EVALUATION OF ANTIFUNGAL ACTIVITY OF THE STEM BARK OF ALSTONIA SCHOLARIS LINN. R.BR

Vishesh Upadhyay1, Harsha Leena K1, Virendra Yadav1 and Richa Upadhyay2

1Vinayaka College of Pharmacy Kullu, HP India 2D.A.V Mohal kullu,HP India

ABSTRACT

Authenticated by Dr. Madhava Chetty, Sri Venkateshwara University, Tirupathi. The antifungal activity of the stem bark of Alstonia scholaris Linn. R. Br. was investigated as the fungal species are gaining resistance to the antibiotics discovered and there is a necessity for the search of new antibiotics. The plants are the cheapest and safe alternative source of antimicrobials. The powder of the dried stem bark was extracted with different solvents in increasing order of the polarity by soxhletion. Antifungal activity was tested against Aspergillus niger, Alternaria alternata, Fusarium solani, Trichoderma virens using agar disc diffusion method. The ethanol and aqueous extracts exhibited significant antifungal activity when compared to other extracts. The results were compared with the standard drug, Ketoconazole.

INTRODUCTION

Alstonia scholaris Linn. R. Br. (Apocynaceae), popularly known as Saptaparni1 is used in traditional medicine as a tonic, antiperiodic, anthelminthic, stimulant, carminative, stomachic and expectorant. The bark is a bitter tonic and febrifuge, useful for the treatment of malaria, diarrhea and dysentery. The decoction of the bark is used to treat asthma, hypertension, lung cancer and pneumonia2,3. The literature suggests that the bark mainly contains alkaloids, steroids and triterpenoids4. The methanolic extract of the bark showed anti stress, antioxidant and nootropic activities5. The butanolic fraction of the methanolic extract of the stem bark was reported to show the antibacterial activity6. The ethanolic and aqueous extracts were reported to promote wound healing activity7 where as the ethanolic extract possessed antioxidant activity8. Hence the present work is done to investigate the antifungal activity of different extracts of the bark of Alstonia scholaris Linn. R. Br.

MATERIALS AND METHODS

Collection of Plant Material

The fresh bark of Alstonia scholaris Linn. R. Br. was collected in the month of June 2010 and authenticated by Dr. Madhava Chetty, Sri Venkateshwara University, Tirupathi.

Preparation of Extracts

The stem bark of Alstonia scholaris Linn. R. Br. was dried in shade for two weeks and powdered. The coarse powder (400gm) was subjected to soxhlet extraction with 1.5 ltrs of various solvents viz., petroleum ether, benzene, chloroform, acetone, methanol, ethanol and distilled water in the ascending order of polarity. Each time before extraction with next solvent, the marc was air dried and the extract was concentrated by distilling the solvent under reduced pressure using rotary flash evaporator. The extracts obtained were suspended in dimethyl sulphoxide (DMSO) to prepare different concentrations ranging from 200 µg/ml to 1200µg/ml and used for screening the antifungal activity.

Phytochemical Screening

All the extracts were screened for the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, steroids, tannins and triterpenoids.

Micro-Organisms Used

Aspergillus niger, Alternaria alternata, Fusarium solani, Trichoderma virens were used for the antifungal screening.

Preparation of Media

The medium was prepared by dissolving saboraud dextrose Agar (HiMedia Laboratories Pvt. Ltd.) in distilled water and autoclaving at 121°C for 15 minutes. It is used for preliminary antifungal study.

Preparation of Inoculum

Stock cultures of Aspergillus niger, Alternaria alternata, Trichoderma virens and Fusarium solani were maintained at 4°C on slants of SDA. Active cultures for experiment were prepared by transferring a loopful of microorganisms from stock cultures to test tubes of SDA slants and incubated for 24 hours at 37°C.

Antifungal Susceptibility Test

The disc diffusion method9 was used to screen the antifungal activity. The SDA plates were prepared by pouring 100 ml of molten media in to sterile petriplates. The plates were allowed to solidify and inoculum suspension was spreaded uniformly with glass spreader. Different extracts were loaded on 6mm
sterile discs till saturation. The loaded discs were placed on surface of medium and compound was allowed to diffuse for 5 minutes. The plates were kept for incubation at 37°C for 24 hours. Zone of inhibition formed around the disc was measured with transparent ruler in mm. These studies were performed in triplicate by using standard drug, Ketoconazole. MIC values were also determined for microorganisms. MIC was defined as the lowest concentration of extract that inhibited the visible growth on agar.

Chemicals Used
- Ketoconazole (standard drug)
- Dimethyl sulphoxide (DMSO)
- S D Agar medium

Statistical Analysis
The values are represented as mean ± standard error of mean (SEM) for triplicate set of experiments.

RESULTS AND DISCUSSION
Preliminary phytochemical screening of the stem bark of *Alstonia scholaris* Linn. R. Br. showed the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, steroids, tannins and triterpenoids. Results of preliminary phytochemical screening are tabulated in table 1.

The zone of inhibition of different extracts and standard were recorded in table 2. All the extracts were found to be active against the test organisms. Of all the extracts, ethanol and aqueous extracts showed significant activity. The antifungal potency of the stem bark of *Alstonia scholaris* Linn. R. Br. maybe attributed to single or the combined effect of the phytoconstituents present in the bark.

The values are represented as mean ± standard error of mean (SEM) for triplicate set of experiments.

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of the stem bark of *Alstonia scholaris* Linn. R. Br. showed the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, steroids, tannins and triterpenoids. Results of preliminary phytochemical screening are tabulated in table 1.

The zone of inhibition of different extracts and standard were recorded in table 2. All the extracts were found to be active against the test organisms. Of all the extracts, ethanol and aqueous extracts showed significant activity. The antifungal potency of the stem bark of *Alstonia scholaris* Linn. R. Br. maybe attributed to single or the combined effect of the phytoconstituents present in the bark.

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of the stem bark of *Alstonia scholaris* Linn. R. Br. showed the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, steroids, tannins and triterpenoids. Results of preliminary phytochemical screening are tabulated in table 1.

The zone of inhibition of different extracts and standard were recorded in table 2. All the extracts were found to be active against the test organisms. Of all the extracts, ethanol and aqueous extracts showed significant activity. The antifungal potency of the stem bark of *Alstonia scholaris* Linn. R. Br. maybe attributed to single or the combined effect of the phytoconstituents present in the bark.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Petroleum ether extract</th>
<th>Benzene extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ : Present; - : Absent

| Table 1 | Preliminary phytochemical evaluation of the stem bark of *Alstonia scholaris* Linn. R. Br |

**Zones of inhibition (mm)**

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Petroleum ether extract</th>
<th>Benzene extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 | Antifungal activity of different extracts of the stem bark of *Alstonia scholaris* Linn. R. Br**

**Table 3 | Antifungal activity of different extracts of the stem bark of *Alstonia scholaris* Linn. R. Br**

**Table 4 | Antifungal activity of the standard drug, Ketoconazole.**

**Table 5 | Minimum inhibitory concentration (MIC) values of Different extracts of the stem bark of *Alstonia scholaris* Linn. R. Br.**

**CONCLUSION**

The findings in the present study offer a scientific support to the use of stem bark of *Alstonia scholaris* Linn. R. Br. as an antifungal in new drugs for therapy as it showed significant antifungal activity compared to standard drug, Ketoconazole.

**Acknowledgement**

The authors are thankful to the chairman, director and the principal, The Oxford College of Pharmacy, Bangalore for providing necessary facilities and support to conduct this work and Dr. Madhava Chetty, Sri Venkateshwara University, for providing the authenticated drug material.

---

835 | Page
References


********