



Antidiarrheal and antimicrobial activities of bark and leaf extracts of *Xylocarpus granatum* Koenig

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Abstract

Objective: To assess the antidiarrheal and antimicrobial activities of ethanolic extracts prepared from bark and leaf of *Xylocarpus granatum*. **Materials and methods:** The effect of ethanolic extracts of bark and leaf of *Xylocarpus granatum* was studied at doses of 500 mg/kg and 1000 mg/kg body weight in 5% gum acacia against castor oil induced diarrhea in rats. The antimicrobial activity of crude ethanolic extracts of bark and leaf of *Xylocarpus granatum* was studied at concentrations of 50 µl of 10 mg/ml, 100 mg/ml and 300 mg/ml of extracts dissolved in 50% dimethyl sulfoxide (DMSO) solution in water against three species of Gram positive bacteria, two species each of Gram negative bacteria, fungi and yeast by agar cup-plate diffusion method. **Results:** The ethanolic extracts of bark and leaf showed antidiarrheal activity in castor oil induced diarrheal rats in a dose-dependent manner. The extracts showed antimicrobial activity against some of the selected species of microorganisms. **Conclusion:** The ethanolic extracts of bark and leaf of *Xylocarpus granatum* showed potential antidiarrheal activity against castor oil induced diarrhea in rats in a dose-dependent manner. The bark extract showed antibacterial activity (Gram positive) and antiyeast activity.

Key words: *Xylocarpus granatum*, antidiarrheal, antimicrobial, purging index.

1. Introduction

The plant *Xylocarpus granatum* Koenig (a mangrove species) is a tree belonging to the family *Meliaceae*. It is distributed along the Coastal areas of India, Sri Lanka, Malaysia, Australia, Africa and South East Asia. Mangroves are economically exploited

especially for timber and fishery products [1]. Interestingly, many mangrove species are used for medicines. Traditionally *Xylocarpus granatum* is used to treat dysentery, cholera, diarrhea, fever and in skin infections [2]. In the present investigation, an attempt has been

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made to study the antidiarrheal and antimicrobial activities of ethanolic extracts of bark and leaf of *Xylocarpus granatum*.

2. Materials and methods

2.1 Plant Material

The plant material of *Xylocarpus granatum* was collected from Shanti Ashram, Visakhapatnam. Dr. K. Hemadri, taxonomist, Regional Research Institute, Botanical Survey of India, Vijayawada identified the plant. The specimens were preserved in the herbarium of our Department.

2.2 Preparation of the Extracts

Dried, powdered bark and leaves of *Xylocarpus granatum* were extracted with ethanol by Soxhlation, until there was no colour to the solvent (6 cycles). The crude extracts of bark and leaves were evaporated to dryness in rotary film evaporator. 1 g of bark extract represents 2.3 g of dried bark and 1 g of leaf extract represents 7.3 g of dried leaf.

2.3 Animals Used

Albino rats of either sex (National Institute of Nutrition, Hyderabad) weighing between 150-200 g were used in the study. They were caged individually and maintained at a room temperature of 25 ± 2 °C with relative humidity of 45% to 55%. They were provided with standard diet (Ratan Brothers, Hyderabad) and water *ad libitum*.

2.4 Microorganisms Used

Twenty four hour old cultures of *Bacillus subtilis* (NCIM 2001), *Bacillus pumilus* (NCIM 2108), *Staphylococcus aureus* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2036) and *Escherichia coli* (NCIM 2035) were used between the Gram positive and Gram negative bacteria. The fungi used were *Aspergillus niger* (NCIM 558) and *Rhizopus oryzae* (ATCC 9363).

Candida albicans (MTCC 1637) and *Saccharomyces cerevisiae* (MTCC 1766) were among the yeast selected for testing antimicrobial activity.

2.5 Antidiarrheal Studies

The method of Niemeegers [3] was adopted. Rats of either sex which responded to castor oil (1 ml/rat) were selected. The rats were divided into six groups (A, B, C, D, E, and F) each group consisting of twelve animals. The animals were fasted overnight. The drugs were prepared as suspension in 5% gum acacia. All groups received treatments orally.

Group A received 0.5 ml of 5% gum acacia and served as control. Group B and C were treated with ethanolic bark extract of *Xylocarpus granatum* (500 mg/kg and 1000 mg/kg) respectively. Group D and E were treated with ethanolic leaf extract of *Xylocarpus granatum* (500 mg/kg and 1000 mg/kg) respectively. Group F received furazolidine (10 mg/kg) and served as standard.

All groups of rats received standard dose of castor oil (1 ml/rat) after one hour of treatment. The animals were examined for presence of diarrhea in each hour upto six hours. Diarrhea is defined as presence of stools of fluid material, which stained the absorbent paper beneath the cage [4]. The number of respondents, the time of onset of diarrhea for the respondents and the number of stools passed during the sixth hour were recorded for each rat. From these parameters, purging index [5] was calculated as

$$\text{Purging Index} = \frac{\text{No. of respondents} \times \text{Average No. of stools}}{\text{Average latent period}}$$

Percent purging indices were calculated taking purging index of control as 100 for better comparison.

Table 1.

Effect of ethanolic extracts of bark and leaf of *Xylocarpus granatum* in castor oil induced diarrheal rats.

Group	Treatment	Percent respondents	Mean No. of stools \pm S.E.M.	Mean latent period \pm S.E.M.	Purging index	Percent purging index
Group A	0.5 ml of 5% acacia + 1.0 ml of castor oil	95	1.90 \pm 0.20	1.28 \pm 0.07	141.01	100
Group B	Ethanolic bark extract of <i>Xylocarpus granatum</i> 500 mg/kg + 1.0 ml castor oil	66.67	1.16 \pm 0.31*	2.41 \pm 0.11*	32.09	22.75
Group C	Ethanolic bark extract of <i>Xylocarpus granatum</i> 1000 mg/kg + 1.0 ml castor oil	58.33	0.83 \pm 0.25*	3.44 \pm 0.07*	14.07	10.30
Group D	Ethanolic leaf extract of <i>Xylocarpus granatum</i> 500 mg/kg + 1.0 ml castor oil	75	1.37 \pm 0.27	2.00 \pm 0.04*	51.56	35.98
Group E	Ethanolic leaf extract of <i>Xylocarpus granatum</i> 1000 mg/kg + 1.0 ml castor oil	62.5	0.75 \pm 0.26*	2.66 \pm 0.05*	17.62	12.29
Group F	Furazolidine 10 mg/kg + 1.0 ml castor oil	70	1.40 \pm 0.25	2.41 \pm 0.11*	40.66	28.83

*P<0.05 compared to Group A (Student's *t*-test).

n = 12 rats in each group.

Table 2.

Effect of ethanolic extracts of bark and leaf of *Xylocarpus granatum* on selected strains of microorganisms

Type	Strain	Zone of inhibition (mm ± S.E.M)							
		Ethanolic extract of <i>Xylocarpus granatum</i> bark			Ethanolic extract of <i>Xylocarpus granatum</i> leaf			Standard*	Control (50% DMSO)
		300 mg/ml	100 mg/ml	10 mg/ml	300 mg/ml	100 mg/ml	10 mg/ml		
Gram positive bacteria	<i>B. subtilis</i> (NCIM 2001)	14.0 ±0.71*	11.0 ± 0.71*	0.0 ± 0.0	13.0 ± 0.71*	11.0 ± 0.71*	0.0 ± 0.0	19.0 ± 0.71*	0.0 ± 0.0
	<i>B. pumilus</i> (NCIM 2108)	14.0 ± 0.71*	12.0 ± 0.71*	0.0 ± 0.0	12.0 ± 0.71*	12.0 ± 0.71*	0.0 ± 0.0	19.0 ± 0.71*	0.0 ± 0.0
	<i>S. aureus</i> (NCIM 2079)	15.0 ± 0.71*	12.0 ± 0.71*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	45.0 ± 0.71*	0.0 ± 0.0
Gram negative bacteria	<i>E. coli</i> (NCIM 2035)	13.0 ± 0.71*	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 0.71*	0.0 ± 0.0	0.0 ± 0.0	45.0 ± 0.71*	0.0 ± 0.0
	<i>P. aeruginosa</i> (NCIM 2036)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	20.0 ± 0.71*	0.0 ± 0.0
Fungi	<i>A. niger</i> (NCIM 558)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	18.0 ± 0.71*	0.0 ± 0.0
	<i>R. oryzae</i> (ATCC 9363)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	15.0 ± 0.71*	0.0 ± 0.0
Yeast	<i>C. albicans</i> (MTCC 1637)	18.0 ± 0.71*	16.0 ± 0.71*	11.0 ± 0.71*	10.0 ± 0.71*	9.0 ± 0.71*	0.0 ± 0.0	18.0 ± 0.71*	0.0 ± 0.0
	<i>S. cerevisiae</i> (MTCC 1766)	15.0 ± 0.71*	12.0 ± 0.71*	10.0 ± 0.71*	9.0 ± 0.71*	0.0 ± 0.0	0.0 ± 0.0	17.0 ± 0.71*	0.0 ± 0.0

a Standard: For antibacterial activity – Ampicillin (10 μ g/ml); For antifungal and antiyeast activities – Ketoconazole (10 μ g/ml).

Significant difference from control: *P<0.01

2.6 Antibacterial Activity

The *in vitro* antibacterial activity of the sample solution was studied by agar cup-plate diffusion method [6]. The test organisms were seeded into sterile nutrient agar medium (Hi-Media) by uniformly mixing one loopful of the inoculum with 20 ml of sterile melted nutrient agar after has been cooled to 48-50°C, poured in a sterile petridish. When the agar solidifies, five holes of uniform diameter (6 mm) were made using a sterile borer.

Then in each cup 50 µl from different concentrations of each extract such as 10 mg/ml, 100 mg/ml and 300 mg/ml were added after dissolving in suitable quantities of 50% DMSO in water solution. Simultaneous controls with 50% DMSO were also studied. Ampicillin (10µg/ml) was used as a standard drug for comparison. The plates were incubated at 37°C for 24 h. The zone of inhibition was calculated by measuring the minimum dimension of bacteria free zone around the cup.

2.7 Antifungal and Antiyeast Studies

The antifungal and antiyeast activities were tested by the same procedure described for testing antibacterial activity using potato dextrose agar medium (Hi-Media). The standard drug to compare the antifungal and antiyeast activities was ketoconazole (50 µl of 10 µg/ml).

2.8 Statistical Analysis

For antidiarrheal studies, results of latent period and number of stools passed during sixth hour were expressed as mean \pm S.E.M. The comparison was done between control and each group employing Student's *t*-test and were considered statistically significant when $P < 0.05$.

For antibacterial, antifungal and antiyeast activities, zone of inhibition was expressed as mean \pm S.E.M. The comparison was done between control and each group employing

Student's *t*-test and were considered statistically significant when $P < 0.01$.

3. Results and discussion

The ethanolic extracts of bark and leaf of *Xylocarpus granatum* were found to be having significant antidiarrheal activity in a dose-dependent manner and the results were shown in Table 1. The purging indices and percent purging indices of 500 mg/kg and 1000 mg/kg doses of the bark extract of *Xylocarpus granatum* were 32.09, 14.07 and 22.75%, 10.30% respectively.

The purging indices and percent purging indices of 500 mg/kg and 1000 mg/kg doses of the leaf extract of *Xylocarpus granatum* were 51.56, 17.62 and 40.66%, 28.83% respectively. The decreased percent purging indices represent the potential antidiarrheal activity of bark and leaf extracts.

The zones of inhibition obtained with different concentrations of alcoholic extracts of bark and leaf of *Xylocarpus granatum* were shown in Table 2. The alcoholic extracts (100 mg/ml and 300 mg/ml) of bark and leaf of *Xylocarpus granatum* were found to be having significant ($P < 0.01$) antimicrobial activity against *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*. The alcoholic extract of bark of *Xylocarpus granatum* showed better antimicrobial activity.

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