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## Urease inhibitory potential of *Zizyphus oxyphylla* Edgew. extracts and isolated compounds

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**Aim:** To determine the urease inhibitory potential of extracts and isolated compounds from *Zizyphus oxyphylla* Edgew.

**Materials and methods:** Methanolic extracts and subsequent fractions of *Z. oxyphylla* stem and 3 isolated compounds from the roots of *Z. oxyphylla* were obtained through column chromatography and subjected to in vitro urease inhibition.

**Results:** Three fractions (n-hexane, ethyl acetate, and butanol fractions) showed good to excellent activity, while the chloroform fraction showed nonsignificant activity. Amongst the isolated compounds, oxyphylline D (1) was the most active of the 3 isolated cyclopeptide alkaloids, followed by nummularin R (3) and nummularin C (2).

**Conclusion:** *Z. oxyphylla* has the potential to be used in the treatment of various complications produced by urease enzymes, such as gastric ulcers.

**Key words:** *Zizyphus oxyphylla*, cyclopeptide alkaloids, urease

### 1. Introduction

Urease is a nickel-containing enzyme and it catalyzes the production of ammonia and carbon dioxide from urea. It is important in the pathogenesis of various diseases in humans as it can act as a virulence factor for various bacteria (1). It plays an important role in the production of gastric ulcers as it locally neutralizes the gastric acid by the production of ammonia, thus providing an appropriate microenvironment for the existence of *Helicobacter pylori*, which is a major cause of illness and death worldwide (2). In addition, it can cause peptic ulcers by the production of a high concentration of ammonia, which disturbs mucosal permeability for hydrogen ions. Previous reports have suggested that urease-producing bacteria play a prominent role in the formation of infection-induced urinary stones (3). Therefore, scientists are constantly searching for compounds that can inhibit urease enzyme, thus avoiding the above-mentioned diseases.

Plants are old friends of humans, who rely on them directly or indirectly for food, shelter, aesthetic

purposes, and for the treatment of disease. Plants can be considered biological factories for the production of various medicinal compounds. Hence plants attract the common man as well as the scientific community for the investigation, authentication, and rationalization of their food and therapeutic effects (4). Pakistan is well known for its luxuriant flora, a major portion of which has not been studied (5), and so there is a need to explore the hidden medicinal potential of these plants.

*Zizyphus*, an important member of the family *Rhamnaceae*, possesses analgesic, antiinflammatory (6–9), antispasmodic (10), hepatoprotective (11), antidiabetic (12), and antioxidant activities (13). It has also been found that some species have antiulcerogenic activity (14). *Zizyphus oxyphylla* Edgew. has already been screened for its antibacterial, antifungal, phytotoxic, cytotoxic, insecticidal, and antinociceptive activities (15–17). Some cyclopeptide alkaloids isolated have exhibited antibacterial activities (18), while oil obtained from its stem and leaves also showed antimicrobial activity (19). However, no work

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exists on the urease inhibition potential of this plant; therefore, the current study was designed to test for this.

## 2. Materials and methods

### 2.1. Plant collection, extraction, and isolation of compounds

Plant material (stem and roots) was collected from the Swat district, Khyber Pukhtun Khwa Province, Pakistan, and identified by a recognized taxonomist, Dr Hasan Sher, Department of Botany, Jehanzeb Postgraduate College, Saidu Sharif Swat.

Air dried powdered roots of *Z. oxyphylla* (8 kg) were macerated in methanol (3 × 7 days × 20 L). On removal of the solvent under vacuum at between 35 and 40 °C, 372 g of crude extract was obtained, which was partitioned between water and dichloromethane. The dichloromethane extract (2.5 g) was subjected to column chromatography (CC) using silica gel (300 mm × 20 mm) and hexanes/acetone/diethylamine (75:25:0.1, 10 L) to afford 8 fractions (A–H). Compounds **1** (10.2 mg) and **2** (8.7 mg) were obtained from fractions D (41.3 mg) and E (53.4 mg) by preparative thin-layer chromatography (TLC) (hexanes/acetone/diethyl amine, 15:10:1). Air dried stem powder (8 kg) was extracted in a similar manner to give 375 g of crude extract, which was partitioned into various fractions, depending upon the polarity of the solvents, and so *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous fractions were obtained as reported previously (15–18). The chloroform extract (11.0 g) was subjected to CC over silica gel (325 mm × 65 mm) with hexanes/acetone/diethylamine (75:25:0.1, 10 L) to afford 8 fractions (A–H). Fraction C (202 mg) was subjected to preparative TLC (hexanes/acetone/diethylamine, 15:10:1) to obtain compound **3** (6.1 mg).

### 2.2. Urease inhibition bioassay

Enzyme (jack bean, 25 mL) and 55 mL of a buffer containing 100 mM of urea were incubated with 5 mL of test samples (0.5 mM concentration) in 96 well plates at 30 °C for 15 min. The indophenols method was used to determine the urease activity by measuring ammonia production. Phenol reagent and alkali reagent were prepared and 45 mL of phenol reagent and 70 mL of alkali reagent were added to every well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Devices, Sunnyvale, CA, USA)

The change in absorbance per minute was noted and the results processed using Soft-Max Pro software (Molecular Devices). All the tests were performed in triplicate. During all this assay pH was maintained at 8.2 by K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O (0.01 M), EDTA (1.0 mM), and LiCl<sub>2</sub> (0.01 M). Thiourea was used as a standard drug. The following formula was used to calculate the percent inhibition (20):

$$\text{Percent inhibition} = 100 - \left( \frac{\text{OD}_{\text{test well}}}{\text{OD}_{\text{control}}} \right) \times 100$$

## 3. Results

The 3 compounds isolated from *Z. oxyphylla* were identified first. Compound **1** was found to be oxyphylline D and compounds **2** and **3** were identified to be nummularin C and R (Figure). In this work we studied the urease inhibitory potential of these 3 compounds as well as crude extract of the plant and various fractions thereof.

The urease inhibitory activity of crude extract from the stem of *Z. oxyphylla* and its various fractions are presented in Table 1. It is obvious from this table that the maximum urease inhibitory activity was shown by the ethyl acetate fraction (% inhibition = 86.7 ± 0.03, IC<sub>50</sub> ± SEM = 43.4 ± 2.01). It was followed by the butanol fraction, which produced a % inhibition of 81.2 ± 0.01 with 49.8 ± 1.06 IC<sub>50</sub> ± SEM value. The crude extract also exhibited good urease inhibitory activity, while the aqueous fraction gave a reading of 63.1 ± 0.02 and 164.7 ± 2.31 for IC<sub>50</sub> ± SEM. The chloroform fraction of the stem exhibited a low urease inhibitory activity, giving a reading of 18.9% ± 0.02, while the *n*-hexane fraction provided a nonsignificant activity of 3.8%.

**Table 1.** Urease inhibitory activity of stem.

Samples	% Inhibition	IC <sub>50</sub> ± SEM
Crude extract	75.8 ± 0.03	105.2 ± 1.61
<i>n</i> -hexane fraction	3.8 ± 0.01	-
Chloroform fraction	18.9 ± 0.02	-
Ethyl acetate fraction	86.7 ± 0.03	43.4 ± 2.01
Butanol fraction	81.2 ± 0.01	49.8 ± 1.06
Aqueous fraction	63.1 ± 0.02	164.7 ± 2.31
standard	98.1 ± 0.02	21 ± 0.011

The urease inhibitory potentials of various cyclopeptide alkaloids from *Z. oxyphylla* are given in Table 2. Compound **1** was the most active of the 3. It exhibited good activity at 58.2 ± 0.02% and IC<sub>50</sub> ± SEM 420.1 ± 1.22, while compound **2** exhibited the lowest. It exhibited a 29% urease inhibitory activity. Compound **3** showed a low to moderate activity of 35.7 ± 0.02.

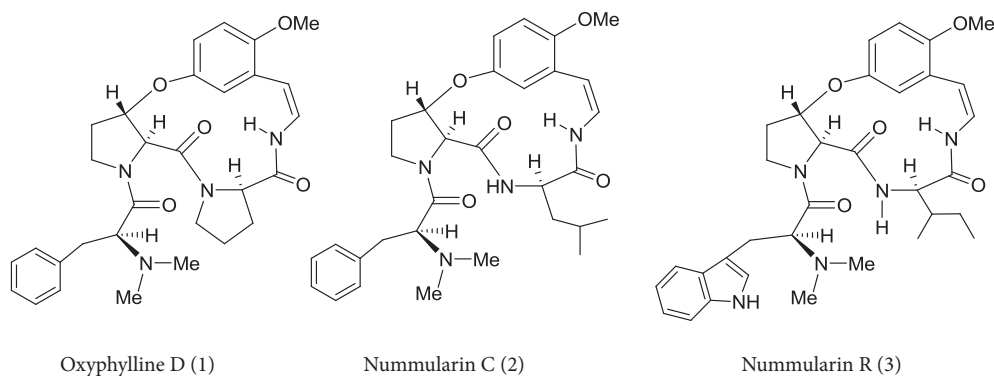
## 4. Discussion

There has been increasing interest in studying the biological properties of plant extracts and isolated compounds globally (18,21–24). Researchers are blending traditional knowledge with experimental methodology for testing the efficacy and safety of these herbal remedies (25,26). The current study is a part of these efforts.

The order of urease inhibitory activity observed for crude extract and fractions obtained from the stem of *Z. oxyphylla* was ethyl acetate > butanol > crude > aqueous > chloroform > *n*-hexane. It is obvious from Table 1 that

**Table 2.** Urease inhibitory activity of isolated compounds.

Compound	Concentration(mM)	% Inhibition	IC <sub>50</sub> ± SEM (µM)
1	0.5	58.2 ± 0.02	420.1 ± 1.22
2		29.3 ± 0.01	-
3		35.7 ± 0.02	-
STD		98.1 ± 0.2	21 ± 0.011

**Figure.** Structure of compounds isolated from *Z. oxyphylla*.

ethyl acetate and n-hexane fractions are the most active and the least active respectively. The activity present in crude extract and fractions is due to the presence of various bioactive constituents (27,28). The distribution of these bioactive constituents in various fractions varies according to the solvents used. The results indicated that the maximum chances of bioactive constituents presence was in the ethyl acetate fraction, and so this fraction was subjected to CC for isolation of compounds. Compound 3 was isolated and identified as numularin R, while compounds 1 and 2 were identified as oxyphylline D and numularin C respectively. The order of urease inhibitory activity observed for these compounds was oxyphylline

D > numularin R > numularin C. It is generally accepted that activities exhibited by isolated compounds depend on the structure of the compound. The current results authenticate the traditional usage of this plant as antiulcer remedy. Other species of this genus *Zizyphus* have been reported to have antiulcer activity (11). Antibiotics are an effective remedy against ulcers produced by *H. pylori* infection but now people are turning towards natural products because of the increasing problem of antibiotic resistance, side effects, and the significant cost of synthetic drugs. This plant has the potential to be used in the treatment of various complications produced by urease enzymes, such as gastric ulcers.

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