EVALUATION OF ANTIFUNGAL ACTIVITY OF ROOTS OF ACHYRANTHES ASPERA FOR RINGWORM INFECTION

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ABSTRACT
The plant Achyranthes aspera is widely used in Ayurvedic system of medicine as Anti-diabetic, Anti-asthmatic, diuretic, Antiviral, Anti-carcinogenic, tooth brush, appetite and cure gastric disorders. Roots are used in treatment of snakebites. Through literature survey it has been revealed that the paste of the roots of the plant is beneficial in treatment of ringworm infection. Thus, the study is carried out in order to determine the antifungal (ringworm) activity of the roots of the plant. The roots of the plant are subjected to extraction with water and ethanol. The antifungal activity was carried out using agar well diffusion method. All the plant extracts were compared with standard Clotrimazole (1\% w/w) as positive control and distilled water, ethanol used as negative control. Accordingly ethanolic extract of the roots was found to more effective against Trichophyton rubrum as compared to aqueous extract.

KEYWORDS: Antifungal, Clotrimazole, Trichophyton rubrum, Achyranthes aspera.

INTRODUCTION
Various species of Achyranthes are used medicinally in India, China and Bangladesh. The plant Achyranthes aspera is also known as apamarg, chichira, and chaff flower. It taxonomically belongs to the family Amaranthaceae. The A. aspera (L.) Pers. is naturalized throughout the hot and moist parts of India.\textsuperscript{[1]}

The leaves used for treatment of Gonorrhea, Bowel complaint, piles and skin eruptions.\textsuperscript{[2]} It also used as diuretic, Anti-periodic, Antimicrobial, Anti-inflammatory, shows Hypoglycemic effects and Anti-carcinogenic effects.\textsuperscript{[3,4,5,6,7,8]} Roots are used in pneumonia, as an astringent to bowels, menstrual disorders, stomachic and reported to have anti-fertility activity.\textsuperscript{[9,10]} Chemically, Achyranthes aspera contains Triterpenoid saponins which possesses oleanolic acid (0.54\%), aglycone A, B, C and D, ecdysterone, long chain alcohol, 17-penta triacanotanol, water soluble base betaine and enzyme level are isolated. Two long chain compound isolated from shoots have been characterized as 27-cyclohexyloctacosan-7-ol and 16-hydroxy-26-methyleneptacosan-2-on by chemical a spectral investigations.\textsuperscript{[11]} The fresh leaves are used very effectively for the treatment of snakebites, dog bites and diarrhea in folk medicines.\textsuperscript{[12]} Literature survey also reveals that paste of roots is beneficial in the treatment of ringworm infection.\textsuperscript{[13]} Although the antifungal activities of extracts of leaves have been reported, no systematic study has been reported on the roots of the plant. Hence the present investigation deals with evaluation of antifungal activity of roots extract Achyranthes aspera against Trichophyton rubrum, one of the causative organisms for Ringworm infection.

MATERIAL AND METHODS
PLANT MATERIAL
The roots of Achyranthes aspera were collected from forest region, cleaned and dried at room temperature in shade and kept away from direct sunlight. The plant was authenticated in the herbarium, Botany department, Govt. Science College Durg, Chhattisgarh, India by comparing morphological features.

DRUGS AND CHEMICALS
The roots of Achyranthes aspera were collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through sieve No.120 meshes to remove fine powders where as coarse powder which remained on sieve was used for extraction. This powder was further subjected to preliminary phytochemical test and thin layer chromatography. Clotrimazole (1\% w/w) is used as standard drug.\textsuperscript{[14]}

EVALUATION OF ANTIFUNGAL ACTIVITY
The in vitro antifungal activity of the Achyranthes aspera root extract was carried out by Agar well diffusion
Clotrimazole (1mg/ml) was used as standard antifungal agent respectively. Antifungal activity was carried out against culture of Trichophyton rubrum, using Sabouraud dextrose agar medium. The microorganism inoculated plates were maintained at room temperature for 2 hours to allow diffusion of the solution into the medium. The petridishes used for antifungal activity were incubated 25°C±1 for 7 days. The diameters of zone of inhibition surrounding each of the wells were recorded.

RESULTS

PHYTOCHEMICAL SCREENING
The extracts were then subjected to preliminary Phytochemical screening to detect the presence of various Phytoconstituents. The results shows that the Ethanolic extract contains steroids, saponins, glycosides, vitamins and aqueous extract contain saponins, glycosides as chemical constituents was confirmed by thin layer chromatography.

ANTIFUNGAL ACTIVITY
The antifungal results reveal that the activity of the crude extracts of Achyranthes aspera plant is encouraging. Antifungal activity was done by using Agar well diffusion method; Clotrimazole were used as standard for comparing results for antifungal activity. The zone of inhibition of Ethanolic extract is 23mm and aqueous extract has 19mm taking 10mg/ml of extract. The zone of inhibition of standard drug Clotrimazole has 21mm. Ethanolic extract shows good antifungal activity. Ethanolic extract shows good antifungal activity against Trichophyton rubrum as compare with aqueous extract respectively (Table1).

Table1: Effect of Achyranthes aspera aqueous and alcoholic extract against Trichophyton rubrum showing antifungal activity.

<table>
<thead>
<tr>
<th>Zone of inhibition (mm)</th>
<th>Extract against Trichophyton rubrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 Mean S.D Result</td>
</tr>
<tr>
<td>Aqueous (5mg/ml)</td>
<td>18 19 18 18 19 18.4 0.5 18.4±0.5</td>
</tr>
<tr>
<td>Alcohol (5mg/ml)</td>
<td>18 19 20 19 19 19.2 0.6 19.2±0.6</td>
</tr>
<tr>
<td>Aqueous (10mg/ml)</td>
<td>19 20 20 19 19 19.4 0.5 19.4±0.5</td>
</tr>
<tr>
<td>Alcohol (10mg/ml)</td>
<td>23 24 23 22 23 0 23.0±0</td>
</tr>
<tr>
<td>Standard</td>
<td>21 21 20 21 21 20.8 0.4 20.8±0.4</td>
</tr>
<tr>
<td>Water (negative) control</td>
<td>12 12 12 12 12 12 0 No activity</td>
</tr>
<tr>
<td>Ethanol (negative) control</td>
<td>12 12 13 13 13 12.6 0.5 12.6±0.5</td>
</tr>
</tbody>
</table>

Diameter of hole ~12mm, S.D – Standard Deviation.

DISCUSSION
Ethanolic extract shows good antifungal activity against Trichophyton rubrum as compare with aqueous extract. Phytoconstituents present in Ethanolic extract are steroids, saponins, glycosides, vitamins and aqueous extract contains saponins, glycosides. The zone of inhibition of standard drug Clotrimazole has 21mm (1mg/ml). When 5mg/ml taken Ethanolic extract gives 19mm and aqueous extract gives 18mm of zone of inhibition. On the basis of zone of inhibition results, Ethanolic extract shows better antifungal activity against Trichophyton rubrum as compare with aqueous extract.

CONCLUSION
From the above results, it is concluded that Achyranthes aspera used traditionally and in backward are as to treat ringworm infection, showed significant antifungal activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as antifungal. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for antifungal activity. Thus the present experiment proved its traditional claim for the beneficial effect in the ringworm from aqueous and alcoholic extract of root of Achyranthes aspera.
ACKNOWLEDGEMENT
We will like to acknowledge the sincere efforts of our research team members Dharmendra Singh Rajput, Pankaj Kashyap and Our Directors of Organization Mr. Sanjay Agrawal, Dr. Manish Jain, Mr. Ashish Agrawal whose contribution helped us in our work.

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