PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND ANTHELMINTIC ACTIVITY OF ACACIA SUMA (ROXB) BARKS

Acharyya Suman1*, Dash Gauri Kumar2, Brahma Dillip Kumar1, Chhetree Rishi Raj3
1Matushree V. B. Manvar College of Pharmacy, Dumiyani, Rajkot, Gujarat, India
2Institute of Pharmacy and Technology, Salipur, Cuttack, Orissa, India
3Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala, West Tripura, India

*Email: sumanjanmenjoy@yahoo.com

ABSTRACT
The present work was conducted to investigate the preliminary phytochemical studies and anthelmintic activities on the bark of Acacia suma (Roxb.) Family- Fabaceae against adult Indian earthworms, Pheretima posthuma. Various concentrations (5-25 mg/ml) of each extract along with the reference samples (Piperazine citrate, Albendazole) were subjected for anthelmintic activity study. The qualitative test revealed that the petroleum ether extracts contained only terpenoids but chloroform and hydroalcoholic (MeTHanol 70% v/v) extracts exhibited the presence of carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins but amino acids and steroids were absent. All the extracts showed anthelmintic activity when compared with petroleum ether and chloroform extracts. The anthelmintic activity of hydroalcoholic extract was comparable with reference drugs.

KEYWORDS: Acacia suma (Roxb.), Pheretima posthuma, Anthelmintic, Hydroalcoholic, Albendazole, Piperazine citrate

INTRODUCTION
Medicinal plants have served through ages, as a constant source of medicaments for the treatment of a variety of diseases1. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides2. A number of medicinal plants have been used to treat parasitic infections in man and animals. Parasitic helminthes effect the human beings as well as animals leading to considerable hardship and stunted growth. The parasitic invasion is caused by mixed infections with several species of stomach and intestinal worms. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. Despite extensive use of synthetic chemicals in modern clinical practices all over the world, interest in exploiting potential use of plants 3 as source of drugs are under study.

Acacia suma (Roxb.) var. Acacia polyacantha (Family-Fabaceae) is a medium sized erect tree; trunk with fissured bark and knobby persistent prickles found in the greater part of India and costal districts of Orissa4,5. The bark is reported to be used as blood purifier5 and possesses anti-cancer, insecticide and astringent properties6-9. The seeds are reported to have hypoglycaemic effect6. The leaves and roots of the plant are reported to be use as insecticide, antifungal, antivenin, aphrodisiac, antimalarial, anticrustacean, stimulant and in the treatment of sores, abscesses and asthma10,11. Presence of proanthocyanidin6, 5,4’-dihydroxy-7,3’-dimethoxyflavone-3-0-D galactopyranoside7,16, gallocatechin-5-7-digallate, quercetin and gallocatechin-7-gallate8 in the barks have been reported earlier. An extensive literature survey does not reveal anthelmintic activity of bark. So the present study was under taken to investigate the preliminary phytochemical screening and anthelmintic activity of bark of Acacia suma.
MATERIALS AND METHODS

Plant Material
The plant material (barks) was collected from the forests of Ganjam district of Orissa during June 2007 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp. No: CNH/ I-I / (17)/2009/Tech.II/28] has been kept in our research laboratory for further reference. After authentication, fresh barks were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powder was stored in a dessicator for further use.

Preparation Of Crude Extracts
The powdered mass was exhaustively extracted successively in soxhlet apparatus using solvents like petroleum ether, chloroform and hydroalcohol (methanol 70 % v/v) based on their polarity. Finally extracts were concentrated under reduced pressure using rotary evaporator and stored for further analysis.

Preliminary Phytochemicals Analysis
During preliminary phytochemical screening tests were mainly concluded to alkaloids, carbohydrates, glycosides, saponins, flavonoids, tannins and terpenoids. The constituents are reported in Table 1.

Preliminary Anthelmintic Activity Screening
The anthelmintic activity was performed according to the method of Ghosh et al. on adult Indian earthworm Pheretima posthuma as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. All the extracts were dissolved in minimum amount of dimethyl sulphoxide and then volume was adjusted with saline water. Three groups were prepared control (saline water), reference samples (piperazine citrate and albendazole at 10 mg/ml and the extracts of (5, 10, 20 and 25 mg/ml). The reference samples and extract solutions were prepared freshly before starting the experiment. Observations were made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color, when dipped in warm water (50°C). The results are shown in Table 2 depict the time taken for paralysis and death of worms after treatment with the extracts at the selected concentrations.

Statistical Analysis
The data on biological studies were reported as mean ± Standard deviation (n = 6). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. The P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION
Preliminary phytochemical screening indicated the presence of carbohydrates, alkaloids, saponins, glycosides, tannins, flavonoids and terpinoids (Table1). All the extracts showed the anthelmintic activity in dose dependent manner at 5 to 25 mg/ml. The chloroform and hydroalcoholic extracts of A. suma revealed significant anthelmintic activity. The hydroalcoholic extract shown better paralytic value and death at 10mg/ml than the standards (Fig 1 and 2). The chloroform extract also showed satisfactory results at concentration of 10mg/ml. The presence of alkaloids, glycosides and tannins may be the responsible chemical constituents for demonstrating anthelmintic activity. The possible mechanism of tannins may to interfere with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface. It was also possible that alkaloids may act on central nervous system and caused paralysis of the Pheretima posthuma worms.

CONCLUSION
It could be concluded and confirmed that the hydroalcoholic extracts of bark of A. suma has anthelmintic activity comparable with standard drugs, which is a significant result. Further studies are required to identify the actual chemical constituents that are present in the crude drug extracts of this plant which are responsible for anthelmintic activity. It is, however, suggested to conduct further research on pure chemical constituents to critically evaluate their activity on a large number of animals.
ACKNOWLEDGEMENTS
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REFERENCES


Table 1: Preliminary phytochemical screening of different extracts of *A. suma* barks.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Carbohydrates</th>
<th>Glycosides</th>
<th>Gums and mucilages</th>
<th>Proteins and amino acids</th>
<th>Tannins</th>
<th>Steroids and sterols</th>
<th>Triterpenoids</th>
<th>Saponins</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. Ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) Present; (-) Absent.

Table 2: Comparative anthelmintic activity of the extracts with reference samples

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Concentration(mg/ml)</th>
<th>Time taken for paralysis(min.) (X±SEM)</th>
<th>Time taken for death(min.) (X±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>137.10 ± 1.89</td>
<td>110.76 ± 0.79</td>
</tr>
<tr>
<td>Pet ether Extract</td>
<td>5</td>
<td>80.2 ± 2.35</td>
<td>79.69 ± 0.96</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>65.02 ± 1.64</td>
<td>59.55 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>59.55 ± 0.82</td>
<td>35.86 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>35.86 ± 0.59</td>
<td>22.57 ± 1.31</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>5</td>
<td>79.69 ± 0.96</td>
<td>33.5 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>59.55 ± 0.82</td>
<td>31.49 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>35.86 ± 0.59</td>
<td>20.92 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>22.57 ± 1.31</td>
<td>10.53 ± 0.78</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>5</td>
<td>35.86 ± 0.59</td>
<td>33.5 ± 0.98</td>
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<tr>
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<td>10</td>
<td>22.57 ± 1.31</td>
<td>31.49 ± 0.82</td>
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<td></td>
<td>20</td>
<td>10.53 ± 0.78</td>
<td>20.92 ± 1.7</td>
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<tr>
<td></td>
<td>25</td>
<td>64.33 ± 1.99</td>
<td>10.53 ± 0.78</td>
</tr>
<tr>
<td>Albendazole</td>
<td>10</td>
<td>32.73 ± 1.5</td>
<td>59.78 ± 0.98</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>10</td>
<td>26.33 ± 1.4</td>
<td>72.67 ± 1.7</td>
</tr>
</tbody>
</table>
Fig 1: Time taken to paralyze by bark extracts of *A. suma* at different concentrations and reference samples.

Fig 2: Time taken to death by bark extracts of *A. suma* at different concentrations and reference samples.

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