



Bioactivity of two medicinal plant *Xylocarpus granatum* Koen. (Meliaceae) and *Sarcolobus globosus* Wall. (Asclepiadaceae) of Sundarbans mangrove forest

M Alamgir*, Ma Rob, DC Kundu, JHK Joy and MM Sarder

Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

SUMMARY

Two medicinal plant of Sundarbans mangrove forest has been tested for the evaluation of growth inhibitory and antibacterial activity. The methanol extract of *Xylocarpus granatum* stem bark showed potent wheat rootlet ($IC_{50} = 0.01 \mu\text{g/ml}$) and shoot ($IC_{50} = 0.23 \mu\text{g/ml}$) growth inhibitory activity in a concentration related manner. The growth inhibitory activity was markedly decreased in residual methanol extract. The methanol extract showed antibacterial activity ($MIC > 3 \text{ mg/ml}$) against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris*. The residual methanol extract did not show any antibacterial activity. The results suggest the bioactive principle(s) of *Xylocarpus granatum* may be relatively non polar compound(s). The methanol extract and residual methanol extract of *Sarcolobus globosus* stem showed poor wheat rootlet and shoot growth inhibitory activity and no antibacterial activity.

Key words: Bioactivity; Sundarbans; Mangrove; Wheat rootlet growth inhibition; Antibacterial activity

INTRODUCTION

The Sundarbans mangrove forest is a globally significant ecosystem rich in plant biodiversity. It has been reported that the Sundarbans provides habitat for around 334 plants (Prain, 1903). In recognition of the global significance of the Sundarbans, UNESCO declared it as a world heritage site in December 1997. The Sundarbans is the largest contiguous block of mangrove forest in the world. Located along the mouth of the Bay of Bengal, it extends over 10,000 square kilometers in Bangladesh and India. Sixty percent of the area belongs to

Bangladesh. The forest lies in the northern south of the tropic cancer between $21^{\circ}30'$ and $22^{\circ}30'$ north latitude and 89° and 90° east longitudes (Chaudhuri and Naithani, 1985).

World wide there are now a growing interest for searching new drug compounds form plants for the treatment of diseases. The natural world, especially the plant kingdom, is recognized as a valuable source of diverse molecules. The Sundarbans reserved forest has a rich biodiversity of flowering plants and many of these have been used in traditional medicine, although the chemistry and bioactivity of the flora remains comparatively uninvestigated scientifically. These plants may represent a valuable source of novel drug compounds for the characterization and subsequent development of pharmaceutical modalities. We have started a systematic screening of the medicinal plants of

*Correspondence: M Alamgir, Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. (Present address) School of Chemistry, University of New South Wales, Sydney-2052, Australia. E-mail: m19alamgir@yahoo.com

Sundarbans with the purpose of discovering new bioactive compounds (Alamgir et al., 2006; Rajia et al., 2006).

Xylocarpus granatum is a moderate size tree and *Sarcolobus globosus* is a large climber that grows in the Sundarbans, the coasts of Indo-China, Malaya, Australia (Prain, 1903; Kirtikar and Basu, 1990). In traditional medicine *Xylocarpus granatum* bark is used for dysentery, diarrhoea and other abdominal troubles, and as febrifuges. The seed ash mixed with sulfur and coconut oil is applied as ointment for itch. Fruit is used as a cure for swellings of the breast and elephantiasis (Yusuf et al., 1994). We have recently reported the antidiarrhoeal activity of *Xylocarpus granatum* (Rouf et al., 2006). Limonoids (Alvi et al., 1991; Kokpol et al., 1996; Wu et al., 2003; Wu et al., 2004a; Wu et al., 2004b), lignins, tannins (Shinoda et al., 1984; Shinoda et al., 1985), alkaloids (Chou et al., 1977), and sterols (Majumdar and Patra, 1976) had been isolated from *Xylocarpus granatum*. *Sarcolobus globosus* has been used in traditional medicine against rheumatism, dengue and fever (Johnson, 1999). Seeds showed cardiotoxic action (Jabbar and Khan, 1982). Glycosidic extract exhibited neuromuscular blocking activity (Mustafa et al., 1990) and smooth muscle inhibitory activity (Mustafa, 1993). Our research collaborator in recent times reported the 15-lipoxygenase inhibitory and radical scavenging activity (Wangensteen et al., 2006) and isolated new rotenoids and isoflavonoids from *Sarcolobus globosus* (Wangensteen et al., 2005). The present study was performed as a preliminary study to investigate the effect of *Xylocarpus granatum* and *Sarcolobus globosus* on wheat rootlet and shoot growth inhibition bioassay and *in vitro* antimicrobial activity.

MATERIALS AND METHODS

Plant materials

Xylocarpus granatum Koen. syn. *Carapa obovata* Blume (Fam: Meliaceae) stem bark and *Sarcolobus globosus* Wall. (Asclepiadaceae) stems were collected from the Burigoalini Range of the Sundarbans

forest, Khulna, Bangladesh, in October 2001 and identified from the Bangladesh National Herbarium (*Xylocarpus granatum*, DACB no. 12791; *Sarcolobus globosus*, DACB no. 16609) Mirpur, Dhaka. The plants were air and oven (40° to 45°C) dried and was grinded by hammer mill to a fine powder.

The extract

The plant was extracted by cold extraction method. The dried powder was soaked in 80% of methanol in a glass container for seven days. The extract was separated from the plant debris by filtration and was concentrated by evaporation using rotary vacuum evaporator. They were dried under vacuum to give *Xylocarpus granatum* (172.8 g; yield 43.2%) and *Sarcolobus globosus* (144.2 g; yield 36.05%). The methanol extract was further partitioned using n-hexane and chloroform which gave the residual methanol fraction. The methanol extract (ME) and residual methanol fraction (RMF) were subjected to the following bioactivity studies.

Wheat rootlet and shoot growth inhibition bioassay

Ten germinated wheat grains (*Triticum awstivum* L., cv Buck Nandti) selected at random, were placed on a filter paper in a Petri dish containing 5 ml of the methanol extract and residual methanol fraction solutions of different concentrations (250, 500, 1,000 and 1,500 µg/ml). The Petri dishes were incubated for 5 days under the normal conditions in a dark place. The longest rootlet length and shoot length of each seed was measured and the inhibition was calculated as a percentage relative to the length of the rootlets and shoots in the controls with tap water. The average rootlet and shoot length of the ten seeds for each concentration was used in the percentage of growth inhibition calculation. The IC₅₀ values were calculated from the straight line drawn from different log concentration vs. percentage inhibition graph (Mongelli et al., 1995).

Antibacterial activity test

The antibacterial activity test was done at Gono

Shasthaya Vaccine Research and Diagnostic Laboratory, Savar, Dhaka using the standard agar dilution method. The sterile Mueller-Hinton agar media was poured into a sterile Petri dish in aseptic condition at room temperature. The medium was incubated at 37°C for 48 h before use to check whether there was any infection in the medium. Culture of the test organism was streaked uniformly over the medium using an inoculating loop in aseptic condition. Concentrations of the extracts used in the test were 1 mg/ml, 2 mg/ml and 3 mg/ml and they were incubated in an incubator (Gallenkamp, Germany) at 37°C for 24 h for culture sensitivity test (Colee, 1967). The microorganisms used in this study were clinical bacterial isolates collected from Gono Shasthaya Hospital, Savar, Dhaka. The microorganisms used were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Bacillus subtilis*.

Phytochemical screening

The phytochemical tests for carbohydrates, alkaloids, saponins, glycosides, tannins, flavonoids were performed according to Ghani (1998).

RESULTS

Wheat rootlet and shoot growth inhibition bioassay

From the wheat rootlet and shoot (Table 1) growth inhibition bioassay we observed that the methanol extract of *Xylocarpus granatum* had potent dose dependent growth inhibitory effect on both the

wheat rootlet and shoot. The IC₅₀ value of the methanol extract was observed 0.01 µg/ml for rootlet and 0.23 µg/ml for shoot. The residual methanol extract of *Xylocarpus granatum* also had a high growth inhibitory effect on both the wheat rootlet and shoot. The inhibition of both the rootlet and shoot was concentration dependent. The IC₅₀ value of the residual methanol extract was 705.16 µg/ml for rootlet and 1403.16 µg/ml for shoot.

The wheat rootlet and shoot (Table 1) growth inhibition bioassay revealed that the methanol extract of *Sarcolobus globosus* had a dose dependent growth inhibitory effect on both the wheat rootlet and shoot. The IC₅₀ value of the methanol extract was 364.88 µg/ml for rootlet and 851.86 µg/ml for shoot. The IC₅₀ of the residual methanol extract was found 557.85 µg/ml for rootlet and 665.49 µg/ml for shoot.

Antibacterial activity test

The antimicrobial study (Table 2) of *Xylocarpus granatum* showed that the methanol extract (ME) had antibacterial activity at the 3 mg/ml concentration level against gram positive bacteria *S. aureus* and *B. subtilis* and gram negative bacteria *P. vulgaris*. The extract was found inactive against *E. coli* and *P. aeruginosa*. The residual methanol extract (RME) was found inactive against the entire tested microorganism. The *Sarcolobus globosus* showed that both the methanol extract and residual methanol extract did not show any antibacterial activity against the gram positive bacteria and gram negative bacteria in our experiment.

Table 1. Wheat rootlet and shoot growth inhibitory activity of *Xylocarpus granatum* and *Sarcolobus globosus*

Experiment	<i>Xylocarpus granatum</i>		<i>Sarcolobus globosus</i>	
	IC ₅₀ (µg/ml)	Regression equation	IC ₅₀ (µg/ml)	Regression equation
Methanol extract				
Rootlet	0.01	Y = 9.87X + 70.03	364.88	Y = 49.13X - 75.88
Shoot	0.23	Y = 13.36X + 8.47	851.86	Y = 88.33X - 208.84
Residual methanol extract				
Rootlet	705.16	Y = 81.65X - 182.58	557.85	Y = 134.41X - 319.16
Shoot	1403.16	Y = 39.67X - 74.87	665.49	Y = 117.10X - 280.59

IC₅₀: Inhibitory concentration 50% of length (µg/ml)

Table 2. Antibacterial activity of *Xylocarpus granatum* and *Sarcolobus globosus* (MIC, g/ml)

Microorganisms	<i>Xylocarpus granatum</i>		<i>Sarcolobus globosus</i>	
	ME	RME	ME	RME
Gram positive bacteria				
<i>Bacillus subtilis</i>	3	-	-	-
<i>Staphylococcus aureus</i>	3	-	-	-
Gram negative bacteria				
<i>Escherichia coli</i>	-	-	-	-
<i>Proteus vulgaris</i>	3	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-

- : Not active; ME: Methanol extract; RME: Residual methanol extract

Phytochemical screening

The phytochemical study of the *Xylocarpus granatum* showed the presence of carbohydrate, saponins, tannins and flavonoids and absence of alkaloids and glycosides. The *Sarcolobus globosus* showed the presence of carbohydrate, saponins, tannins, glycosides and flavonoids and absence of alkaloids.

DISCUSSION

During the rapid growth of the root and shoot from seeds the cells are divided by mitosis. We have performed the wheat rootlet and shoot growth inhibition bioassay to observe the effect of the methanol extract and residual methanol extract on the mitotic cell division of the rootlet and shoot of wheat grain. Wheat rootlet growth inhibition bioassay has been previously reported as a monitor for detecting biological activities (Puyvelde *et al.*, 1988; Gonzalez *et al.*, 1993; Mongelli *et al.*, 1995). Root and shoot growth in plants also involves the action of specific growth factors and hormones, and thus the mechanism of action of the extract on growth inhibition may not necessarily reflect a direct inhibition of mitosis alone

The ethanol extract and residual methanol extract of *Xylocarpus granatum* (Meliaceae) showed a potent growth inhibitory effect on both the wheat rootlet and shoot growth inhibition bioassay in a dose dependent manner. Whereas the residual methanol extract exhibited less inhibitory effect

than the methanol extract. However, both the extracts indicated a more pronounced inhibitory effect on the rootlet growth than the shoot growth. Removal of the non polar compounds (by n-hexane and chloroform) from the methanol extract reduced the inhibitory activity both on the rootlet and shoot growth, suggests the growth inhibitory active principle(s) may be relatively non polar. Meliaceae contains a variety of compounds types but specially pentacyclic triterpenoids, limonoids. A considerable number of quite different functions have been ascribed to plants terpenoids. Their growth regulating properties are very well documented, two of the major classes of growth regulators are the sesquiterpenoid abscisins and the diterpenoid gibberellins (Harborne, 1973). We can assume that the limonoids isolated previously from *Xylocarpus granatum* (Alvi *et al.*, 1991; Kokpol *et al.*, 1996) may cause this wheat rootlet and shoot growth inhibitory effect by interfering with the mitotic cell division.

The methanol extract and residual methanol extract of *Sarcolobus globosus* showed a poor growth inhibitory effect on the wheat rootlet and shoot growth inhibition bioassay (Table 1). The methanol extract displayed a higher inhibitory effect than the residual methanol extract on wheat rootlet growth. Removal of the non polar compounds (by n-hexane and chloroform) from the methanol extract increased the inhibitory activity on the shoot growth, but decreased activity on the rootlet growth compared to residual methanol extract. In

this short study it is not possible to mention which type of compound is responsible for this activity, but we can assume saponins or flavonoids present in the extracts may cause this inhibition.

In particular, the search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Davies, 1994). Remarkable diversity of chemicals present in biological samples has tremendous potential in search of new antimicrobial agents (Rahman *et al.*, 2001). The methanol extract of *Xylocarpus granatum* showed antibacterial activity, but residual methanol extract was found inactive against the tested microorganisms. Fractionation of the methanol extract (by n-hexane and chloroform) removes the antibacterial activity, suggests that the active antibacterial principle(s) may be relatively non polar compound(s). It is also known that triterpenes have a protective function in repelling insect and microbial attack (Harborne, 1973). Thus the triterpenes may be involved in the antimicrobial activity of *Xylocarpus granatum*. Whereas, *Sarcolobus globosus* did not show any antibacterial activity against the microorganisms tested in our experiment. Resistance of the clinical bacterial isolates could be the cause of this ineffectiveness.

The antibacterial activity of *Xylocarpus granatum* provides some basis of the treatment for diarrhea in traditional medicine. In addition, *Xylocarpus granatum* possess potential to be a source of cytotoxic molecule(s) than *Sarcolobus globosus*. The mechanism underlying for these two activity might be for single compound or synergistic effect of multiple compounds in the extracts Further studies are required to explore the activity on different models and to isolate the active component(s) responsible for this antibacterial and growth inhibition activity.

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