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RESEARCH ARTICLE

COMPARATIVE STUDY OF ANTIBACTERIAL, ANTIFUNGAL AND CYTOTOXIC EFFECTS OF DIFFERENT EXTRACTS OF *DILLENIA INDICA* THUNB AND *ABROMA AUGUSTA* LINN

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The present study was designed to evaluate *in vitro* antibacterial, antifungal and cytotoxic effects of ethanolic and petroleum ether extracts of two Bangladeshi medicinal plants *Dillenia indica* and *Abroma augusta*. Aiming to investigate antibacterial and antifungal activities, disc diffusion method was followed using eleven pathogenic bacteria and six fungi as test organisms. The plant extracts (400 μg/disc) showed moderate antibacterial activities (zone of inhibition (zoi): 8-15 mm) which was compared with standard kanamycin (30 μg/disc), while extracts showed positive antifungal activities (zoi: 10-18 mm) and griseofulvin (1.0 μg/disk) was used as standard antifungal agent. During evaluation of *in vitro* cytotoxicity effects of the plant extracts, brine shrimp lethality bioassay was performed observing mortality rate of brine shrimp nauplii (*Artemia salina*) and the LC_{50} value observed by probity analysis as 574.926, 334.284, 380.875 and 307.459 for DIET, DIPE, AAET and AAPE respectively. Current studies indicated that both plant extracts possessed moderate antimicrobial activities and good cytotoxic properties.

Key words: *Dillenia indica*, *Abroma augusta*, Antimicrobial activity, Cytotoxicity, Disc diffusion method.

INTRODUCTION

At present, herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants having folklore reputation in a more intensified way. A huge number of the world's population have exclusively been used medicinal plants for centuries as remedies for human diseases (Nostro *et al* 2000; Arokiyaraj *et al* 2008). As a result, different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects (Ali *et al*, 2001; El-Fiky *et al* 1995). Some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Barbour *et al* 2004; Redo *et al* 1989; Cragg *et al* 1997). Recently, multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995) making it a global growing-problem. Meanwhile, over 50% of all drugs in clinical trials for anticancer activity were isolated from natural sources or are related to them (Cragg and Newman, 2000). Hence, Brine shrimp lethality test (BST) has been employed as an alterantive bioassay technique to screen the plant extracts (Meyer *et al* 1982; Mitscher *et al* 1972). *Dillenia indica* Thunb (Family: Dilleniaceae) locally named Chalta, Chalita (Bangali), Dillenia (English), Bhavya, Bharija (Sanskrit) that is widely distributed in sub-Himalayan tract including Bangladesh, India (Garhwal to Assam, Arunachal Pradesh, Manipur,

Tripura, West Bengal, Orissa, Bihar, Central and South India); Nepal and Sri Lanka (**Figure 1**). The fruits are tonic and laxative; used in diarrhoea, dysentery and burns in Khagrachari (Yusuf *et al* 1994). Seed extract possess antimicrobial activity (Asolkar *et al* 2009). *Abroma augusta* Linn (Family: Sterculiaceae), commonly known as Ulatkambal (Bengali and Hindi), widely distributed (native or cultivated) throughout the hotter parts of India (UP, Sikkim, Khasia Hills, Assam) and hill tract area of Bangladesh (**Figure 2**). The fresh viscid sap of the root bark is considered to be a valuable emmenagogue and uterine tonic, useful in the congestive and neuralgic varieties of dysmenorrhoea (Kirtikar and Basu, 1998). In present research work, we examine the antimicrobial and cytotoxic effect of the ethanolic extracts of stem bark of *Dillenia indica* Thunb and *Abroma augusta* Linn.

Fig. 1. Photograph of *Dillenia indica* Thunb

Fig. 2. Photograph of *Abroma augusta* Linn.

MATERIALS AND METHODS

Collection of plant material

The stem bark of *Dillenia indica* Thunb and *Abroma augusta* Linn were collected from Ramgarh, Chittagong. Using standard taxonomical methods, the Bangladesh Forest Research Institute (BFRI), Chittagong, identified the plant's parts used in this project. The samples were then separated and cleaned from impurities.

Extraction of plant material

The stem barks were separated and washed with tap water to remove the impurities. The stem barks were cut into small pieces and were subjected to air dry for 10 days. The air-dried samples were then transferred into oven for drying and then were crushed. The crushed powders of the plants were soaked into two aspiratory bottles with ethanol and pet ether. The amounts of solvents were 2 liters per bottles for the first time. After six times extraction, the extracts were collected and concentrated under reduced pressure at below 50°C through rotary vacuum evaporator. The solid mass was then dried at 70°C.

Antibacterial and antifungal assay

The antimicrobial activity of the crude extracts were determined by the disc diffusion method (Bauer *et al* 1966; Rahman and Rashid, 2008) against the microbial strains listed in **Table 1** whereas kanamycin (30 µg/disc) and griseofulvin (1 µg/disc) were used as the standard for antibacterial and antifungal respectively. The extracts were dissolved separately in suitable solvent and applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent. These plates were then kept at low temperature (4°C) for 24 h to allow maximum diffusion of the test materials, kanamycin and griseofulvin. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Collection of test organisms

The bacterial species used in the current study were *S. dysenteriae, S. typhi, P. aeruginosa, S. aureus, B. cereus, B. subtilis, E. coli, Klebsiella sp, S. lutea, S. sonnei* and *B. megaterium* whereas the fungi used were *M. phaseolina, F. solani, Curvularia, Botryodiplodia, Altenaria, C. trycomonus.* The test organisms were collected

from Department of Pharmacy, Rajshahi University, Rajshahi, Bangladesh.

Cytotoxicity screening

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer *et al* 1982; Zhao *et al* 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial sea water (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method (Meyer *et al* 1982).

The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, 600 µg/ml, 700 µg/ml, 800 µg/ml, 900 µg/ml and 1000 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. The number of the nauplii that died after 24 h was counted. Then, % of mortality was plotted against respective concentrations used and from the graph LC_{50} was calculated.

RESULT AND DISCUSSION

Antibacterial assay

The extracts of the sample were tested for antibacterial activity against a number of both gram positive and gram negative bacteria. Standard antibiotic disk of kanamycin at 30 µg/disk was used for comparison purposes. The extracts showed antibacterial activity against limited number of the test organisms. The results of the antibacterial activity measured in terms of diameter of zone of inhibition in mm are showed in **Table 1**. One concentration of the extracted sample 400 µg/disc was used for antibacterial activity.

Table 1. *In vitro* antibacterial activity of extracts of stem bark of *Dillenia indica* and *Abroma augusta*

Sample code	Bacterial species	Diameter of zone of inhibition (mm)				
		Extracts (400 µg/disc)				Standard (30 µg/disc)
		DIET	DIPE	AAET	AAPE	Kanamycin
B 01	*Shigella dysenteriae*	-	-	-	-	27
B 02	*Salmonella typhi*	12	10	-	-	22
B 03	*Pseudomonas aeruginosa*	-	-	9	10	30
B 04	*Staphylococcus aureus*	13	11	-	-	27
B 05	*Bacillus cereus*	-	-	-	-	18
B 06	*Bacillus subtilis*	-	-	10	8	27
B 07	*Escherichia coli*	-	-	-	-	32
B 08	*Klebsiella spp*	11	12	-	-	28
B 09	*Sarcina lutea*	-	-	9	10	30
B 10	*Shigella sonnei*	12.5	-	-	-	30
B 11	*Bacillus megaterium*	15	-	-	-	28

*DIET, DIPE, AAET and AAPE denotes for *Dillenia indica* ethanolic extract, *Dillenia indica* petroleum ether extract, *Abroma augusta* ethanolic extract and *Abroma augusta* petroleum ether extract

Antifungal assay

The extracts of the sample were tested for antifungal activity against a number of fungi. Standard disk of griseofulvin at 0.1 µg/disc was used for comparison purposes. The extracts showed little antifungal activity against the test organisms. The results of antifungal activity measured in terms of diameter of zone of inhibition (ZOI) are shown in the **Table 2**.

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay of the ethanolic and petroleum ether extracts of *Dillenia indica* Thunb (**Table 3**, **Figure 1**) and *Abroma augusta* Linn. (**Table 4**, **Figure 2**) were tested by following the procedure of Meyer where DMSO used as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality or not. For the extract, the number of

Table 2. *In vitro* antifungal activity of extracts of stem bark of *Dillenia indica* and *Abroma augusta*

Sample code	Fungal species	Diameter of zone of inhibition (mm)				
		Extracts (400 µg/disc)				Standard (0.1 µg /disk)
		DIET	DIPE	AAET	AAPE	Griseofulvin
F 01	*Macrophomina phaseolina*	12	10	-	-	58.7
F 02	*Fuserium solani*	-	-	17.5	15	32
F 03	*Curvularia*	-	-	-	-	37
F 04	*Botryodiplodia*	-	-	-	-	29.5
F 05	*Altenaria*	-	-	-	-	26
F 06	*Colitrio trycomonus*	13	-l	18	16	35

*DIET, DIPE, AAET and AAPE denotes for *Dillenia indica* ethanolic extract, *Dillenia indica* petroleum ether extract, *Abroma augusta* ethanolic extract and *Abroma augusta* petroleum ether extract

nauplii died and percent mortality was counted. We have observed that the LC_{50} value of the ethanolic and petroleum ether extracts of *Dillenia indica* Thunb were 574.926 µg/ml and 334.283 µg/ml respectively whereas it was 380.875 µg/ml and 307.458 µg/ml respectively for ethanolic and petroleum ether extracts of *Abroma augusta* Linn (**Table 3**).

Table 3. *In vitro* cytotoxic activity of extracts of stem bark of *Dillenia indica* Thunb

Conc. (µg/ml)	Log C	% Mortality		Probit		LC_{50} (µg/ml)	
		DIET	DIPE	DIET	DIPE	DIET	DIPE
100	2.00	10	30	3.72	4.48	574.926	334.284
200	2.30	20	30	4.16	4.48		
300	2.47	20	40	4.16	4.75		
400	2.60	40	50	4.75	5		
500	2.69	30	60	4.48	5.25		
600	2.77	50	60	5	5.25		
700	2.84	60	80	5.25	5.84		
800	2.90	80	100	5.84	5.27		
900	2.95	100	100	5.25	5.25		
1000	3.00	100	100	5.84	5		

Table 4. *In vitro* cytotoxic activity of extracts of stem bark of *Abroma augusta* Linn

Conc. (µg/ml)	Log C	% Mortality		Probit		LC_{50} (µg/ml)	
		AAET	AAPE	AAET	AAPE	AAET	AAPE
100	2.00	20	30	4.16	4.48	380.875	307.459
200	2.30	30	30	4.48	4.48		
300	2.47	30	40	4.48	4.75		
400	2.60	40	50	4.75	5		
500	2.69	50	50	5	5		
600	2.77	60	70	5.25	5.52		
700	2.84	70	90	5.52	6.28		
800	2.90	90	100	6.28	4.38		
900	2.95	100	100	5.25	5		
1000	3.00	100	100	5.52	4.75		

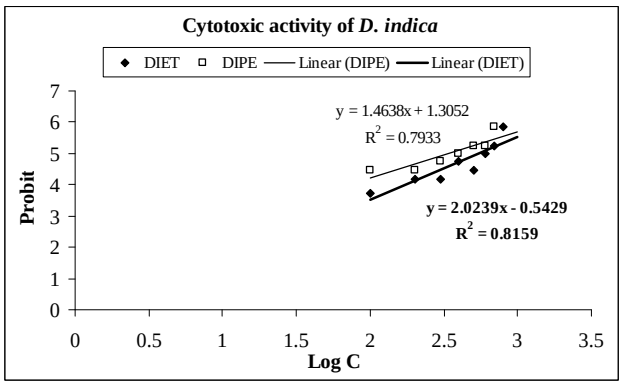

Fig. 1. *In vitro* cytotoxic activity of extracts of stem bark of *Dillenia indica*

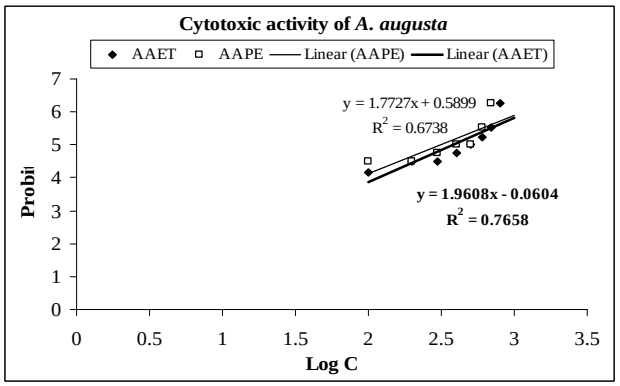

Fig. 2. *In vitro* cytotoxic activity of extracts of stem bark of *Abroma augusta*

CONCLUSION

Considering the results of the conducted study, it can be inferred that the ethanolic and petroleum ether extracts of *Dillenia indica* Thunb and *Abroma augusta* Linn possessed moderate zone of inhibition towards different gram positive and gram negative organisms as an antimirobial effect while the extracts showed considerable cytotoxic effect. However, there is plenty of scope for further studies for the better understanding of the pharmacological activities, mechanism of action as well as the active compounds responsible for actions exerted by these plant extracts.

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