *m*, H-14), 1.05 (1H, *m*, H-22b), 1.07 (1H, *m*, H-1b), 1.09 (1H, *m*, H-15b), 1.21 (1H, H-23), 1.65 (1H, *m*, H-15a), 1.67 (1H, *m*, H-2b), 1.69 (1H, *m*, H-25), 1.81 (1H, *m*, H-16a), 1.84 (1H, *m*, H-1a), 1.89 (1H, *m*, H-2a), 1.96 (1H, *m*, H-8), 2.12 (1H, *m*, H-12a), 2.46 (1H, *t*, H-4b), 2.72 (1H, *m*, H-4a), 3.93 (1H, H-2'), 3.97 (1H, *m*, H-5'), 4.05 (1H, *t*, J=8.1 Hz, H-3'), 4.26 (1H, *m*, H-4'), 4.31 (1H, *m*, H-3), 4.41 (1H, *dd*, J=5.1, 11.7 Hz, H-6'b), 4.56 (1H, *dd*, H-6'a), 5.33 (1H, H-1'); ^{13}C NMR (pyridine-d5, 125MHz): δ 11.97 (C-18), 12.15 (C-29), 19.00 (C-21), 19.2 (C-27), 19.17 (C-19), 19.97 (C-26), 21.27 (C-11), 23.37 (C-28), 24.50 (C-15), 26.36 (C-23), 28.54 (C-16), 29.44 (C-25), 30.24 (C-2), 32.04 (C-7), 32.16 (C-8), 34.19 (C-22), 36.38 (C-20), 36.91 (C-10), 37.47 (C-1), 39.32 (C-4), 39.93 (C-12), 42.47 (C-13), 46.02 (C-24), 50.33 (C-9), 56.23 (C-17), 56.81 (C-14), 62.81 (C-6'), 71.67 (C-4'), 75.33 (C-2'), 78.09 (C-5'), 78.48 (C-3'), 78.60 (C-3), 102.56 (C-1'), 121.92 (C-6), 140.80(C-5).

Compound **6**: **swertiamarin**, ^{1}H NMR (MeOD, 500 MHz): δ 1.75 (2H, *d*, J=13.2 Hz, H-6a), 1.92 (2H, *ddd*, J = 5.0, 13.4, 13.6 Hz, H-6b), 2.92 (1H, *dd*, J = 1.4, 9.4 Hz, H-9), 3.18 (1H, *dd*, J=8.0, 8.8 Hz, H-2'), 3.28 (1H, H-4'), 3.36 (1H, H-5'), 3.40 (1H, H-3'), 3.67 (2H, *dd*, J=5.6, 12.0 Hz, H-6'b), 3.89 (2H, *dd*, J=2, 12.0 Hz, H-6'a), 4.35 (2H, *m*, H-7a), 4.64 (1H, *d*, J=8.0 Hz, H-1'), 4.76 (2H, *ddd*, J=2.8, 11.0, 12.6 Hz, H-2'), .31 (2H, *m*, H-10), 5.45 (1H, *m*, H-8), 5.73 (1H, *d*, J=1.2, H-1), 7.64 (1H, *s*, H-3); ^{13}C NMR (MeOD, 125 NMR): 33.75 (C-6), 51.99 (C-9), 62.59 (C-6'), 64.28 (C-5), 65.96 (C-7), 71.43 (C-4'), 74.47 (C-2'), 77.83 (C-3'), 78.57 (C-5'), 99.09 (C-1), 100.21 (C-1'), 108.93 (C-4), 121.18 (C-10), 133.85 (C-8), 154.77 (C-3), 168.02 (C-11).

The results of biochemical analysis showed α-amylase inhibitory activity of all plant fractions (IC_{50} 16.8 - 37.0 mg/ml) (Fig. 2). As it is obvious in Fig. 2, methanol and chloroform fractions showed more enzyme inhibition than other fractions. Aqueous fraction was the weakest one for α-amylase inhibition. In the hexane and chloroform fractions, the inhibitory effects decreased by increasing the concentration. In fact, a reverse concentration-dependent inhibition was observed for various concentrations of the extracts.

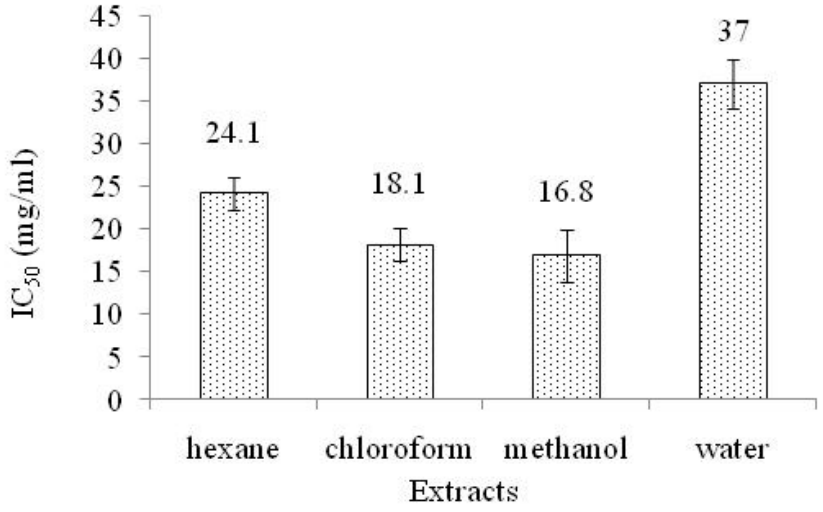


Fig. 2. α-Amylase inhibition activity of different fractions of *Swertia longifolia*.

Among examined pure compounds, daucosterol showed the highest inhibitory activity (57.5 ± 3.1% at concentration of 10 mg/ml). Enzyme inhibition percentage of β-sitosterol and a mixture of α- and β-amyrin were found to be 25.5 ± 3.5% and 30.1 ± 4.2% at concentration of 10 mg/mL, respectively. Acarbose was employed as a positive control (IC_{50} 1.8 mg/ml).

DISCUSSION

Nowadays usage of medicinal plants for treatment of various disorders especially chronic disorders such as diabetes has been increased (24). α-Amylase inhibition has been considered as one of important mechanisms involved in controlling diabetes. Several plant species have been introduced to contain constituents acting as α-amylase inhibitor and might be used for control of the diabetes (18). *Swertia* species have a long history in control of diabetes in traditioanal medicine (8).

In the present study, α-amylase inhibitory activity of *S. longifolia* extracts and constituents have been established. The inhibitory effects decreased by increasing the concentration of the extract in the hexane and chloroform fractions. Reverse dose-dependent activity of an extract might be due to the conformational changes from binding of compounds to the enzyme by increasing the concentration as has been previously reported (25).

Methanol and aqueous extracts showed dose-dependent α-amylase inhibition. Regarding to the α-amylase inhibitory effects of all fractions of the plant, it could be concluded that different compounds with various structures and polarities are involved in α-amylase inhibitory properties of *S. longifolia*. Therefore, it is suggested that the whole extract could be used in further diabetes studies.

Some phytochemicals from natural products are able to control diabetes, of which triterpenes are well-known to create insulin like effects and decrease blood glucose, which is a useful mechanism in treatment of diabetes (26). Hexane and chloroform fractions of *S. longifolia* contained famous triterpenes α- and β-amyrin, ursolic acid, and also β-sitosterol and its glucoside, daucosterol, as well as scoiridoid swertiamarin. All the purified compounds had terpenoid structure and were obtained from the plant for the first time but they had already been isolated from other species. α-amylase inhibitory activity of some mentioned compounds has been already reported.

The comparison of α-amylase inhibitory effects obtained from different investigations, helps to observe significant differences in inhibition percentage for the same compound.

This is due to various methods for assay of enzyme inhibition (27,28).

Among the isolated compounds from *S. longifolia* in the present study, α- and β-amyrinare are bioactive compounds commonly found in the leaves, barks and resins of various plants. Extensive investigations over the last years have identified α- and β-amyrinin in some species of *Swertia* genus including *S. corymbosa*, *S. chirata* and *S. paniculata,* and the pure compounds have demonstrated anti-microbial, anti-inflammatory, and other interesting biological activities (29-31). But there is no report on α-amylase inhibitory activity of these compounds. In the present study, a mixture of the compounds proved weak enzyme inhibition (30.1%). *β*-Sitosterol is a common steroid, which has been isolated from *S. ciliata*, *S. przewalskii*, *S. speciosa*, *S. franchetiana*, *S. chirata* and *S. bifolia* (32). It is an anti-diabetic (33,34), antioxidant (35), cytotoxic (36,37), anti-ulcer (38), anti-inflammatory (39), and analgesic agent (40). Recent studies demonstrated good α-amylase inhibitory activity of the compound. In an investigation, β-sitosterol inhibited the enzyme with IC_{50} 300 μM (41). Kumar and coworkers demonstrated that the compound had 48.8% inhibition effects at concentration of 50 μg/ml (42). Moreover, *in silico* studies exhibited the potent inhibition of β-sitosterol on human pancreatic amylase (43). These results are not in agreement with the present results that suggested weak activity for this compound (25.5% at concentration of 10 mg/ml). The difference may be due to the enzyme source or different conditions of experiments. Therefore, more investigations are recommended to disclose the real potency of the plant sterols and triterpenes. Meanwhile, for the future, a standardized protocol to study potential inhibitors maybe designed in order to minimize the differences between the results obtaining in various studies. Ursolic acid has been isolated from *S. david*, *S. przewalskii*, *S. corymbosa*, *S. speciosa*, and *S. thomsonii* (32). This compound can strongly inhibit α-glucosidase activity (44). The α-amylase inhibitory effects of the compound has been already esblished (3). Daucosterol, a glycosidic form of β-sitosterol, has been isolated from *S. bifolia* (32). Although strong α-glucosidase inhibitory effect of daucosterol has already been established (45), α-amylase inhibitory effect of the compound is now being reported by us for the first time and was found to be higher than other tested compounds. Swertiamarin has been isolated from *S. franchetiana*, *S. pseudochinensis*, *S. punicea, S. davidi*, *S. mussotii*, *S. nervosa, S. chirata*, *S. binchuanensis, S. delavayi* (32), while there are no report on its α-amylase inhibitory effect. Regarding the enzyme inhibition of the plant extracts and some purified compounds, it is concluded that some synergistic effects may be involved in α-amylase inhibitory activity of the plant which have induced desirable α-amylase inhibition.

Terpenoid compounds in hexane and chloroform fractions might be considered as the active components of the extracts for enzyme inhibition; while, other compounds such as xanthones which have been already found in polar fractions (12,13) may be involved in α-amylase activity of methanol and water fractions needing more investigation.

CONCLUSION

in the present study, α-amylase inhibitory effects of hexane, chloroform, methanol and water extracts of *S. longifolia* and some of terpenoid constituents isolated and identified from these fractions were proved. Therefore, this plant species could have a good potential for the treatment of diabetes. However more *in vitro, in vivo* and clinical studies are warranted for further evaluation of antidiabetic activities of this plant. Indeed, α-amylase inhibition was a preliminary test for screening the plant materials for future investigations.

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