SHORT REPORT

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Antidiabetic Flavonol Glycosides from *Eryngium caeruleum*

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Abstract: Phytochemical investigation of the aerial parts of *Eryngium caeruleum* led to the isolation of two new source flavone glycosides (**1** and **2**). The structures of these compounds were determined with the help of one- and two-dimensional (1D- and 2D-) NMR techniques including ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, ^{1}H-^{1}H COSY, and NOESY experiments. The compounds were studied for their *in vitro* aldose reductase (ALR1 and ALR2) and glucosidase (α and β) inhibitory activities, and antiglycation potential. Both the compounds showed higher inhibition potential against ALR1 than ALR2. Compound **2** showed three fold higher potency against ALR2 than the reference drug Sorbinil. *In silico* studies were performed to understand the binding mechanism of these compounds to aldose reductase.

Keywords: *Eryngium caeruleum*; flavone glycosides; aldose reductase; glucosidase; antiglycation; molecular docking.

1. Plant Source

Flavonoids are very common secondary metabolites in plants which are responsible for various important biological activities [1]. Flavonoids have been studied for their *in vitro* antidiabetic activities. Different flavonoids like rutin, kaempferol, quercetin, and quercetrin have been studied for their excellent aldose reductase and glucosidase inhibitory activities. These flavonoids and some others have shown excellent antidiabetic potential by inhibiting the aforementioned enzymes [2, 3].

Aldose reductase is a key enzyme involved in polyol pathway and responsible for the conversion of glucose into sorbitol which then converts into fructose by sorbitol dehydrogenase. Metabolism of

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sorbitol into fructose is a slow process [4], hence, the excessive accumulation of sorbitol creates osmotic imbalance, thus causing cellular damage [5]. Chronic diabetic patients have complications in the organs which are insulin-insensitive such as retinopathy, cataracts, and neuropathy [6]. Decrease in sorbitol accumulation has been observed by using aldose reductase inhibitors [7]. Blockage of aldose reductase is the potential target to prevent diabetic complications [8]. Aldose reductase (ALR) has two types, i.e. ALR1 and ALR2. ALR1 is aldehyde reductase while ALR2 is aldose reductase enzyme. Both of these enzymes are immunologically non-related and ALR1 in different species is different. ALR2, on the other hand, has evolutionary relatedness among different species. Inhibition of ALR2 in animals has shown a possible method for preventing diabetic complications.

Non-enzymatic and non-specific reaction of a protein or a lipid molecule with a sugar molecule is called glycation which ends up in the formation of protein aggregates and highly reactive fluorescent entities, referred to as Advanced Glycation End-products (AGEs) [9, 10]. AGEs are responsible for the acceleration of age-related diseases such as diabetes, neurodegenerative disorders, end stage renal failure, and atherosclerosis [11]. In diabetic patients, AGEs accumulate in tissue proteins and plasma; promoting the injurious effects of type-2 diabetes [12, 13]. Hence, inhibition of protein glycation may be one of the ways of minimizing pathogenesis of secondary complications of diabetes. Glucosidase enzymes have also been involved with diabetic complications. α-glucosidase is an important enzyme which digests complex carbohydrates and breaks them into simple sugars. In diabetic patients, the inhibition of α-glucosidase slows down the digestion of carbohydrates in intestines, thus reducing hyperglycemia [14].

Eryngium caeruleum (Apiaceae) is an important plant used in folk medicine [15]. The aerial parts of *E. caeruleum* were collected during its flowering season (May-June) from Swabi, K.P.K., Pakistan and authenticated by Dr. Muhammad Rasheed Awan, Department of Botany, Hazara University, Mansehra, K.P.K., Pakistan. A voucher specimen (# 3533) has been deposited in herbarium of the same department.

2. Previous Studies

Flavonoids, triterpenoids, steroids, coumarins, and saponins have been extracted from *E. caeruleum* in past [16, 17]. The whole plants of *E. caeruleum*, especially the roots, are stimulant, hematinic, expectorant, and nervine in nature. Its ashes are very useful for the treatment of haemorrhoids [15]. Old people use the plant to prepare a recipe for strengthening their nerves, brain, and memory. In northern Iran *E. caeruleum* has been used as food vegetable and for flavoring in the preparation of several foods [18]; its leaves have been used in soup or mixed with yogurt and used in the treatment of several skin diseases [19]. *E. caeruleum* has enforcing generative power, lenitive, diuretic, and appetizer [20]. Essential oils of *E. caeruleum* are found to enhance the permeation of piroxicam through rat skin [18].

3. Present Study

Keeping in view medicinal importance of *E. caeruleum* with the fact that a very little phytochemical work has been done on it previously, we carried out phytochemical investigation of the plant leading to the isolation of two new source flavone glycosides (**1-2**) (Fig. 1). Due to excellent antidiabetic activities of flavonoids present in literature and the fact that no biological studies have been done on flavonoids **1** and **2**, we investigated them for their *in vitro* aldose reductase and glucosidase inhibition, antiglycation potential, and *in silico* studies for aldose reductase binding to the compounds.

The air dried aerial parts of *E. caeruleum* (15.5 kg) were grinded and extracted with methanol (30 L × 3) at room temperature. The methanolic extract was evaporated under reduced pressure to yield the residue (994 g) which was partitioned between *n*-hexane (163 g), chloroform (98 g), ethyl acetate (20 g), *n*-butanol (249 g), and the remainder was the water soluble portion. The ethyl acetate soluble fraction was subjected to column chromatography (CC) over silica gel using *n*-hexane with gradient of ethyl acetate by increasing the percentage of ethyl acetate up to 100% and followed by MeOH/$CHCl_3$ mixture with increasing polarity up to 100% MeOH. Sixteen sub-fractions were collected from the chloroform

fraction (EC-1–EC-16). Fractions EC-3 and EC-11 were further subjected to CC using flash silica (70-230 mesh) and eluted with a gradient of *n*-hexane/ethyl acetate (100:0–0:100) which yielded the pure compounds **1** (8.9 mg) and **2** (13 mg), respectively (Fig. 1). The purity of the compounds was monitored on TLC and HPTLC.

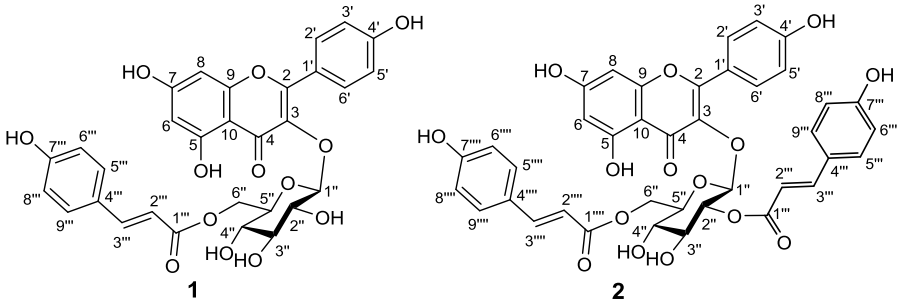

Figure 1. Structures of compounds **1** and **2**, isolated from *E. caeruleum.*

3.1. In vitro Aldose Reductase Inhibition Assay Results

Compounds **1** and **2** were analyzed *in vitro* against ALR1 and ALR2. Compound **2** was more potent against both the enzymes showing dual inhibitory activity but the selectivity was less as both ALR1 and ALR2 have almost 65% structural homology [21]. In terms of potency the inhibitory activity towards ALR1 was more as compared to ALR2. The results are shown in Table 1.

Table 1. Aldose reductase/glucosidase inhibition and antiglycation activity data of **1** and **2**

Compounds	ALR1	ALR2	SI[c]	*α*-glucosidase	*β*-glucosidase	Antiglycation Activity
	IC_{50} [a] ± SEM (µM) % Inhibition[b]			(% Inhibition[b])		(% Inhibition[b])
1	12.22[a] ± 4.32	2.54[a] ± 0.98	4.81	33.11[b]	6.32[b]	50.5[b]
2	1.31[a] ± 0.67	0.93[a] ± 0.04	1.41	23.74[b]	4.03[b]	18.2[b]
Valproic acid	57.4[a] ± 0.89	-	-	-	-	-
Sorbinil	-	3.14[a] ± 0.02	-	-	-	-
Rutin	-	-	-	-	-	81.2[a] ± 7.1

[a]IC_{50} is the concentration at which 50% of the enzyme activity is inhibited.
[b]The % inhibition of the enzyme activity caused by 200 µM of the tested compounds.
[c]Selectivity Index

For both ALR1 and ALR2, Valproic acid and Sorbinil were used as positive controls, respectively. Both compounds **1** and **2** were more active against ALR2 than ALR1 as shown by their IC_{50} values. Compound **2** was found more active against ALR1 as well as ALR2 with IC_{50} values 1.31 ± 0.67 and 0.93 ± 0.04, respectively. Compound **2** was three fold more potent as compared to the known reference inhibitor Sorbinil against ALR2.

3.2. In vitro Glucosidase Inhibition Assay Results

The compounds **1** and **2** were less potent in inhibition against both *α*- and *β*-glucosidase (Table 1).

3.3. Antiglycation Assay Results

Both the compounds were tested for their antiglycation activity. Compound **1** was found more potent with maximum percent inhibition of 50% as compared to compound **2**. Rutin was used as a reference drug for antiglycation assay (Table 1).

3.4. Molecular docking studies

In order to investigate the possible ligand interactions with ALR1 and ALR2, molecular docking studies were performed using Autodock 4.2 [22] to generate the binding modes. For ALR1, the compounds were docked to the selected PDB ID 3FX4 template of porcine [23, 24] X-ray structure because the human ALR1 X-ray structure of complex is not available; whereas for ALR2, the compounds were docked to PDB ID 1US0 [25].

Table 2. Docking scores for compounds **1** and **2** screened against ALR1 and ALR2

Compounds	ALR1		ALR2	
	Binding Energy (Kcal/mol)	**K_i (µM)**	**Binding Energy (Kcal/mol)**	**K_i (µM)**
1	-10.07	41.88	-10.50	42.62
2	-10.23	31.66	-10.72	13.79

The results showed that structures of the compounds were well prepared and the native bound conformations are reproduced well by the docking program of co-crystallized bound ligands. The docking results for both ALR1 and ALR2 were obtained showing different conformations (poses) predicted for both the compounds in which all the available water molecules and crystallographic ligand from the X-ray structures were removed prior to docking. Crystallographic position of NADPH was maintained by keeping it as rigid structure. Standard Autodock 4.2 parameters were used for the docking of selected inhibitors in the active sites of both ALR1 and ALR2 templates and Discovery Studio 4.0 Visualizer was used for the visualization of results. Table 2 shows the docking scores obtained with Autodock 4.2, generated binding free energy (ΔG) which provides a good quantitative assessment of the docking results.

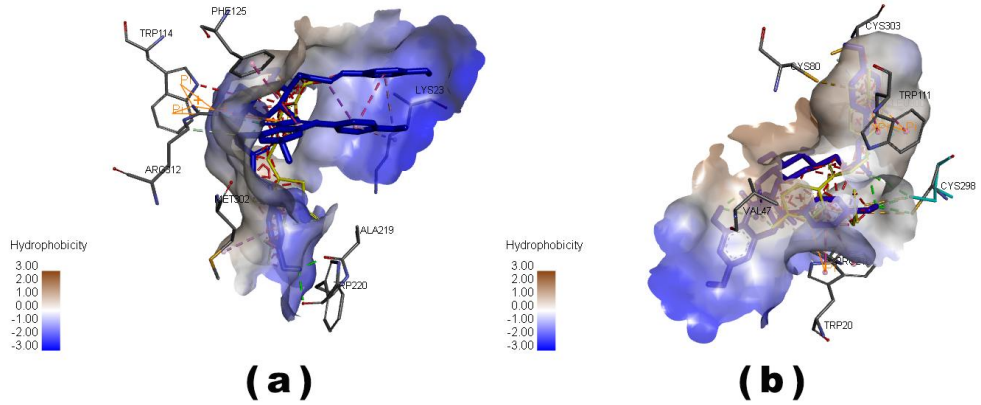

Figure 2. (a): Modeled and observed binding modes. Putative binding mode of compound **2** (blue) within the active site of ALR1 (grey) with the co-crystalized inhibitor FX4401 (yellow). Putative binding mode of compound **2** (blue) within the active site of ALR2 (grey) with the co-crystalized inhibitor IDD594 (yellow). (b): ALR2 co-crystallized with compound **2** and IDD594 inhibitor. The Trp111 and Thr113 residues form hydrogen bond interactions with the hydroxyl group of phenyl (ring B) moiety. The residue Tyr48 interacts *via* weak van der Waal interactions with the oxygen of chromen-3-yl moiety. Hydrophobic interactions of hydroxyl groups of compound **2** with Trp111 and Phe115 residues

Compound **2** was the best inhibitor against both ALR1 and ALR2 as shown in Figure 2a along with the inhibitor FX4401 co-crystallized in the active site of ALR1 (Fig. 2b). The chromen-3-yl moiety interacts *via* strong hydrogen bonding with the residue Trp22 and Trp220. Residue Arg312 also forms hydrogen bonding with the oxygen of chromen-3-yl moiety. Met302 and Trp220 form weak van der Waal interactions with the hydroxyl group attached to the pyran ring.

4. Conclusion

Due to no detailed phytochemical work on *E. caeruleum*, an indigenous plant, it was investigated phytochemically to obtain two new source flavone glycosides which have not been reported from the genus *Eryngium* before. Both the compounds (**1** and **2**) were investigated for *in vitro* antidiabetic potential against aldose reductases (ALR1 and ALR2), glucosidases (α and β), and their antiglycation potential. Both the compounds showed higher inhibition potential against ALR2 than ALR1. Compound **2** showed three fold higher potency against ALR2 than the reference drug Sorbinil. *In silico* studies have also been performed for understanding the binding mechanism of these compounds.

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Supporting Information

All the experimental details and protocols, discussion on structure elucidation of the compounds, and NMR spectra of the compounds are available in the Supporting Information which accompanies this paper on http://www.acgpubs.org/RNP.

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