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## Phytochemical Screening, Cytotoxicity and Antibacterial Activities of Two Bangladeshi Medicinal Plants

<sup>1</sup>Ajoy Roy, <sup>2</sup>Subrata Kumar Biswas, <sup>2</sup>Anusua Chowdhury, <sup>3</sup>Manik Chandra Shill,  
<sup>4</sup>Sheikh Zahir Raihan and <sup>4</sup>Md. Abdul Muhit

<sup>1</sup>Department of Pharmacy, East West University Bangladesh, Dhaka, Bangladesh

<sup>2</sup>Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh

<sup>3</sup>IPD Pharmacy, Square Hospitals Limited, Dhaka, Bangladesh

<sup>4</sup>Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy,  
University of Dhaka, Bangladesh

**Abstract:** The objectives of the present study were to investigate phytochemical screening and to assay cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Family: Araceae) and *Lannea grandis* Engl. (Family: Anacardiaceae) available in Bangladesh. The brine shrimp lethality bioassay showed that the ethanolic extracts of *Aglaonema hookerianum* and *Lannea grandis* possessed cytotoxic activities with  $LC_{50}$  5.25 ( $\mu\text{g mL}^{-1}$ ) and 5.75 ( $\mu\text{g mL}^{-1}$ ) and  $LC_{90}$  10.47 ( $\mu\text{g mL}^{-1}$ ) and 9.55 ( $\mu\text{g mL}^{-1}$ ), respectively. Two extracts obtained from leaves were examined for their antibacterial activities against some gram positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium* and *Staphylococcus aureus*, also gram negative strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi* and *Vibrio cholerae*. Agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that both plant extracts ( $500 \mu\text{g disc}^{-1}$ ) displayed antibacterial activity against all of the tested microorganisms. These results were also compared with the zones of inhibition produced by commercially available standard antibiotic, Amoxicillin at concentration of  $10 \mu\text{g disc}^{-1}$ . Observed antibacterial properties of the ethanolic extract of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl. showed that both plants might be useful sources for the development of new potent antibacterial agents.

**Key words:** Antibacterial, cytotoxic, bioassay, amoxicillin, phytochemical

### INTRODUCTION

*Aglaonema* Schott is included in the family of Araceae. This medicinal plant is widely distributed in tropical Asia. Malay, Archipelago and Papuasias (Mayo *et al.*, 1997). The genus *Aglaonema* was revised by Nicolson (1969) who recognized 21 species and 2 sections. The genus *Aglaonema* is known as *Aglaonema hookerianum* Schott in Bangladesh (Ara *et al.*, 2005). The plant is available in Chittagong Hill Tracts and Sylhet. The root infusion of *Aglaonema hookerianum* Schott taken orally is used for the treatment of conjunctivitis and constipation (Rahman *et al.*, 2007). On the other hand, the local name of *Lannea grandis* Engl is Jikkha belonging to the family of Anacardiaceae. The leaves of *Lannea grandis* Engl were used as diuretic in case of urinary problems. This medicinal plant is also utilized by the Garo Tribal Healers of Netrokona District in Bangladesh

(Rahmatullah *et al.*, 2009). The meristem of *Lannea grandis* was used to treat dog bites, while the gum of the same plant was mixed with seeds of *Asterantha longifolia* to treat low sperm count (Rahmatullah *et al.*, 2010). Due to lack of scientific data, the present study was conducted to investigate the cytotoxic and antimicrobial activities of the leaves of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl.

### MATERIALS AND METHODS

**Plant materials and preparation of the extract:** The medicinal plants *Aglaonema hookerianum* Schott and *Lannea grandis* Engl selected for this study were collected from Sylhet and Netrokona, respectively during the months of September-October (2009) at day time. The leaves of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl. were dried at room temperature

under shade and were ground into a coarse powder with the help of a suitable grinder. About 200 g of powdered material of each medicinal plant was taken in clean, flat bottomed glass container and soaked in 1000 mL of 99.8% ethanol. The containers with its contents were sealed and kept for a period of 14 days accompanied by continuous shaking with a shaker. The solvent extracted materials were filtered and the resulting filtrates were concentrated under reduced pressure. Finally, the extracts of both plants were preserved until required.

**Culture media and test microorganisms:** The media used for bacteria was Nutrient agar and the test organisms used in this study included both gram-positive bacteria such as (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*) and gram negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi* and *Vibrio cholerae*).

**Standard drug:** Amoxicillin was used as a standard drug in this research work and the drug was collected from Square Pharmaceuticals Limited, Dhaka, Bangladesh.

**Preliminary phytochemical screening:** The ethanolic extracts of the leaves of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl. were subjected to preliminary phytochemical screening as per procedure to identify the presence of various phytochemicals. Phytochemical screening of the extracts were performed using the following reagents: Alkaloids with Dragendroff's reagent and Mayer's reagent, Steroids with sulphuric acid, Glycosides with sodium hydroxide, Tannins with ferric chloride and potassium dichromate solutions, Reducing sugars with Fehling's solution (A and B) and Benedict's solution, Saponins with the ability to produce foams. Gum was also tested using Molish reagent and concentrated sulphuric acid (Trease and Evans, 1989).

**Cytotoxic activity:** The cytotoxic activity of the crude ethanolic extracts of leaves of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl was tested on brine shrimp nauplii according to brine shrimp lethality bioassay (Meyer *et al.*, 1982). From this study, the lethal concentrations  $LC_{50}$  ( $\mu\text{g mL}^{-1}$ ) and  $LC_{90}$  ( $\mu\text{g mL}^{-1}$ ) of the ethanolic extracts were determined.

**In-vitro antimicrobial assay:** The extracts obtained from the leaves of *Aglaonema hookerianum* Schott. and *Lannea grandis* Engl. were studied for antimicrobial activity using the agar disc diffusion method (Bauer *et al.*, 1966). The concentrations of ethanolic extracts of the plants and the standard drug, Amoxicillin used in this study were 500 and 10  $\mu\text{g disc}^{-1}$ , respectively.

## RESULTS AND DISCUSSION

**Phytochemical screening:** The results of phytochemical screening of the ethanolic extracts of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl were summarized in Table 1. Phytochemical study of ethanolic extracts of *Aglaonema hookerianum* Schott revealed the presence of alkaloid, glycoside, tannin, reducing sugar, saponin and gum. Alkaloid, steroid, tannin, reducing sugar, saponin and gum were also identified in the ethanolic extract of *Lannea grandis* Engl.

**In-vitro antimicrobial activities:** The ethanolic extracts of leaves of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl were used in the present study to investigate their antimicrobial potentials where both gram-negative and gram-positive bacteria were used. The results of antimicrobial activities of extracts were shown in Table 2 and 3. The ethanolic extracts of leaves of *Aglaonema hookerianum* Schott showed significant antimicrobial activity (zone of inhibition:  $15.08 \pm 0.45$  mm to  $20.37 \pm 0.45$  mm) against all tested bacterial strains and the highest zone of inhibition was observed against *S. paratyphi* ( $20.37 \pm 0.45$  mm). The ethanolic extracts of *Lannea grandis* Engl leaves also showed significant activity against all tested bacteria with zone of inhibition ranging from  $13.93 \pm 0.09$  mm to  $18.25 \pm 0.54$  mm.

**Cytotoxic activities:** The results obtained from the brine shrimp lethality bioassay showed that the ethanolic extract of both plants possessed cytotoxic activities and the values of  $LC_{50}$  ( $\mu\text{g mL}^{-1}$ ) of *Aglaonema hookerianum* Schott and *Lannea grandis* Engl were  $5.25$  ( $\mu\text{g mL}^{-1}$ ) and  $5.75$  ( $\mu\text{g mL}^{-1}$ ), respectively whereas  $LC_{90}$  ( $\mu\text{g mL}^{-1}$ ) values of *Aglaonema hookerianum* Schott. and *Lannea grandis* Engl. were  $10.47$  ( $\mu\text{g mL}^{-1}$ ) and  $9.55$  ( $\mu\text{g mL}^{-1}$ ), respectively (Table 4 and 5). The values of  $LC_{50}$  ( $\mu\text{g mL}^{-1}$ ) and  $LC_{90}$  ( $\mu\text{g mL}^{-1}$ ) were deduced from the best-fit line slope.

Table 1: Results of preliminary screening of ethanolic extracts of leaves of the medicinal plants

Ethanolic extract	Alkaloid	Steroid	Glycoside	Tannin	Reducing sugar	Saponin	Gum
<i>A. hookerianum</i>	+	-	+	+	+	+	+
<i>L. grandis</i>	+	+	-	+	+	+	+

Table 2: Antimicrobial activities of ethanolic extract of *Aglaonema hookerianum* Schott. and standard drug, Amoxicillin

Test organisms	Blank	Diameter of zone of inhibition (mm)	
		Ethanol extract of <i>A. hookerianum</i> (500 µg disc <sup>-1</sup> )	Standard drug, Amoxicillin (10 µg disc <sup>-1</sup> )
<b>Gram positive</b>			
<i>B. subtilis</i>	-	18.08±0.82	32.73±0.49
<i>B. megaterium</i>	-	16.93±0.13	33.00±0.94
<i>S. aureus</i>	-	16.02±0.02	32.93±1.53
<b>Gram negative</b>			
<i>E. coli</i>	-	15.08±0.12	31.90±1.27
<i>P. aeruginosa</i>	-	15.58±0.79	34.33±0.77
<i>S. typhi</i>	-	17.43±1.21	32.75±0.54
<i>S. paratyphi</i>	-	20.37±0.45	32.60±0.75
<i>S. dysenteriae</i>	-	18.25±0.54	32.70±1.17
<i>Vibrio cholerae</i>	-	16.12±0.83	33.25±0.89

Table 3: Antimicrobial activities of ethanolic extract of *Lansea grandis* Engl. and standard drug, Amoxicillin

Test organisms	Blank	Diameter of zone of inhibition (mm)	
		Ethanol extract of <i>A. hookerianum</i> (500 µg disc <sup>-1</sup> )	Standard drug, Amoxicillin (10 µg disc <sup>-1</sup> )
<b>Gram positive</b>			
<i>B. subtilis</i>	-	14.43±0.52	33.15±0.49
<i>B. megaterium</i>	-	15.20±0.25	32.75±0.94
<i>S. aureus</i>	-	15.13±1.03	33.05±1.53
<b>Gram negative</b>			
<i>E. coli</i>	-	16.83±0.12	33.70±1.27
<i>P. aeruginosa</i>	-	13.93±0.09	34.15±0.77
<i>S. typhi</i>	-	14.75±0.54	33.00±0.54
<i>S. paratyphi</i>	-	15.20±0.19	31.55±0.75
<i>S. dysenteriae</i>	-	18.25±0.54	34.35±1.17
<i>Vibrio cholerae</i>	-	15.92±0.72	34.00±0.89

Table 4: Brine shrimp lethality bioassay of ethanolic extract of *Aglaonema hookerianum* Schott. leaves

Conc. of samples (µg mL <sup>-1</sup> )	Log Conc	No. of alive shrimp				Mortality (%)	LC <sub>50</sub> (µg mL <sup>-1</sup> )	LC <sub>90</sub> (µg mL <sup>-1</sup> )
		Test-1	Test-2	Test-3	Avg.			
2.50	0.40	8	7	8	7.67	23.33	5.25	10.47
4.00	0.60	6	7	6	6.33	36.67		
5.50	0.74	4	5	5	4.67	53.33		
7.00	0.85	5	3	2	3.33	66.67		
8.50	0.93	3	2	2	2.67	73.33		
10.00	1.00	1	2	1	1.33	86.67		
11.50	1.06	1	0	0	0.33	96.67		
13.00	1.11	0	0	0	0.00	100.00		
14.50	1.16	0	0	0	0.00	100.00		

Table 5: Brine shrimp lethality bioassay of ethanolic extract of *Lansea grandis* Engl. leaves

Conc. of samples (µg mL <sup>-1</sup> )	Log Conc	No. of alive shrimp				Mortality (%)	LC <sub>50</sub> (µg mL <sup>-1</sup> )	LC <sub>90</sub> (µg mL <sup>-1</sup> )
		Test-1	Test-2	Test-3	Avg.			
2.50	0.40	8	8	8	8.33	16.67	5.75	9.55
4.00	0.60	8	7	8	7.67	23.33		
5.50	0.74	6	5	5	5.33	46.67		
7.00	0.85	5	3	2	3.33	66.67		
8.50	0.93	3	2	2	2.33	76.67		
10.00	1.00	1	0	1	1.00	93.33		
11.50	1.06	1	0	0	0.33	96.67		
13.00	1.11	0	0	0	0.00	100.00		
14.50	1.16	0	0	0	0.00	100.00		

## DISCUSSION

Very few research works were done on the cytotoxicity and antibacterial activities of the ethanolic extracts of the plants. The phytochemical screening showed the presence of alkaloid, glycoside, tannin, reducing sugar, saponin and gum in the ethanolic extracts of *Aglaonema hookerianum* Schott. Alkaloid, steroid, tannin, reducing sugar, saponin and gum were also identified in the ethanolic extract of *Lansea grandis* Engl. The present study revealed significant antibacterial activities of the medicinal plants against all of tested bacterial strains. The results of antimicrobial activities showed that *S. paratyphi* and *S. dysenteriae* were the most sensitive bacterial strains to the ethanolic extracts of *Aglaonema hookerianum* Schott and *Lansea grandis* Engl with significant zone of inhibition (20.37±0.45 and 18.25±0.54), respectively. In addition to this, the study also confirmed the cytotoxic activities of the ethanolic extracts of *Aglaonema hookerianum* Schott and *Lansea grandis* Engl showing LC<sub>50</sub> values 5.25 and 5.75 µg mL<sup>-1</sup> and LC<sub>90</sub>, 10.47 and 9.55 µg mL<sup>-1</sup>, respectively. Finally, it was concluded that both of the plants possessed cytotoxic and antibacterial activities.

## CONCLUSION

In conclusion, the results of this study have shown that both plants are potentially good sources of antimicrobial agents and support the traditional medicinal application of the tested plants. The broad spectra of activity of both plants extracts are promising and the isolation of active constituents of each extract can be the subject of the future researchers.

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