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Short communication

ANTIBACTERIAL ACTIVITY OF THE CRUDE ETHANOLIC EXTRACT OF *XYLOCARPUS GRANATUM* STEM BARKS

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ABSTRACT

Antimicrobial effect of the crude organic extract of *Xylocarpus granatum* stem barks was studied in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, during the period from October to December 2003. Disc diffusion method has been adopted in this study and petri dishes (120 mm in diameter) containing nutrient agar medium seeded with the test organism was used for antimicrobial screening. Test materials diffuse from the discs to the surrounding medium of the plate. The plates are then kept in an incubator (37º) for 18 hours to allow the growth of the microorganisms. The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter. Antimicrobial screening showed that the crude ethanol extract and other partially extracted fraction of the barks of *Xylocarpus granatum* possess antimicrobial activity against most of the test organisms depending upon the nature of their active ingredients in the extract and capacity of diffusion into the agar medium. Among the test organisms, the extract showed significant antimicrobial activity against *Staphylococcus epidermis*, *Staphylococcus aureus*, *Shigella boydii*, and *Proteus* spp. and moderate activity against *Escherichia coli, Streptococcus pyogenes*, and no activity against *Shigella dysentery, Enterococci, Salmonella typhi.*

Key words : Antibacterial activity, *Xylocarpus granatum*, kanamycin, disc diffusion

INTRODUCTION

Scanty literature is available on the antibacterial activity of mangroves. However, studies of other biological activities in general are available. The study of Premnathan *et al*. (1992, 1996) revealed that the mangroves were found highly effective for antiviral activity as compared to seaweeds and sea grasses. Kokpal *et al*. (1990) had also reported the bioactive compounds from mangrove plants. Some mangroves had shown insecticidal activity (Miki *et al.,* 1994, Ishibashi *et al*., 1993). Wu *et al.* (1997) reported the cytotoxic and antiplatelet aggregation activity of methanol extract of *Aglaia elliptifolia*. *Xylocarpus granatum*. Koen (Bengali- Dhundul) is a moderate sized evergreen tree with their grey berks, usually grows in coastal forests of Bangle, Andaman's, Burma, The Malay peninsula and island of Australia and Africa. (Ghani, 1998; Kirtikar and Basu, 1980).The berks are astringent, and are used for dysentery, Diarrhea, and other abdominal troubles and as febrifuge. (Ghani, 1998) The seed ash mixed with sulphur and coconut oil is applied as ointment for itch. (Ghani, 1998) Fruit is used as a cure for swelling of the breast and elephantiasis. (Ghani, 1998) Chemical investigation of *X. granatum* afforded a number of alkaloids (Chou *et al.,* 1977), limonoids (Cui *et al*., 2005; Alvi *et al*., 1991; Connolly *et al.,* 1976; Kokpol *et al*., 1996; NgAng and Fallis, 1979) etc. N-methylflindersine was identified as the component responsible the antifeedant activity of the exists towards army worms (Spodoptera *exempta* & *S. littoralis*́) and Mexican been beetles (*Eplachna varivestis*́). (Chou *et al.,* 1977) As a part of our study for the search of bioactive secondary metabolites from local medicinal plants we have investigated an ethanolic extract of *X. granatum* for potential antibacterial activity. A preliminary screen revealed that the crude and partially extracted fraction markedly inhibit the growth of the microorganisms at a dose of 400 µg/ml.

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MATERIALS AND METHODS

Antimicrobial effect of the crude organic extract of *Xylocarpus granatum* stem barks was studied in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, during the period from October to December 2003. Antimicrobial assay was measured using the methods described by Yongabi, (2003); Mokbel and Hashinaga (2005); Bauer *et al.,* 1966; Brghe and Vlientinek, 1991).

Plant materials

Xylocarpus granatum was collected from the Sundarbons of Karomjol, Dacope region. The time of collection was October 2003 at day time. The steam berks were collected from the fresh tree from the bank of the river. The plant was identified at Bangladesh National Herbarium where a voucher specimen was deposited.

Preparation of extracts

A 100 g amount of the pulverized dried stem bark was continuously extracted with solvent in a soxhlet extractor for 2½ h and the solvent distilled off in the rotatory evaporator. The extract was then poured into a weighed flask and further dried in a desiccating chamber to a constant weight. The dried extract was exposed to UV fractions were quantitatively evaluated for activity against rays for 24 hours and checked for sterility by streaking on nutrient agar plate.

Microorganism used for the activity test

Both gram positive and gram negative bacterial strains were taken for the test. The bacterial strains used for the investigation are listed in Table 1. These organisms were collected from the Microbiology Laboratory of Pharmacy Discipline, Khulna University, Khulna.

Preparation of the seeded test plates

Each of the test organisms was transferred to the test tube containing 16 ml previously autoclaved media with the help of the sterilized inoculating loop at 45° C under laminar air flow unit. The test tubes were shaken by rotation to get a uniform suspension of organism. The bacterial suspensions were immediately transferred to the sterile petri-dishes aseptically. The petri-dishes are rotated several times to assure homogeneous distribution of the test organisms. The medium was poured into petri dishes in such a way as to give a uniform layer of depth of approximately 4 mm. After the medium become cooled to room temperature, it was stored in a refrigerator (4º C).

Preparation of the discs

The agar disc diffusion techniques involved placing sterile paper discs (Whitman No. 1 filter paper) of 5 mm diameter impregnated with different crude extracts and dried in a hot air oven at 60°C on agar plates seeded with the test organism. Three types of discs were used for antimicrobial screening; sample discs, standard discs and blank discs. Then the sample disc was prepared by applying sample solution of the desired concentration on the sterile filter paper discs (5 mm in diameter) with the help of a micropipette in an aseptic condition. Similarly blank discs and other discs were prepared to serve as negative control and test sample respectively. In this investigation kanamycin (30 µg/disc) standard disc was used as reference and methanol was used as blank. These discs were left for few minutes in aseptic condition under UV light for complete sterilization.

Antibacterial activity test

Sample impregnated discs, standard disc and negative control discs were placed gently on the solidified agar plates, freshly seeded with the test organisms with the help of a sterile forceps to assure complete contact with medium surface. The special arrangement of the discs was such that the discs were not closer than 15 mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in refrigeration for about 24 hours at 4º C. This was sufficient time for the material to diffuse into a considerable area of the medium. The antibiotic Kanamycin and normal saline were used as positive and negative controls respectively. The whole set-up was incubated as 37°C for 18 h after (Yongabi, 2003) after which diameter of zones of inhibition were measured.

Table 1. *In vitro* antibacterial screening of crude ethanolic, pet ether, carbon tetrachloride and chloroform fractions of ethanolic extract of *Xylocarpus granatum*

Bacterial strain	Diameter of zone of inhibition in millimeter				
	Kanamycin 30 µg/disc	Ethanol extract 400 µg/disc	Pet-ether fraction 400 µg/disc	CCl_4 fraction 400 µg/disc	$CHCl_3$ fraction 400 µg/disc
Staphylococcus aureus	30	15	17	13	15
Staphylococcus epidermis	32	25	20	23	24
Escherichia coli	39	10	11	14	11
Shigella dysentery	34	00	00	00	00
Shigella sonnei	29	00	00	00	00
Salmonella typhi	30	00	00	00	00
Proteus spp.	33	16	15	13	13
Streptococcus pyogenes	32	10	12	13	12
Shigella boydii	38	20	23	23	22
Enterococci	36	00	00	00	00

'0' indicates no sensitivity or zone of inhibition lower than 5 mm.

RESULTS AND DISCUSSION

Antimicrobial activity of ethanolic and partially extracted products of *X. granatum* were examined and found to exhibit good antibacterial activity at 400 µg/disc dose level against most of the gram positive and gram negative organisms which has been depicted in the Table 1. Among the test organisms the extract showed good antimicrobial activity against *Staphylococcus epidermis*, *Staphylococcus aureus*, *Shigella boydii*, and *Proteus* spp. and moderate activity against *Escherichia coli, Streptococcus pyogenes* and no activity against *Shigella dysentery, Enterococci, Salmonella typhi.* The result of the antimicrobial activity expressed in term of diameter of zone of inhibition in millimeter. The economical uses of products from mangrove ecosystems are many and varied. Traditionally, the mangroves have been exploited for firewood and charcoal. Use has also been found for mangroves in the construction of dwellings, furniture, boats and fishing gear, tannins for dyeing and leather production. The mangroves provide food and wide variety of traditional products and artifacts for the mangrove dwellers. Extracts and chemicals from mangroves are used mainly in folkloric medicine (e.g. bush medicine), as insecticides and pesticides and these practices continue to this day. However the extraction of novel natural chemical compounds from mangroves, in addition to those already known to the pharmacopoeia of the people is in its infancy. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of already known biologically active compounds. *Xylocarpus granatum* belongs to the family Meliaceae and the family Meliaceae includes many plants that are sources of valuable timber and many that have wide ranging uses in ethnomedicine (Ambrozin *et al.*, 2006). The family is distinguished by the occurrence of characteristic substances called limonoids (Ambrozin *et al.*, 2006). These substances have wide spectrum of biological activities, particularly insecticidal action (Ambrozin *et al.*, 2006). Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Okeke *et al*., 2001; Ebi and Ofoefule *et al*., 1997). *Xylocarpus granatum* also possess alkaloidal substances which also have biological activities (Chou *et al.,* 1977). In our study, some of the bacterial strains did not respond to crude extracts, whereas the fractions showed broad-spectrum activity against multiple strains. This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract or synergism by the presence of some compounds or factors in the extract. The variation of antibacterial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Similar observations were made by Vlachos *et al.* (1997) who found that fractionation of crude extracts tested enhanced their activity against both

Gram negative as well as the resistant Gram positive pathogens. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from Sundarbon region. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

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