Research Article

Urease Inhibitory Activity of Aerial Parts of Artemisia scoparia: Exploration in an In Vitro Study

Murad Ali Khan,1 Haroon Khan,2 Shafiq Ahmad Tariq,3 and Samreen Pervez4

1 Department of Chemistry, Kohat University of Science and Technology, Kohat 26000, Pakistan
2 Department of Pharmacy, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan
3 Institute of Basic Medical Sciences, Khyber Medical University, Peshawar 25210, Pakistan
4 Department of Pharmacy, University of Peshawar, Peshawar 25210, Pakistan

Correspondence should be addressed to Haroon Khan; hkdr2006@gmail.com

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Artemisia scoparia has been used in the treatment of different disorders including ulcers. The current study was therefore designed to investigate the aerial parts of Artemisia scoparia (crude extract, total sterol and flavonoidal contents, and aqueous fraction) for its urease inhibitory potential. The crude of the plant evoked marked attenuation on urease activity, when tested in various concentrations with IC50 values of 4.06 mg/ml. The inhibitory potential was further augmented in the aqueous fraction (IC50: 2.30 mg/ml) of the plant. When the total sterol and flavonoidal contents were challenged against urease, both showed concentration dependent activity; the latter showed maximum potency with IC50 values of 8.04 and 2.10 mg/ml, respectively. In short, the aerial parts of the plant demonstrated marked antagonism on urease and thus our study validated the traditional use of Artemisia scoparia in the treatment of ulcer.

1. Introduction

Urease (urea amidohydrolase) is usually found in different bacteria, fungi, algae, and plants, an enzyme that catalyzes the hydrolysis of urea to ammonia and carbamate, which is the final step of nitrogen metabolism in living organisms [1]. Carbamate rapidly and spontaneously decomposes, yielding a second molecule of ammonia. These reactions may cause significant increase in pH and are responsible for negative effects of urease activity in human health and agriculture [2, 3].

From the medical viewpoint, infections produced by these bacteria such as Helicobacter pylori and Proteus mirabilis usually have a high urease activity. Urease is central to H. pylori metabolism and virulence, is necessary for its colonization of the gastric mucosa, and is a potent immunogen that elicits a vigorous immune response. This enzyme is used for taxonomic identification and for diagnosis and followup after treatment and is a vaccine candidate. Urease represents an interesting model for metalloenzyme studies. Before the discovery of H. pylori, humans were thought to produce “gastric urease.” It is now known that the source of this notable protein is this bacterium, which colonizes the gastric mucosa of humans. It contributes in urinary tract and gastrointestinal infections, probably augmenting the severity of several pathological conditions like peptic ulcers and stomach cancer as in the case of H. pylori. Ureases are also involved in the development of different human and animal pathogenicity of urolithiasis, pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation [4–6].

Being involved in the pathophysiology of multiple human and animal disorders, targeting urease for treating pathogenic disorders caused by urease-producing bacteria has already opened a new line of treatment for infections caused by such bacteria. Indeed more effective and potent compounds are required with a whole new level of safety and specificity. In this regard, urease inhibitors have gained tremendous attention over the years which resulted into the discovery of numerous inhibitors [5, 7–11].
The genus *Artemisia* L. consists of approximately 522 species found throughout the northern half of the world [12]. The aerial parts of the plant have been widely used for their hypoglycemic, hypolipidemic, diuretic, antulcer and anti-inflammatory activities [13] and antisipetic, antibacterial, cholagogue, diuretic, purgative, and vasodilator properties [14, 15]. The essential oils of *A. scoparia* showed strong insecticidal activity against stored-product insects. Similarly, the aerial parts of plant exhibited significant anti-malarial, free radical scavenging, and insecticidal activities [16].

Phytochemical analysis of the genus led to the isolation of several coumarins, flavonoids, phenylpropanoids, sterols and terpenoids (specially sesquiterpenes and monoterpenes), and their glycosides [16]. Keeping in view the antiulcer use of the plant, the current study was designed to investigate urease inhibitory properties of crude methanolic extract, total flavonoidal and sterol contents, and aqueous fraction of aerial parts of the plant *in vitro*.

## 2. Materials and Methods

### 2.1. Plant Material

Fresh plant of *Artemisia scoparia* was collected from Parachinar Valley, Pakistan. The taxonomic identity of the plant was determined by a qualified plant taxonomist at Department of Botany Kohat University of Science and Technology, Pakistan. The plant was washed 2-3 times with running tap water followed by shade-drying. The plant material was powdered for extraction.

### 2.2. Chemicals Used

- Urea (Sigma-Aldrich), Sodium Nitroprusside, Phenol Red (BDH Chemicals Ltd, England), Thiourea, Sodium Dihydrogen Phosphate, (Merck, Germany), Urease Jack beans (Avonchem Ltd, UK), Sodium Hypochlorite (HC Haq Chemicals, Pakistan), and Dimethyl Sulfoxide (UNI-Chem).

### 2.3. Preparation of Solvent Extraction

2 kg of the shade-dried powder of plant materials was soaked separately in methanol for 10 days, extracted three times at room temperature in the same solvent, and then filtered. The diluted extracts were concentrated on the vacuumed rotary evaporator under reduced pressure at a temperature of 46°C to give a residue (extract), which was further suspended in water and partitioned to get aqueous fraction. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these extracts was weighed and stored in airtight bottles for further use.

### 2.4. Extraction of Total Flavonoid Contents

Flavonoids content of the crude extract of the aerial parts of *A. scoparia* was estimated using our previously reported method [17]. Briefly, 10 g was extracted repeatedly with 10 mL of the 80% aqueous methanol at room temperature. The resulting solutions were filtered through Whatman filter paper No. 42 (125 mm). The filtered was then transferred into a crucible and evaporated at water bath and weighted which account for total flavonoid contents.

### 2.5. Extraction of Total Sterol Contents

For the determination of total sterol contents, powdered plant material of aerial parts of *A. scoparia* was extracted with methanol 3 times and was concentrated. Then it was suspended in 5% methanol and filtered. Aqueous extract was exhaustively extracted with hexane [18]. The resulting hexane soluble extract was evaporated and dried which represents the total sterol contents.

### 2.6. Urease Assay

Exactly 25 μL of enzyme (Jack Bean Urease) solution and 5 μL of test compounds (0.5 mM concentration) were incubated for 15 min at 30°C [9]. The aliquot was taken after 15 min and again incubated with 55 μL of buffers containing 100 mM urea for 15 min at 30°C. Ammonia production was measured as a urease activity by indophenol method as described earlier [19]. Final volumes were maintained as 200 μL by adding 45 μL 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). The increase in absorbance was measured at 630 nm after 50 min at pH 8.2. The results (change in absorbance per min) were calculated spectrometrically on different concentrations of drugs in the absence and presence of ascorbic acid. Thiourea was used as the standard inhibitor and percentage inhibitions were calculated as follows:

\[
\%\text{Inhibition} = 100 - \left( \frac{OD_{\text{test well}}}{OD_{\text{control}}} \right) \times 100. \tag{1}
\]

The IC₅₀ values were calculated using statistical software, GraphPad PRISM 6.

### 2.7. Statistical Analysis

The resulting data were expressed as the mean ± SEM (n = 3) in each group. To determine the differences between groups, one way analysis of variance (ANOVA) was performed (GraphPad PRISM 6, San Diego, CA, USA) using the least significant difference (LSD) test at \( P < 0.05 \) or \( P < 0.01 \).

## 3. Results

### 3.1. Effect of Total Sterol and Flavonoidal Contents

As shown in Figure 1, the crude extract contained significant quantity of both sterols and flavonoidal contents 12.67 and 14.99%, respectively.

### 3.2. Effect of Crude Extract of *A. scoparia* on Urease Inhibition

The effect of urease inhibition on the crude extract of the aerial parts of *A. scoparia* is present in Figure 2. The urease inhibition was observed on various concentrations of the extract with maximum antagonism (60.65%) at 10 mg/mL. The estimated IC₅₀ value of crude form of the aerial parts of the plant was 4.06 mg/mL (Table 1).

### 3.3. Effect of Total Sterol Contents of *A. scoparia* on Urease Inhibition

As shown in Figure 3, the total sterol contents of *A. scoparia* provoked concentration dependent urease inhibition. The maximum activity (54.13%) was observed
3.4. Effect of Total Flavonoidal Contents of A. scoparia on Urease Inhibition. The results of urease inhibition of total of flavonoidal contents of aerial parts of A. scoparia are shown in Figure 4. It had attenuated urease in a concentration dependent manner. The maximum antagonism (86.17%) was obtained at the concentration of 10 mg/mL, while the IC\textsubscript{50} value was calculated as 2.10 mg/mL (Table 1).

3.5. Effect of Total Aqueous Fraction of A. scoparia on Urease Inhibition. Urease inhibition caused by the aqueous fraction of A. scoparia is illustrated in Figure 5. It demonstrated marked attenuation of urease in a concentration dependent manner with maximum inhibition of 81% at 100 mg/mL. The estimated IC\textsubscript{50} value for aqueous was 2.30 mg/mL (Table 1). The standard used in assay, Thiourea, showed dose dependent activity with maximum inhibition 91% at 100 μg/mL (Figure 6).

4. Discussion

The results of our study showed profound inhibition of urease (Jack Bean) of the extract, total flavonoidal and sterol contents, and aqueous fraction of A. scoparia in a concentration dependent manner. The results indicated that the total flavonoidal contents were most potent followed by the aqueous fraction and crude extract of the plant, respectively. However, the sterol contents were least potent at a dose of 100 mg/mL. It had a significant IC\textsubscript{50} value of 8.04 mg/mL (Table 1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drugs</th>
<th>IC\textsubscript{50} (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crude methanolic extract</td>
<td>4.06 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>Sterols</td>
<td>8.04 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>2.10 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous</td>
<td>2.30 ± 0.03</td>
</tr>
</tbody>
</table>

The estimated IC\textsubscript{50} values of tested drugs.

Table 1: The estimated IC\textsubscript{50} values of tested drugs.

FIGURE 1: The total sterols and flavonoidal contents (%) in the crude extract of Artemisia scoparia.

FIGURE 2: The percent effect of crude extract of Artemisia scoparia against urease. The resulting data were shown as mean ± SEM of three independent assays. One way one analysis of variance (ANOVA) was carried out for the determination of difference between groups. \( P < 0.5 \) was considered as significant.

FIGURE 3: The percent effect of total sterol contents of Artemisia scoparia against urease. The resulting data were shown as mean ± SEM of three independent assays. One way one analysis of variance (ANOVA) was carried out for the determination of difference between groups. \( P < 0.5 \) was considered as significant.

FIGURE 4: The percent effect of total sterol contents of Artemisia scoparia against urease. The resulting data were shown as mean ± SEM of three independent assays. One way one analysis of variance (ANOVA) was carried out for the determination of difference between groups. \( P < 0.5 \) was considered as significant.

FIGURE 5: The percent effect of total sterol contents of Artemisia scoparia against urease. The resulting data were shown as mean ± SEM of three independent assays. One way one analysis of variance (ANOVA) was carried out for the determination of difference between groups. \( P < 0.5 \) was considered as significant.

FIGURE 6: The percent effect of total sterol contents of Artemisia scoparia against urease. The resulting data were shown as mean ± SEM of three independent assays. One way one analysis of variance (ANOVA) was carried out for the determination of difference between groups. \( P < 0.5 \) was considered as significant.
potent in urease inhibition. It reflects that polar constituents of the plant were responsible for the current urease inhibitory effect.

In summary, the aerial parts of A. scoparia possessed potent urease inhibitory constituents that could be possibly flavonoids in nature. Thus the study provided scientific evidence to the traditional uses of the plant in the treatment of ulcers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


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