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Short Communications

Antibacterial and cytotoxic activities of *Spondias pinnata* (Linn. f.) Kurz fruit extract

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The plant *Spondias pinnata* (Linn. f.) Kurz belonging to Anacardiaceae family, is used as a medicinal agent in Bangladesh. In the present investigation, attempt was undertaken to study the antibacterial potency and cytotoxic activity of 80% ethanol extract of its fruit. The antibacterial activity was performed by the disc diffusion method and cytotoxicity was observed by brine shrimp lethality bioassay. The fruit extract exhibited mild to potent antibacterial activity against some Gram-positive and Gram-negative bacteria at a concentration of 500 µg/disc. Among them *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* shows promising result. The ethanolic extract revealed strong cytotoxicity having LC_{50} of 2.12±0.09 µg/ml.

Keywords: *Spondias pinnata,* Anacardiaceae, Antibacterial, Cytotoxic, Fruit

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Introduction

Medicinal plants are potential sources of drugs and are used as a source of medicine in developing countries to treat serious diseases. About 80% people of the rural areas of underdeveloped countries still depend on medicinal plants. Studies revealed that there are more traditional medicine providers than the allopathic practitioners especially in the rural areas (WHO, 2002)[1]. Bangladesh has a good number of medicinal plants and these plants have many biologically active compounds[2-4]. Researchers now become concerned about natural products of higher plants due to novel source of antimicrobial agents[5].

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Spondias pinnata (Linn. f.) Kurz (Family–Anacardiaceae) is a deciduous tree distributed in India, Sri Lanka and South-East Asian countries. The genus *Spondias* Linn. includes 17 species, 7 of which are native to the neotropics and about 10 are native to tropical Asia[6]. The phytochemistry of this plant has been studied and it is found that this plant contains sterols, flavonoids and gums[7]. The gum exudate of the species contained acidic polysaccharides[8]. Previously isolated compounds are β-amyrin, oleanolic acid and amino acids (alanine, leucine)[9]. There are reports which showed that fruits are astringent and antiscorbutic and used in bilious dyspepsia. Bark astringent and refrigerant, used in diarrhoea and dysentery; a paste is applied in rheumatism. Roots are employed for regulating menstruation[10]. In ethno-medicine, equal quantities of bark juice of *S. pinnata* and *Syzygium cuminii* (Linn.) Skeels are prescribed as a remedy for dysentery[11]. The fruit is a useful antiscorbutic and its pulp which is acidic and astringent, cures rheumatism and is used in bilious dyspepsia[12].

Since, no report has been found on the antibacterial and cytototoxic activity of the fruits of this plant, present study was conducted to evaluate these properties.

Materials and Methods

Plant material

S. pinnata was collected from Miksimil, Dumuria, Khulna, Bangladesh and identified and authenticated by the experts at Bangladesh National Herbarium (BNH), Mirpur, Dhaka (Accession number-3132). A voucher specimen was also deposited in the same herbarium.

Test organisms

Six Gram negative and four Gram positive pathogenic bacteria (Table 1) were used in this experiment. Bacterial strains were collected from the Microbiology Lab. of Square Pharmaceutical Limited, Pabna.

Preparation of fruit extract

The fruits were collected and sun-dried for one week. The shade dried fruits were ground into a

coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 650 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 21 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

The filtrate (ethanol extract) thus obtained was evaporated using a rotavapour and in water-bath until dried. It produced a gummy concentrate of reddish black color and it was designated as crude ethanol extract.

Determination of antimicrobial activity

Disk diffusion assay

The antimicrobial activity of the extract was determined against the test organisms (Table 1) by the disc diffusion method[13]. Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diam) were then impregnated with known amounts of the test substances using micropipettes and the residual solvents were completely evaporated. Discs containing the test materials were placed onto nutrient agar medium uniformly seeded with the test microorganisms. Standard discs of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were kept at low temperature (4ºC) for 24 h to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms. The antimicrobial activity of the test agents was determined by measuring the diameter (mm) of zone of inhibition. The experiment was carried out in triplicate and the mean values were taken.

Brine shrimps lethality bioassay

Brine shrimp lethality bioassay technique[14-17] was applied for the determination of preliminary cytotoxic activity of the fruit extractive. DMSO solutions of the samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For this experiment, 4 mg of ethanol crude extract was dissolved in DMSO and the solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml) were obtained by serial dilution. Vincristine sulphate was used as positive control. The experiment was carried out in triplicate and the mean values were taken.

Table 1—Anti-microbial activity of the ethanol extract of the fruits of *S. pinnata*

Bacterial strains	Diameter of zone of inhibition (mm)	
	Kanamycin (30µg/disc)	Ethanolic extract (500µg/disc)
Gram negative		
Shigella boydii (ATCC 9361)	25± 0.57	7± 0.64
S. flexneri (ATCC 12022)	17± 1.15	10± 0.70
S. sonnei (ATCC 25931)	22± 0.62	10± 1.35
Salmonella typhi (ATCC 14612)	22± 0.23	12± 0.58
Pseudomonas aeruginosa (ATCC 25619)	29± 1.52	21± 1.00
Vibrio cholerae (ATCC 14035)	22± 0.50	9± 1.61
Gram positive		
Staphylococcus epidermidis (ATCC 12228)	28± 1.41	15± 1.42
S. pyogenes (ATCC 19615)	24± 1.35	8± 1.00
S. saprophyticus (ATCC 15305)	24± 0.24	10± 0.57
S. aureus (ATCC 6538)	21± 2.08	12± 0.62

Results and Discussion

From the zone of inhibition produced by the 80% ethanolic extract of fruits (Table 1) it was found that the extract showed activity against all of the test organisms. Among the bacterial strains tested *Shigella boydii* (7 mm), *Staphylococcus pyogenes* (8 mm), *Vibrio cholerae* (9 mm), *Shigella flexneri* (10 mm), *Shigella sonnei* (10 mm), and *Staphylococcus saprophyticus* (10 mm) showed mild sensitivity whereas *Salmonella typhi* (12 mm), and *Staphylococcus aureus* (12 mm) showed moderate sensitivity against *S. pinnata*. However, significant activity was exhibited by *Pseudomonas aeruginosa* (21 mm), and *Staphylococcus epidermidis* (15 mm) against the same. The results indicated that the extract of the fruits of *S. pinnata* is effective against both Gram-positive and Gram-negative microorganisms.

The lethality of ethanolic crude extract to brine shrimp was determined after 24 h of exposure. The LC_{50} were found to be 2.12±0.09, and 0.32±0.05 µg/ml for ethanol crude extract and the positive control (vincristine sulphate), respectively. The

cytotoxicity exhibited by the ethanol crude extract was found to be significant.

From this study, it can be concluded that the extractive of the fruits of *S. pinnata* showed significant biological activity and further investigation is required to isolate the bioactive principles.

Conclusion

The bioactivities shown by extracts of *S. pinnata* fruits support the traditional medicinal uses of this plant in gastrointestinal disorders and infectious diseases. Further investigations are required to isolate the active principles.

References

1 WHO *Traditional Medicine* Strategy 2002–2005*,* Regional committee fifty fifth session, 11-13 September 2002.

2 Shilpi JA, Rahman MTU, Uddin SJ, Alam MS, Sadhu SK and V´eronique S, Preliminary pharmacological screening of *Bixa orellana* L. leaves, *J Ethnopharmacol,* 2006, **108**, 264-271.

3 Rahman S, Hasnat A, Hasan C M, Rashid M A and Ilias M, Pharmacological evaluation of Bangladeshi medicinal plants-A review, *Pharmac Biol,* 2001, **39**, 1-6.

4 Yusuf M, Chowdhury JU, Yahab MA and Begum J, Medicinal plants of Bangladesh (BCSIR Laboratories, Bangladesh), 1994, 38.

5 Fabricant DS and Farnsworth N R, The value of plants used in traditional medicine for drug discovery, *Environm Health Persp Suppl*, 2001, **109**, 69-75.

6 Ghani A, Medicinal plants of Bangladesh, 2nd edn (Asiatic Society of Bangladesh, Dhaka), 2003, 127.

7 Tandon S, and Rastogi RP, Studies on the chemical constituents of *Spondias pinnata*, *Planta Med,* 1976, **29**, 190-192.

8 Ghosal PK and Thakur S, Structural features of the acidic polysaccharide of *Spondias pinnata* gum exudates, *Carbohydr Res,* 1981, **98,** 75-83.

9 Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal Plants, Publications and Information Directorate, New Delhi, 1993, 643.

10 The Useful Plants of India, National Institute of Science Communication, CSIR., 2000, 595.

11 Mahanta RK, Rout SD and Sahu HK**,** Ethnomedicinal plant resources of Similipal biosphere reserve, Orissa, India, *Zoos Print J,* 2006, **21**(8), 2372-2374.

12 Kirtikar KR and Basu BD, Indian Medicinal Plants, Vol. III. 2nd edn, International Book Distributors, India, 1987, 2013-2014.

13 Bauer AW, Kirby WMM, Sherries JC and Truck M, Antibiotic susceptibility testing by standard single disc diffusion method, *Am J Clin Pathol,* 1966, **45,** 426-493.

14 Meyer BN, Ferrigni NR, Puam J E, Lacobsen L B, Nichols D E and McLaughlin J L, Brine shrimp: A convenient general bioassay for active constituents, *Planta Med,* 1982, **45,** 31-32.

15 McLaughlin JL and Rogers LL, The use of biological assays to evaluate botanicals, *Drug Inf J*, 1998, **32**, 513-524.

16 Culkin F, The major constituents of seawater in Chemical Oceanography, J.P. Riley and G. Skirrow eds. Academic Press, 1965, 121-61.

17 Persoone G, Proceedings of the international symposium on brine shrimp, *Artemia salina*, University Press, Wittern, Belgium, 1988, p. 1-3