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ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF *ASTERACANTHA LONGIFOLIA* NEES.

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ABSTRACT

The antibacterial and antifungal activity of methanolic extract of *Asteracantha longifolia* Nees has been evaluated against 4 Gram positive bacteria, 7 Gram negative bacteria and 7 fungi. Ciprofloxacin and fluconazole used as standards for bacteria and fungi, respectively. The extract showed varying degrees of antimicrobial activity with zone of inhibition ranging from 15.0 to 26.0 mm. *A. longifolia* demonstrated significant zone of inhibition against all experimental bacteria, namely, *B. cereus*, *B. megaterium*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. paratyphi*, *S. typhi*, *Sh. dysenteriae*, *Sh. Sonnei* and *V. cholerae* and four fungi, namely, *A. niger, B. dermatitidis, Microsporum* spp. and *Trichophyton* spp. The MICs of the plant extract was found to be 31.25 µg/ml against *B. cereus, S. typhi, Sh. Sonnei, V. cholerae, A. niger and B. dermatitidis*. In the brine shrimp lethality bioassay, the LC_{50} and LC_{90} of *A. longifolia* were found to be 6.1 & 12.2 µg/ml, respectively.

INTRODUCTION: The use of plants in various parts of the world for both preventive and curative purposes is an ancient tradition and is increasing pragmatically. With this upsurge however, a thorough scientific investigation of these medicinal plants is imperative, based on the need to provide information on their efficacies and toxicity risk. One such plant *Asteracantha longifolia* Nees is used in the folklore medicine of Bangladesh as an oral remedy for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction and pain without scientific evaluation of its efficacy.

A. longifolia Nees belongs to the family (Acanthaceae) is a common weed growing in marshy and water logged areas [1].

The plant is an important medicinal herb, widely distributed in Indian subcontinent and is used by local population for different medicinal purposes [2].

The roots, seeds and ashes of the plant are extensively used in traditional system of medicine for various ailments like jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infections, edema and gout. It is classified in Ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of premeham (diabetes), athisaram (dysentery), etc [3, 4]. Literature survey revealed that there are no scientific studies carried out regarding antimicrobial and cytotoxic activities on the *Asteracantha longifolia* to authenticate their therapeutic claim. Hence, in the present study the methanolic extracts of *Asteracantha longifolia* was examined for its antimicrobial and cytotoxic property.

MATERIALS AND METHODS:

Collection and identification: The plant selected for the present work, *Asteracantha longifolia* Nees (Family: Acanthaceae), was collected from Rajasthali, Rangamati, Bangladesh in May, 2011 and were identified at the Bangladesh Forest Research Institute, Chittagong, Bangladesh. A voucher specimen has been preserved there for future reference.

Extraction: The plant materials were subjected to drying in an oven below 40^{o}C. Then the crude dried plant was ground into coarse powder and subjected to hot extraction with 97% methanol by using a Soxhlet apparatus. The extraction was carried out about 18 hrs and filtered through a cotton plug followed by Whatman filter paper number #1. The extract was then concentrated by using rotary evaporator.

Antimicrobial screening: The antibacterial and antifungal activities of the crude extracts were evaluated by the disc diffusion method [5] against 4 Gram positive bacteria, 7 Gram negative bacteria and 7 fungi (**Table 1**). Ciprofloxacin and fluconazole were using as standards against bacteria and fungi respectively. The organisms were obtained as pure culture from the Department of Microbiology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was expressed by measuring the diameter of zone of inhibition expressed in mm. The experiments were carried out in triplicate.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the extract was determined by the serial dilution technique [6] in nutrient broth medium, containing graded concentration of the plant extract and inoculated test organisms.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay [7, 8, 9] technique was applied for determination of general toxic property of the plant extract.

RESULTS AND DISCUSSIONS:

1. **Antimicrobial screening:** The extract showed varying degrees of antimicrobial activity with zone of inhibition ranging from 15.0 to 26.0 mm (Table 1). *A. longifolia* demonstrated significant zone of inhibition against all experimental bacteria and four fungi, namely, *A. niger, B. dermatitidis, Microsporum* spp. and *Trichophyton* spp. Ciprofloxacin and fluconazole were taken as standards for antibacterial and antifungal test.

TABLE 1: ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *ASTERACANTHA LONGIFOLIA* NEES (500µg/disc) And Standard (50µg/disc).

Test microorganisms	Zone of inhibition (mm)	
	MeOH extract of *A. longifolia*	Standard
Gram positive bacteria		**Ciprofloxacin**
Bacillus cereus	23.0 ±0.34	28.0 ±0.34
B. megaterium	21.0 ±1.17	25.0 ± 0.67
B. subtilis	20.0 ±0.34	24.0 ±0.34
Staphylococcus aureus	24.0 ±1.17	27.5 ±0.34
Gram negative bacteria		
Escherichia coli	22.0 ± 0.58	28.0 ±0.34
Pseudomonas aeruginosa	23.0 ±1.21	31.0 ±0.34
Salmonella paratyphi	20.0 ±0.34	23.5 ±0.34
S. typhi	23.67±0.89	23.67±0.89
Shigella dysenteriae	23.5 ± 0.58	32.0 ± 0.34
Sh. sonnei	23.0 ± 0.34	27.0 ± 0.58
Vibrio cholerae	19.33±0.34	24.5±0.34
Fungi		**Fluconazole**
Aspergillus niger	26.0±0.58	27.0 ±0.34
Blastomyces dermatitidis	25.0 ±0.34	22.0 ±0.34
Candida albicans	15.0±0.58	26.0 ±0.58
Cryptococcus neoformans	15.0 ±0.89	21.0 ±0.34
Microsporum spp.	20.0 ±0.34	23.0 ±0.34
Pityrosporum ovale	19.0 ±0.34	22.0 ±0.34
Trichophyton spp.	24.0±0.58	29.0 ±0.34

2. **Minimum inhibitory concentration (MIC):** During the MIC determination, the methanol extract of *A. longifolia* inhibited the growth of test organisms between 31.25-62.50 µg/ml (**Table 2**). The low MIC values of the extract, against *B. cereus, S. typhi, S. sonnei, V. cholerae, A. niger* and *B. dermatitidis* and suggest the presence of strong antimicrobial compounds in the extract.

   Here, ciprofloxacin and fluconazole were taken as standard antibacterial and antifungal agent, respectively.

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (MIC) OF METHANOLIC EXTRACT OF *ASTERACANTHA LONGIFOLIA* NEES

Test organisms	Minimum inhibitory concentration (µg/ml)
Bacteria	
Bacillus cereus	31.25
Escherichia coli	62.50
Staphylococcus aureus	62.50
Salmonella paratyphi	-
S. typhi	31.25
Shigella dysenteriae	-
Sh. sonnei	31.25
Vibrio cholerae	31.25
Fungi	
Aspergillus niger	31.25
Blastomyces dermatitidis	31.25
Candida albicans	62.50
(-): MIC>100 µg/ml	

Brine Shrimp Lethality Bioassay: In brine shrimp lethality bioassay, the LC_{50} and LC_{90} of methanol extracts were found to 6.1 and 12.2 µg/ml, respectively as compared to 0.44 and 0.82 µg/ml for standard Vincristine sulphate (**Table 3**).

TABLE 3: BRINE SHRIMP LETHALITY BIOASSAY OF METHANOL EXTRACT OF *ASTERACANTHA LONGIFOLIA* NEES

Sample	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Vincristine sulphate	0.44	0.82
A. longifolia	6.1	12.2

CONCLUSION: From the study, it is evident that, the methanolic extract of *Asteracantha longifolia* Nees showed moderate to strong antimicrobial activity against selected bacteria and fungi species. The extract also demonstrated significant cytotoxicity against brine shrimp nauplii. Further investigation is required to isolate the bioactive moieties. Bioactivities demonstrated by the extracts support the traditional uses of the plant in various diseases.

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