Short Communication

Preliminary phytochemical screening and evaluation of the antibacterial and mutagenic activity of *Rhynchocorys elephas* (L.) Griseb

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Traditional medicine has a key role in health care worldwide. Obtaining scientific information about the efficacy and safety of the plants is one of the researcher’s goals. In this research, the flowering aerial parts of *Rhynchocorys elephas* (L.) Griseb (Scrophulariaceae) were collected from Siah Bisheh (Mazandaran, Iran) in May 2009. Extract was tested for its antibacterial activity using 3 Gram-positive bacteria strains (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus cereus*) and 2 Gram-negative bacterial strains (*Escherichia coli* and *Salmonella typhi*) by cup plate method. Its mutagenic activity was also investigated using Ames test on *Salmonella typhi* TA100. Here we report for the first time the activity of *R. elephas* against *S. aureus* (MIC = 31.25 mg/ml), *S. epidermidis* (MIC = 31.25 mg/ml), *B. cereus* (Minimum inhibitory concentration (MIC) = 62.5 mg/ml), *E. coli* (MIC = 62.5 mg/ml) and *S. typhi* (MIC = 62.5 mg/ml). Furthermore the mutagenic potential of *R. elephas* using Ames test studied and no mutagenic effects was detected. The results of preliminary phytochemical study showed the presence of saponins, flavonoids and tannins in the methanolic extract of flowering aerial parts of this plant.

Key words: *Rhynchocorys elephas* (L.) Griseb., scrophulariaceae, antibacterial effect, mutagenic activity.

INTRODUCTION

The genus *Rhynchocorys* (family Scrophulariaceae) comprises of 5 species which are naturalized in Europe and some parts of Asia. Two of these species grow wild in Iran. *Rhynchocorys elephas* (L.) Griseb. is one of the species which grow in north of Iran (Saeidi, 2006). There is no evidence of its traditional use in folk medicine. Since the literature survey revealed that there is no work on phytochemical and biological activities of *R. elephas*, we were prompted to investigate the antibacterial activity of methanolic extract of flowering aerial parts of the plant using the Cup Plate method and its mutagenic effects by using the Ames mutagenicity test. A preliminary phytochemical screening, using standard phytochemical reaction methods, was also done to determine the main active principles of *R. elephas*.

MATERIALS AND METHODS

Plant material

The flowering aerial part of *R. elephas* (L.) Griseb was collected from Siah-Bisheh (Mazandaran, Iran) in May 2009 and identified by Dr. G. H. Amin at the Pharmacognosy Department, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen (NO. 178) has been deposited in the herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Azad Islamic University (AIU), Tehran, Iran.

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Extraction and isolation

The dried ground material was extracted by percolator apparatus using methanol. The extract was concentrated by Rotary Evaporator apparatus and the solvent removed to produce a dark green gummy solid. The resulting extract was kept in a sterile vial in a dark and cool place for further test study.

Test microorganisms

Gram-positive bacteria including Staphylococcus aureus (PTCC 1112), Staphylococcus epidermidis (PTCC 1435) and Bacillus cereus (PTCC 1015), and Gram-negative bacteria including Escherichia coli (PTCC1330) and S. typhi (PTCC1639) were obtained from Persian type culture collection (PTCC) of Iranian Research Organization for Science and Technology.

Antibacterial assay

Antibacterial activity of the methanolic crude extract of R. elephas was investigated against 5 bacterial strains by the cup plate method (Fazly et al., 2005). An overnight bacterial culture equal to 0.5 Mac Farland standard (1.5 x 10^8 CFU/ml) was used to culture on Muller-Hinton agar plates. The wells were made on agar plates with 5 mm diameter. The methanolic extract at 150, 100, 50 and 25 mg was separately dissolved in 1 ml methanol and was filtered, and 80 µl of each solution was added to each well. Simultaneously, one well penicillin (50 mg/ml) was used as positive control and in another well 80 µl of pure methanol served as negative control. The plates were incubated at 37°C for 24 h. The diameter of the zone of inhibitions was detected in each plate. The experiments were carried out 4 times and the results were presented as mean ± SD.

Minimum inhibitory concentration (MIC)

MIC of the extract was determined by testing 8 concentrations of the extract against gram-positive and Gram-negative bacteria, by micro dilution method and using Muller Hinton broth. The reconstituted extract was diluted to give concentrations of 250, 125, 62.5, 31.25, 15.625, 7.812, 3.906 and 1.953 mg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC (Mehregan et al., 2008). As the same penicillin and pure methanol were used as positive and negative control, respectively.

Mutagenic activity assay

Chemicals and positive mutagens

D-biotin, glucose, 1-histidine-HCl monohydrate, crystal violet and sodium chloride were purchased from Sigma Chemicals (UK). Ampicillin trihydrate was from Fluka (Germany), oxoid agar, oxoid nutrient broth No. 2 from oxoid Limited (UK) and magnesium chloride, potassium phosphate, potassium chloride, citric acid monohydrate, sodium ammonium phosphate, sodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Merck (Germany). The positive mutagens sodium azide (NaN₃) and 2-Aminoanthonacene (AA) were purchased from Sigma Chemicals (UK).

Ames mutagenicity test

The Salmonella mutagenicity assay was carried out according to the method described by Maron and Ames (1983). Oxid nutrient broth No. 2 was used for overnight culture. For plate incorporation assays, 0.1 ml of bacterial strain (TA100), 0. 25 ml of S9 mix if appropriate and the sample to be tested were added to 2 ml of molten top agar. The content were mixed and poured on agar plates. After 48 to 72 h of incubation, revertant colonies were counted (Claxton et al., 1987). At least 3 plates were used for each dose and each experiment was repeated 2 times. The tester strain was checked routinely for ampicillin resistance, ultraviolet-light sensitivity, crystal-violet sensitivity, histidin requirement and spontaneous reversion rate.

Phytochemical screening

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods of Aiyegoro et al. (2009) and Pascual et al. (2002) with little modifications. Exactly 1.0 g of plant extract was dissolved in 10 ml of distilled water and filtered (using Whatman No. 1 filter paper) A blue coloration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% hydrogen chloride (HCl) on steam bath. 1 ml of the filtrate was treated with few drops of Dragendorff’s reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloid. About 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicated as the flavonoids. Freshly prepared 7% blood agar plate was used and wells were made in it. The crude extract dissolved in 10% methanol was used to fill the wells bored in the blood agar plates. 10% methanol was used as a negative control while commercial saponin solution was used as a positive control. The plates were incubated at 35°C for 6 h, complete haemolysis of the blood around the extract was indicative of saponin. About 0.5 g of the extract was dissolved in 3 ml of chloroform and filtered. Concentrated H₂SO₄ was carefully added to the filtrate to form lower layer. A reddish brown color at the interface was taken as positive for steroid ring.

RESULTS

The inhibitory effects of methanolic extract of R. elephas (L.) Griseb against different microorganisms are shown in Table 1. The extract indicated significant antibacterial activity (inhibition zone diameters ranging from 9 to 32 mm) against gram-positive bacteria including S. aureus, S. epidermidis and B. cereus and gram-negative bacteria including E. coli and S. typhi. After detection antibacterial effect in methanolic extract of R. elephas by cup plate method, the MIC of the extract was also determined using the microdilution method for both gram-positive and gram negative tested bacteria. However, the methanolic extract of R. elephas had antibacterial effect on both gram positive and negative bacteria, it’s effect on S. aureus and S. epidermidis was more with MIC 31.25 mg/ml compare to B. cereus, E. coli and S. typhi with MIC 62.5 mg/ml (Table 2). The results of Ames test showed that R. elephas has no mutagenic effect. Through the phytochemical screening, the presence of flavonoids, saponins and tannins has been indicated in the floral.
Table 1. Antibacterial activity of *R. elephas* methanolic extract.

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Diameter of inhibition zone (mm)</th>
<th>Pen</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>22.5</td>
<td>30.7</td>
</tr>
<tr>
<td>S. epidermis</td>
<td>-</td>
<td>9.2</td>
<td>15.2</td>
</tr>
<tr>
<td>S. typhi</td>
<td>-</td>
<td>-</td>
<td>11.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
</tr>
<tr>
<td>B. cerus</td>
<td>-</td>
<td>10.1</td>
<td>11.2</td>
</tr>
</tbody>
</table>

ª Mean value of four independent experiments; Pen (Penicillin) (50 mg/ml) was used as positive control; Methanol was used as negative control; -, no inhibition.

Table 2. Minimum inhibitory concentration (MIC) of *R. elephas* methanolic extract.

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>MIC (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>R. elephas</td>
</tr>
<tr>
<td>S. aureus</td>
<td>31.25</td>
</tr>
<tr>
<td>S. epidermis</td>
<td>31.25</td>
</tr>
<tr>
<td>S. typhi</td>
<td>62.5</td>
</tr>
<tr>
<td>E. coli</td>
<td>62.5</td>
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<tr>
<td>B. cerus</td>
<td>62.5</td>
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</table>

ª All determinations were done in triplicate.

**DISCUSSION**

Since through the phytochemical screening, the presence of only flavonoids, saponins and tannins has been indicated in the flowerial aerial parts of the plant, we suggest the strong antibacterial effect due to flavonoids because it is more likely to possess antibacterial activity. Flavonoids antibacterial activity is probably due to their ability to form complex with extra-cellular and soluble proteins, as well as bacterial cell wall. Lipophilic flavonoids may also disrupt bacterial membranes (Tsuchiya et al., 1996). As no mutagenic effect was observed for *R. elephas* at different solutions, we can suggest this plant as an antibacterial agent which has passed one of its safety parameters. Based on the results of this study, further in *vivo* and *ex vivo* confirmatory tests are recommended.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


