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New flavonoid glycosides from two *Astragalus* species (Fabaceae) and validation of their antihyperglycaemic activity using molecular modelling and *in vitro* studies

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ABSTRACT

Phytochemical investigation of the methanol extracts of the aerial parts of *Astragalus turkestanus* and *A. xanthomeloides* using various chromatographic techniques resulted in the isolation and structural elucidation of two new flavonoid glycosides, namely, 7-methoxy kaempferol-3-*O*-α-L-arabinosyl-(1 → 6)-*β*-D-galactopyranoside **(3)** from the former and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranosyl-(1 → 6)-*β*-D- galactopyranoside **(6)** from the latter. In addition five other known compounds were isolated for the first time from these species. *In silico* molecular modeling study was carried out on α-glucosidase (PDB ID 3TOP; 2.88 A°) to assess the α-glucosidase inhibitory activity of the isolated compounds. Both compounds **3** and **6** displayed the highest fitting score with ΔG equals to −82.10 and −67.66 Kcal/mol in the pH-based ionization method and −68.55 and −67.83 Kcal/mol in the rule-based ionization method, respectively. α-Glucosidase inhibitory activity was further asserted *in vitro* using an ELISA assay that is measured colorimetrically at 405 nm. Compound **3** showed a significant α-glucosidase inhibitory activity displaying IC_{50} of 50.31 μg/mL approaching that of acarbose (IC_{50} = 30.57 μg/mL). All the tested samples showed no significant cytotoxic activity against Caco-2 (human epithelial colorectal adenocarcinoma) cells using the MTT assay (IC_{50} > 400 μg/mL). Thus; both new compounds could offer promising natural antihyperglycaemic entities without any marked toxicity, which could be useful in the pharmaceutical industry.

1. Introduction

Diabetes mellitus has recently been recognized as one of the most popular spreading metabolic dysfunction with an evident occurrence of morbidity and mortality all over the globe. It is characterized by the consequent emergence of various complications on the micro- and macrovascular levels as neuropathy, retinopathy, and nephropathy as well as cardiovascular complications and ulceration, (Singab et al., 2014; Youssef et al., 2017).

Although, insulin and synthetic drugs that control hyperglycaemia are considered the primary ways for proper adjustment of blood glucose levels, they showed vigorous undesirable side effects with concomitant failure to prohibit the complications associated with diabetes. Thus, the need for novel antidiabetic entities of herbal origin for effective combating of hyperglycaemia is felt mandatory worldwide (Youssef et al., 2017).

Pharmaceutical industry worldwide is based mainly upon formulating the synthetic raw materials produced by companies that are of prominent side effects. Herein, an excellent opportunity is provided to use the treasure supplied by the natural environment as raw material to be formulated in a suitable dosage form by the pharmaceutical companies. These pharmaceutical products will be used by diabetic patients with minimum cost and accepted by several categories in the society than synthetic drugs.

Astragalus represents one of the popular genera of flowering plants belonging to the family Fabaceae. It comprises nearly about 2000–3000 species of annual or perennial herbs, subshrubs, or shrubs that are prevalent in temperate and arid areas (Li et al., 2014). Traditionally, many of these species were widely adopted for the relief of depression and as diuretics and tonics (Avunduk et al., 2007; Choudhary et al.,

Abbreviations: AG, α-glucosidase enzyme; AT, methanol extracts of the aerial parts of *A. turkestanus*; AX, methanol extracts of the aerial parts of *A. xanthomeloides*; PPARα, peroxisome proliferator-activated receptor *alpha*; PPARγ, peroxisome proliferator-activated receptor *gamma*

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2008). Recently, many of the *Astragalus* species have shown many biological activities, in particular, antiviral and hepatoprotective properties in addition to boosting the immune response (Linnek et al., 2011). Undoubtedly, this could be attributed to the existence of many bioactive secondary metabolites, including alkaloids, anthraquinones, flavonoids and saponins (Ibrahim et al., 2013). Meanwhile, many of the species are used as nutrients, substituting agents for many beverages as tea or coffee as well as medicinal agents and cosmetics relying on their richness by amino acids, polysaccharides and metallic elements (Li et al., 2014). In addition some *Astragalus* species have been reported to exert a potent antihyperglycaemic activity particularly the radix of many varieties belonging to *Astragalus membranaceus* that proved a high efficacy with a satisfying safety margins. This is relied upon their richness with polysaccharides and isoflavones. The polysaccharides resulted in a notable decline in serum glucose levels, triglycerides, and low density lipoproteins as well as in insulin resistance with concomitant elevation in high density lipoproteins in streptozotocin- induced diabetes in rats (Fu et al., 2014). However, the isolated isoflavones from *Astragalus membranaceus* showed a good antihyperglycaemic amelioration *via* in *vitro* activation of PPARα and PPARγ in addition to promoting adipocyte differentiation (Shen et al., 2006).

Astragalus turkestanus Bge. and *A. xanthomeloides* Eug. Korr. et M. Pop. are endemic to the Uzbek flora. Tracing the current literature, nothing was found regarding either the chemical composition or the biological activities of *A. turkestanus.* However, only two compounds namely, D-pinitol (3-*O*-methyl-D-chiro-inositol) and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside were reported earlier from *A. xanthomeloides* by one of the authors (Janibekov et al., 2015; Janibekov et al., 2016).

Herein we reported the isolation and structural elucidation of two new antihyperglycaemic flavonoid glycosides from the aerial parts of *Astragalus turkestanus* and *A. xanthomeloides*, which are 7-methoxy kaempferol-3-*O*-α-L-arabinosyl-(1 → 6)-*β*-D-galactopyranoside **(3)** and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranosyl-(1 → 6)-*β*-D-galactopyranoside **(6).** In addition, five other compounds namely; daucasterol **(1)** and stigmasterol glucoside **(2),** D-pinitol **(4),** azukisaponin V **(5)** and 7-methoxy kaempferol-3-*O*-*β*-D-glucopyranoside **(7)** were also isolated. Molecular modeling of the isolated compounds within the active sites of α-glucosidase was done followed by *in vitro* evaluation of their α-glucosidase inhibitory activity to validate their bioactivity. In addition, their cytotoxic effect on Caco-2 cell line was reported.

2. Materials and Methods

2.1. Plant material

The aerial parts of *Astragalus turkestanus* Bge. and *Astragalus xanthomeloides* Eug. Korr. et M. Pop. were collected from the Qashqadaryo and Tashkent regions of Uzbekistan in 2015. The authenticated voucher specimens of the species (Accession no. 20081101 and 20081106, respectively) are kept in the Department of Herbal Plants (Institute of the Chemistry of Plant Substances, Uzbekistan).

2.2. Extraction and isolation

The air-dried powdered aerial parts of *A. turkestanus* and *A. xanthomeloides* (2.5 kg each) were macerated in neat methanol (5 × 12 L) at room temperature till exhaustion (no considerable yield was further obtained) i.e. the extraction was carried out five times, using 12 L of neat methanol each. The solvent was evaporated at 40 °C using a rotary vacuum evaporator (BÜCHI Labortechnik AG, Switzerland) and concentrated to give 233 and 190 g dried methanol extracts, respectively. Consequently, the dried methanol extracts were successively fractionated using chloroform then *n*-butanol. The combined *n*-butanol fractions were then concentrated at 42 °C under reduced pressure to yield 152 g and 64 g, respectively.

150 g of the dried *n*-butanol fraction of *A. turkestanus* were chromatographed over a silica gel column using the dry loading method. The column was then eluted with $CHCl_3/CH_3OH$ (9:1, v/v) accompanied by monitoring using TLC on silica gel 254 plates to give subfractions **A1- A26** which contain a mixture of less polar compounds. Consequently elution with $CHCl_3/CH_3OH/H_2O$ in the ratio of 4:1:0.1, v/v followed by 70: 23:3, v/v accompanied by monitoring using TLC on silica gel 254 plates with subsequent pooling of similar subfractions was done in which the polar subfractions were collected in order to be **B1-B20** and **C1-C25**, respectively. Subfractions **B12-B19** were pooled together and then subjected to column chromatography using silica gel with subsequent elution with $CHCl_3/CH_3OH$ (6:1, v/v) to give a mixture (16 mg) of compounds **1** and **2.** Additionally, the subfractions C7-C10 were collected together, subjected to silica gel column chromatography and eluted using $CHCl_3/CH_3OH/H_2O$ (70:23:3 v/v) to give compound **3** (7 mg) and compound **4** (13.5 mg).

In addition, 60 g of the dried *n*-butanol fraction of *A. xanthomeloides* were subjected to silica gel column and subsequently eluted using the following solvent systems successively $CHCl_3/CH_3OH$ (9:1, v/v), followed by a mixtures of $CHCl_3/CH_3OH/H_2O$ (70:12:1, v/v), (4:1:0.1, v/v), (70:23:3, v/v), (70:28:8, v/v) and (60:35:8, v/v) and then monitored by TLC on silica gel 254 plates with subsequent pooling of similar subfractions and gave **X1-97** subfractions. Subfractions **X71-77** were collected together and separated by silica gel column chromatography, then eluted with $CHCl_3/CH_3OH/H_2O$ (70:28:8, v/v) and followed by further purification to yield compound **5** (15 mg). Separation of sub fractions **X88-90** by silica gel column chromatography using solvent system (60:35:8, v/v) resulted in the isolation of compound **6** (15.5 mg) and compound **7** (12 mg).

TLC plates were visualized under UV light (λ = 254 and 365 nm) and by spraying with phosphorus wolfram acid solution followed by heating at 105° C for 10 min. A scheme summarizing the isolation of secondary metabolites from both *Astragalus species* was added in the in the supplementary data (Fig. S1).

2.3. General experimental procedures

^1H and ^{13}C (APT) NMR analyses were carried out on a Bruker Ascend 400/R spectrometer (Burker Avance III, Fallanden Switzerland) at the operating frequencies of 400 and 100 MHz, respectively. Chemical shifts were expressed in δ ppm and were related to that of the solvents. The measured samples were dissolved in various deuterated solvents (Sigma Aldrich, Germany) depending upon their solubility and transferred to 3 mm NMR tubes (Bruker). Spectra were recorded at 25 °C; δ ppm rel. to Me4Si as internal standard. Two-dimensional (2D) NMR experiments (H, H −COSY; H, C HSQC; H, C HMBC) were performed utilizing the pulse sequences from the Bruker user library. ESI–MS analysis was done on a Waters Xevo TQD mass spectrometer with UPLC Acquity mode (Milford, USA). HR-ESI–MS analyses were performed using Bruker micro-TOF-Q Daltonics (API) Time-of-Flight mass spectrometer (Bremen, Germany) applying the method previously described (Nováková et al., 2010). Normal phase column chromatography was performed using silica gel (63–100 μm; Tianjin, Sinomed Pharmaceutical Co. Ltd., China). TLC analysis was done utilizing normal phase silica gel precoated plates F254 (Merck, Germany).

2.4. Compound characterization

2.4.1. 7-Methoxy kaempferol-3-O-α-L-arabinosyl-(1 → 6)-β-D-galactopyranoside (3)

Yellowish amorphous powder; UV (MeOH) λ_{max}: 267 and 349 nm; ^1H (400 MHz) and ^{13}C NNR (100 MHz) in DMSO see Table 1; HR-ESI–MS $[M-H]^-$ *m/z* 593.1530 (calculated. for *m/z* $C_{27}H_{30}O_{15}$, 593.1584). Compound different spectra are available in the