SUPPLEMENTARY MATERIAL

Urease Inhibitory profile of extracts and chemical constituents of *Pistacia atlantica* ssp. *cabulica* Stocks

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Abstract

The current study was designed to evaluate the urease inhibitory profile of extract and fractions of *Pistacia atlantica* ssp. *cabulica* Stocks followed by bioactivity guided isolated of compounds. The crude extract was found significantly active urease inhibitor (95.40% at 0.2 mg/ml) with IC₅₀ values of 32.0 ± 0.28 µg/ml. Upon fractionation, ethyl acetate fraction displayed 100% urease inhibition with IC₅₀ values of 19.9 ± 0.51 µg/ml at 0.2 mg/ml. However, *n*-hexane and chloroform fractions exhibited insignificant urease inhibition. Similarly, the isolated compound, transilutin (**1**) and dihydro luteolin (**2**) demonstrated marked urease attenuation with 95 and 98% respectively, at 0.15 mg/ml. Both the isolated compounds showed marked potency with IC₅₀ values of 8.54 ± 0.54 and 9.58 ± 2.22 µg/ml, respectively. In short, both the extract and fractions and isolated compounds showed marked urease inhibition and thus could a useful natural source of urease inhibition.

Key words: *Pistacia atlantica* ssp. *cabulica* Stocks; chemical constituents; urease inhibitory activity.

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Experimental

Plant Material

Pistacia atlantica ssp. *cabulica* Stocks parts such as bark, leaves, roots and fruits were collected from Drosh Distt. Chitral, The plant materials were identified by plant Taxonomist (Ghulam Jilani) Department of Botany, University of Peshawar, Pakistan where the voucher specimen number Bot.20092(Pup) was deposited.

Extraction and Fractionation

Shade dried plant material was subjected to cold extraction with methanol for nine days at room temperature. The extract was filtered and then concentrated under reduce pressure at 40°C using rotary evaporator The crude methanolic extract was suspended in water and successively partitioned with *n*-hexane, chloroform, ethyl acetate to get their respective fractions.

Isolation

The ethyl acetate fraction is subjected for column chromatography and two compounds, transilutin (**1**) and dihydro luteolin (**2**) were isolated and the structures of the compounds 1 and 2 were characterized by comparing their spectra data with reported which coincide well (Jyh-horng et al., 2008; Yamashita 2000).

***In-vitro* urease assay**

Enzyme (jack bean urease) 25 µL solution and 55 µL buffers containing 100 mM urea were incubated with 5 µL of test samples (extracts and pure compounds) (0.5 mM concentration) at 30°C for 15 min in 96-well plates. Indophenol method was used to determine the urease activity by measuring the production of ammonia (Khan et al., 2014). Briefly, 45 µL each phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent

(0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μ L. The results (change in absorbance per min) were processed by using soft Max Pro software (molecular Device, USA). The entire assays were performed at pH 6.8.(Khan et al., 2013, Khan et al., 2014) Thiourea was used as the standard inhibitor of urease Percentage inhibitions were calculated by using the following formula

$$\text{Inhibition (\%)} = 100 - \frac{\text{Optical density of test well}}{\text{Optical density of control well}} \times 100$$

Molecular docking simulation

The crystal structure of Urease enzyme (PDB accession code of 4GY7 from jack bean) was retrieved from protein data bank (PDB). Energy refinement of crystal structure was done through swiss pdb viewer v4.1.0 program (Guex and Peitsch, 1997). The docking simulation was carried out through Autodock Vina (Trott and Olson, 2010) and i-GEMDOCKv 2.1 software (Hsu et al., 2011). At the start of compound **1-2** docking, both docking software procedures were optimized through co-crystallized ligand of receptor. Autodock vina was connected with PyRex tools (Yellamma et al. 2010). All the water molecules from urease receptor was removed, hydrogen were added and gasteiger charges calculation was done (Chang et al., 2010).The macromolecule, compound **1-2** and standard thiourea were uploaded in PyRex tool (Jacob et al., 2012). The conversion of receptor and compound files was take place into pdbqt format. Grid center was focus on already co-crystallized ligand of the urease enzyme, with a grid box of center x = 20 y = -57 z = -22 and size of x = 25Å y = 25Å z = 25Å with an exhaustiveness global search algorithm of 8 (Kumar et al., 2013). The docking study was also performed by i-GEMDOCKv2.1 software.

(Kumar et al., 2012) The docking was carried out a setup of 80 generations per compound with the population size of 800 random individuals and 10 solutions. The binding pocket of urease enzyme was identifying with a co-crystallized ligand at a distance of 12Å. The scoring function of iGEMDOCK is comprises of: $\text{Fitness} = \text{vdW} + \text{Hbond} + \text{Elec}$. The terms vdW, H bond and Elec are stand for vander Waal energy, hydrogen bonding energy and electro statistic energy respectively. The complex (urease + compounds) interactions were analyzed through LIGPLOT+ version v.1.4.5 (Laskowski and Swindells, 2011), PyMOL version 1.7.2. (DeLano, 2002) and Discovery studio visualizer version 4.0 software.

Figure S1: The surface overview of active site from urease enzyme having superimposition of standard thiourea shown by ball and stick (cyan color) while red color ball and stick indicate co-crystallized ligand and the docked compound **1-2** are shown by sticks green color.

Figure S2: The 2D and 3D schematic presentation of Compound 1 binding interactions with active site of urease enzyme.

Figure S3. The 2D and 3D schematic presentation of Compound 2 binding interactions with active site of urease enzyme

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