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Bioactivity of the methanol extract of *Excoecaria agallocha* Linn. (Euphorbiaceae)

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SUMMARY

The methanol extract and residual methanol fraction of *Excoecaria agallocha* L. (Euphorbiaceae) stem bark was investigated in this study by wheat rootlet and shoot growth inhibition, and antimicrobial bioassay. The methanol extract and residual methanol fraction showed high inhibitory effect on both the wheat rootlet (82 - 89%) and shoot growth (85 - 90%) compared to control. The methanol extract showed a better and dose related inhibition on both the rootlet and shoot growth compared to residual methanol fraction. The IC_{50} value of methanol extract for rootlet and shoot were 2.88 μg/ml and 2.32 μg/ml, and of residual methanol fraction for rootlet and shoot were 7.91 μg/ml and 4.45 μg/ml. The methanol extract and residual methanol fraction did not show any antibacterial activity against the tested microorganisms of clinical isolates *Escherichia coli, Staphylococcus aureous, Pseudomonas aeruginosa, Proteus vulgaris* and *Bacillus subtilis.* The plant has the potential to be a source of novel cytotoxic compound(s).

Key words: *Excoecaria agallocha*; Euphorbiaceae; Wheat rootlet and shoot growth inhibition; Bioassay; Atibacterial activity

INTRODUCTION

Excoecaria agallocha L. (Family: Euphorbiaceae) is distributed on seashores and edge-mangroves throughout tropical Africa, Asia, and northwest Australia. The species is very common in the Sundarbans and other coastal areas of Bangladesh (Siddiqi, 2001). The *Excoecaria* genus is well known to contain skin irritants (Konishi *et al*., 2003a). The milky latex exuded from the bark of *E. agallocha* may cause blindness or blistering of the skin (Jayaweera, 1980), has been used in the past as a poison for fish and as poison applied to arrowheads. In traditional Thai medicine the bark and wood of this plant is used against flatulence (Karalai *et al*., 1994). In Sri Lanka the smoke of the burning wood has been used in the treatment of leprosy, while the root pounded with ginger has been used as an embrocation for swelling hands and feet (Jayaweera, 1980). In Bangladesh the plant is used as caustic in obstinate ulcers and leprous sores. It is used as purgative and alternative. Soft reddish substance obtained from the lower part of the trunk and roots is aphrodisiac. Latex is abortifacient, boiled in oil is applied in rheumatism, leprosy and paralysis. Decoction of leaves is used in epilepsy and ulcers. Bark is used as purgative and emetic (Ghani, 1998).

The first work on their chemical constituents lead the isolation of exocarol, agalocol, and isoagalocol

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from latex (Qudrat *et al*., 1964), later epitaraxerol was obtained from leaves (Hui and Sung, 1968). Phorbol ester was reported to be the cause of the skin irritation (Erickson *et al*., 1995). A novel piperidine alkaloid and tetramethoxychalcone (Prakash *et al*., 1983), taraxerol and acetoxytaraxeroic acid (Anjaneyulu *et al*., 1993), cycloartenol (Kawashima *et al*., 1971), glycosides (Konishi *et al*., 2003b) was reported. From stem, root and wood several new diterpenes excoecarins and agallochins has been isolated (Konishi *et al*., 1996a,b, 1998a,b, 1999, 2000a,b, 2003a,b,c; Anjaneyulu and Rao, 2000; Anjaneyulu *et al*., 2002).

Diterpenes from wood of *E. agallocha* showed significant antitumor activity (Konishi *et al*., 1996c, 1998c; Konoshima *et al*., 2001). Anti-HIV activity (Erickson *et al*., 1995), antioxidant activity (Masuda *et al*., 1999), anti-termite activity (Miki *et al*., 1994) and antifungal activity (Sakaki *et al*., 1994) has been reported.

Present study was performed to investigate the bioactivity of the methanol extract of *Excoecaria agallocha* L. stem bark by wheat rootlet and shoot growth inhibition and antimicrobial assay.

MATERIALS AND METHODS

Plant materials

Excoecaria agallocha L. stem bark was collected from the Sundarbans of Burigoalini region. The plant was identified by the Bangladesh National Herbarium, Dhaka. A voucher specimen was deposited at the Pharmacy Discipline, Khulna University. The bark was air dried for two days before dried in an oven with strict temperature between 40 to 45°C 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 six days. The extract was separated from the plant debris by filtration and was concentrated by evaporation using rotary vacuum evaporator. The methanol extract (ME) was further partitioned using n-hexane and chloroform which gave the residual methanol fraction (RMF). The ME and 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 ME and RMF 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 IC_{50} 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

Table 1. Effect of methanol extract and residual methanol fraction of *Exceocaria agallocha* on wheat rootlet growth inhibition bioassay[a]

Conc. (mg/ml)	Control length	Methanol Extract		Residual Methanol Fraction	
		Rootlet length	% Inhibition	Rootlet length	% Inhibition
250	40.9 ± 12.93	5.67 ± 1.36	86.14	7.12 ± 1.84	82.59
500		5.27 ± 1.32	87.11	6.46 ± 1.11	84.20
1,000		4.63 ± 1.67	88.68	6.61 ± 1.85	83.83
1,500		4.45 ± 1.60	89.12	6.64 ± 2.00	83.76

[a]Values are expressed as Mean ± S.D. (*P* value), lengths are in mm

Table 2. Effect of methanol extract and residual methanol fraction of *Exceocaria agallocha* on wheat shoot growth inhibition bioassay[a]

Conc. (mg/ml)	Control length	Methanol Extract		Residual Methanol Fraction	
		Shoot length	% Inhibition	Shoot length	% Inhibition
250	56.6 ± 10.15	7.67 ± 1.29	86.45	7.98 ± 0.90	85.93
500		7.55 ± 1.32	86.66	7.56 ± 2.09	86.64
1,000		6.46 ± 3.23	88.58	6.95 ± 2.08	87.72
1,500		5.41 ± 3.04	90.44	7.60 ± 0.92	86.57

[a]Values are expressed as Mean ± S.D. (*P* value), lengths are in mm

microorganisms used in this study were clinical bacterial isolates collected from Gono Shasthaya Hospital, Savar, Dhaka. The microorganisms used were *Escherichia coli, Staphylococcus aureous, Pseudomonas aeruginosa, Proteus vulgaris* and *Bacillus subtilis*.

RESULTS AND DISCUSSION

During the rapid growth of the root and shoot from seeds the cells are divised by mitosis. We have performed the wheat rootlet and shoot growth inhibition bioassay to observe the effect of the methanol extract and residual methanol fraction on the mitotic cell division of the root and shoot of wheat grain. Wheat rootlet growth inhibition bioassay has been previously reported as a monitor for detecting biological activities (Van 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.

From the wheat rootlet (Table 1) and shoot (Table 2) growth inhibition bioassay we observed that the ME of *Excoecaria agallocha* showed high inhibitory effect on both the wheat rootlet and shoot growth. It inhibited the growth of wheat rootlet about 86 - 89% and shoots 86 - 90% at different concentrations (250, 500, 1,000 and 1,500 μg/ml) when compared to control. The inhibition of both the rootlet and shoot growth observed was concentration dependent. The Pearson correlation coefficient (R^2) value of the primary methanol extract was observed high as 0.9873 for rootlet and 0.8651 for shoot. The IC_{50} value (Table 3) of methanol extract was found 2.88 μg/ml for rootlet and 2.32 μg/ml for shoot.

The RMF of *Excoecaria agallocha* also showed considerable inhibitory effect on both the wheat rootlet and shoot growth. It inhibited the growth of wheat rootlet about 82 - 84% (Table 1) and shoots 85 - 88% (Table 2) at different concentrations (250,

Table 3. IC_{50} and R^2 value of methanol extract and residual methanol fraction of *E. agallocha* on wheat rootlet and shoot growth

Experiment	IC_{50} (μg/ml)	Regression equation	R^2
Methanol Extract			
Rootlet	2.88	4.035X + 76.39	0.9873
Shoot	2.32	5.104X + 73.65	0.8651
Residual Methanol Fraction			
zRootlet	7.91	1.126X + 79.94	0.4008
Shoot	4.45	1.351X + 82.91	0.3846

Table 4. Antimicrobial activity of methanol extract and residual methanol fraction of *Exceocaria agallocha*

Microorganism	Methanol Extract / Residual Methanol Fraction		
	1 mg/ml	2 mg/ml	3 mg/ml
Gram positive bacteria			
Bacillus subtilis	-	-	-
Staphylococcus aureus	-	-	-
Gram negative bacteria			
Escherichia coli	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas aeruginosa	-	-	-

- inactive

500, 1,000 and 1,500 μg/ml) when compared to control. The inhibition of both the rootlet and shoot growth was not concentration dependent. The Pearson correlation coefficient (R^2) value of the residual methanol fraction (Table 3) was observed low as 0.4008 for rootlet and 0.3846 for shoot. The IC_{50} value (Table 3) of residual methanol fraction was found 7.91 μg/ml for rootlet and 4.45 μg/ml for shoot.

Both the methanol extract and residual methanol fraction of *Exceocaria agallocha* showed a high inhibition on both the wheat rootlet and shoot growth inhibition bioassay. The methanol extract showed a better and dose related inhibition on both the rootlet and shoot growth compared to residual methanol fraction. After 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 non polar fractions may contain growth inhibitory active principle(s). Classical plant hormones auxins, cytokinins, gibberellins and abscisic acid have growth regulatory biological effects (Gaspar *et al*., 1996). From the literature study we have seen that the plant possesses terpenoids which have growth regulatory properties. We can assume terpenoids may be responsible for the current wheat rootlet and shoot growth inhibitory effect. But the exact mechanism and reason is not known, which needs further studies.

A large number of human, animal and plant disease are caused by pathogenic microbes, unfortunately human struggle against pathogenic microbes is far from over due to many reasons. Most important of them time to time discovery of new pathogens and remarkable abilities of microbes to develop resistance against used antibiotics. The discovery and development of new antimicrobial agents is therefore an on going process. Remarkable diversity of chemicals present in biological samples has tremendous potential in search of new

antimicrobial agents (Rahman *et al*., 2001). Considering Bangladesh is a tropical country with high susceptibility to protozoal diseases, which are a major health care problem of our country. We have performed some antimicrobial activity tests by agar dilution method on some clinical isolates. From the Table 4 we can observed that the methanol extract did not show any antibacterial activity against the tested microorganisms: gram positive bacteria, *S. aureus* and *B. subtilis,* and gram negative bacteria *E. coli, P. aeruginosa* and *P. vulgaris* at the different concentrations used (1 mg/ml, 2 mg/ml and 3 mg/ml). As the test organisms were clinical isolates, bacterial resistance could be the cause of this ineffectiveness. However further studies should be done with a wide range of microbes.

CONCLUSION

In conclusion we can say that the methanol extract and residual methanol fraction of *Exceocaria agallocha* stem bark showed high inhibitory effect on both the wheat rootlet and shoot growth. Although it failed to show antibacterial activity against the clinical isolates tested, considering the previous study the plant has the potential to be a source of novel cytotoxic compound(s). Further bioassay guided studies required to isolate the active molecule(s).

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