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Phytochemical Investigation with Assessment of Cytotoxicity and Antibacterial Activities of the Ethanol Extract of *Elaeocarpus serratus*

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ABSTRACT

The present study was designed to detect the presence of phytochemical compounds and to evaluate the cytotoxic profiles and antibacterial activities of the ethanol extract of the leaves of *Elaeocarpus serratus*. The phytochemical investigation was done according to the standard procedures. The phytochemical analysis of the extract showed the presence of alkaloid, glycoside, tannin, saponin, flavonoid and carbohydrate. The antibacterial tests of the plant extract and the standard drug, Levofloxacin were conducted against eight bacterial strains using agar disc diffusion technique. The extract at 500 μg disc^{-1} produced significant zone of inhibition against *Bacillus subtilis* (25.82±0.61mm), *Bacillus megaterium* (30.52±0.43 mm), *Pseudomonas aeruginosa* (20.35±0.51), *Salmonella typhi* (32.95±0.39 mm) and *Vibrio cholerae* (16.93±0.14) but no zone of inhibition was detected against *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysenteriae*. The obtained results were also compared with the standard drug, Levofloxacin used at the concentration of 10 μg disc^{-1}. Subsequently, the cytotoxicity test of the plant sample was performed using brine shrimp lethality bioassay which showed lethality against the brine shrimp nauplii with LC_{50} 141.25 and LC_{90} 870.96 μg mL^{-1}. Finally, it was concluded that the ethanol extract of the leaves of *Elaeocarpus serratus* possessed significant antibacterial and cytotoxic properties.

Key words: *Elaeocarpus serratus*, cytotoxicity, antibacterial, brine shrimp lethality

INTRODUCTION

Different types of plant and plant-derived compounds are used in folk medicine for the treatment of different ailments (Pareta *et al*., 2011). The medicinal plant, *Elaeocarpus serratus* Linn belonging to the family of Elaeocarpaceae is widely distributed in Chittagong region of Bangladesh. The plant also grows in many other parts of Bangladesh, India, Pakistan, Srilanka, East Africa, Tropical Australia, Subtropical and Tropical Asia (Ghani, 2003). The plant is commonly known as Singhali Jolpai in Bangladesh (Ghani, 1998). A number of glycosides such as myricitrin, mearnsetin 3-O-β-D-glucopyranoside, mearnsitrin, tamarixetin 3-O-α-L-rhamnopyranoside were isolated from the leaves of *Elaeocarpus serratus*. The plant extract possessed antioxidant properties due to the presence of myricitrin (Jayasinghe *et al*., 2011). Sriti *et al*. (2011) reported the presence of alkaloids and anthraquinone glycosides in the chloroform extract of the leaves of

the plant. They also detected flavonoids in petroleum ether extract of the same plant. Rheumatism and poisoning are treated by the extract of the leaves and on the other hand, fruits are used to treat diarrhea, dysentery and to increase appetite in patients by the stimulation of taste buds (Sharker and Shahid, 2010).

From literature survey, it was found that few works had been done on the cytotoxicity and antibacterial activities of the crude extracts of leaves and fruits of the plant. This stimulated interest to further investigate this plant with a view to determine the antibacterial and cytotoxic properties of the ethanol extract of *Elaeocarpus serratus*. The study was also designed to detect the phytochemical composition of the crude extract.

MATERIALS AND METHODS

Collection and preparation of plant extract: The leaves of *Elaeocarpus serratus* were collected from Sitakunda of Bangladesh in the month of October, 2010 at day time. After collection, they were washed by running tap water and were kept under shadow and air-dried for 21 days. Then, the dried leaves were cut into small pieces and ground into a course powder and stored in an air-tight container until the analysis started. About 338 g of the powdered materials were soaked in 1400 mL of 70% ethanol in a glass container and kept for a period of 21 days with continuous shaking. The whole mixture was then filtered by a piece of clean and white cotton materials. Finally, the filtrate was evaporated until dried to render to a reddish black color of the crude ethanol extract of the plant and the yield value of the ethanol extract of leaves of *Elaeocarpus serratus* was 3.57%.

Chemicals and standard drug: Ethanol was collected from LOBA Chemicals Pvt. Ltd., India and levofloxacin was obtained from Incepta Pharmaceuticals Ltd., Bangladesh. All the chemicals used in the whole study were of analytical grade.

Preliminary phytochemical screening: Preliminary phytochemical analysis of the ethanol extract was carried out to identify the presence of several types of phytochemical compounds such as alkaloid, cardiac glycosides, steroid, flavonoid, tannin, reducing sugar, saponin and gum according to the standard procedures (Trease and Evans, 1989).

Test microorganisms: The test microorganisms used in this study were *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae* and *Vibrio cholerae*.

Cytotoxic activity: The cytotoxic activity of the ethanol extract was tested on brine shrimp nauplii according to brine shrimp lethality bioassay (Meyer *et al*., 1982). From this study, LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) of the ethanol extract were determined.

***In vitro* antimicrobial assay:** The antimicrobial activity of the ethanol extract was investigated using agar disc diffusion method (Bauer *et al*., 1966) where the concentrations of the ethanol extract and the standard drug, levofloxacin were 500 and 10 μg $disc^{-1}$, respectively.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the results of the experiment were expressed as Means±Standard Deviation (SD).

RESULTS

Preliminary phytochemical analysis: The phytochemical screening studies showed the presence of alkaloid, glycoside, tannin, saponin, flavonoid and carbohydrate. The results of phytochemical analysis are shown in Table 1.

Cytotoxic activity: The brine shrimp lethality bioassay showed the cytotoxic properties of the ethanol extract of the plant. The LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) were deduced from the best-fit line slope where the values of LC_{50} (μg mL^{-1}) and LC_{90} (μg mL^{-1}) were 141.25 and 870.96 μg mL^{-1}, respectively which are shown in Table 2.

Antibacterial activity: In agar disc diffusion method, three gm (+) and five gm (-) bacterial strains were used to observe the antibacterial actions of the ethanol extract of the plant at the concentration of 500 μg $disc^{-1}$. Table 3 showed the results of antibacterial activity of the extract with significant zone of inhibition. It was observed that the extract was effective against *B. subtilis*, *B. megaterium*, *P. aeruginosa*, *S. typhi* and *V. cholerae* with zone of inhibition between 16.93±0.14 to 32.95±0.39 mm.

Table 1: Results of preliminary phytochemical investigation of ethanol extract of the leaves of *Elaeocarpus serratus*

Alkaloid	Glycoside	Steroid	Gum	Tannin	Saponin	Flavonoid	Carbohydrate
+	+	-	-	+	+	+	+

+: Positive; -: Negetive

Table 2: Brine shrimp lethality bioassays of ethanol extract of the leaves of *Elaeocarpus serratus*

Conc. (μg μL^{-1})	Log conc.	%Mortality	LC_{50} (μg mL^{-1})	LC_{90}(μg mL^{-1})
50	1.70	3.33	141.25	870.96
75	1.88	26.67		
125	2.10	46.67		
250	2.40	63.33		
500	2.70	73.33		
750	2.88	80.00		
1000	3.00	100.00		
1250	3.10	100.00		

Table 3: Antimicrobial activity of ethanol extract of the leaves of *Elaeocarpus serratus*

		Diameter of zone of inhibition (mm)	
Test organisms	Negative control (Blank)	Ethanol extract of *E. serratus* 500 (μg $disc^{-1}$)	Standard drug, Levofloxacin (10 μg $disc^{-1}$)
Gram positive			
B. subtilis	-	25.82±0.61	33.83±1.03
B. megaterium	-	30.52±0.43	32.60±0.43
S. aureus	-	-	32.77±0.56
Gram negative			
E. coli	-	-	34.08±0.51
P. aeruginosa	-	20.35±0.51	32.60±0.43
S. typhi	-	32.95±0.39	32.98±0.43
S. dysenteriae	-	-	32.12±0.44
V. cholerae	-	16.93±0.14	33.50±0.41

Data were represented as Mean±SD of triplicate determination; (-): No inhibition

The results also showed that the extract did not elicit antibacterial activity against *S. aureus*, *E. coli* and *S. dysenteriae*. The maximum zone of inhibition was recorded against *S. typhi* (32.95±0.39 mm) which was almost similar to that of the standard drug, levofloxacin.

DISCUSSION

The results of phytochemical analysis showed the presence of alkaloid, glycoside, tannin, saponin, flavonoid and carbohydrate. It was reported that flavonoids in the plants could possess antioxidant and free radical scavenging potentials (Middleton and Kandaswami, 1992). On the other hand, it was stated that antinociceptive activities could be elicited by flavonoids (Zakaria *et al*., 2006) and tannins (Rahman *et al*., 2011; Ramprasath *et al*., 2006). Moreover, flavonoids also act as antioxidant in biological systems as scavengers of free radicals (Rice-Evans *et al*., 1997; Jorgensen *et al*., 1999). Alkaloids are well known for their ability to inhibit pain perception (Uche *et al*., 2008). In addition to these, tannins could be indicated in the prevention of urinary tract infection (Agbafor *et al*., 2011). Thus, it could be demonstrated that the ethanol extract of the leaves of *Elaeocarpus serratus* might possess analgesic, antioxidant and antibacterial activities which need to be investigated in future. The present study confirmed the antibacterial properties of the plant extract.

A study carried out by Sharker and Shahid (2010) demonstrated the antibacterial effects of the ethanol extract of the leaves of *Elaeocarpus serratus* at 600 μg disc^{-1} against *S. typhi* but no antibacterial activity was found against *S. aureus*. The present study showed the similar results against *S. typhi* and *S. aureus* at 500 μg disc^{-1} of the plant extract. Sriti *et al*. (2011) also studied the antibacterial activities of the petroleum ether, chloroform, benzene and acetone extracts of the leaves of the plant which did not show any antibacterial activity against *S. aureus*, *E. coli* and *B. subtilis* at 200 μg mL^{-1}. However, our study confirmed the absence of antibacterial potentials against *S. aureus*, *E. coli*, where different observation was found in case of *B. subtilis* which was contradictory to the results obtained by Sriti *et al*. (2011) and it might be due to different concentrations of the plant extracts used in the studies. The study results also showed no antimicrobial activity against *S. aureus*, *E. coli* and *S. dysenteriae*. The insensitivity of these test microorganisms on the ethanol extract of the plant could be due to short time of exposure (Duru and Onyedineke, 2010). The sensitivity test results demonstrated that the ethanol extract was less potent than the standard drug, levofloxacin used in the study. Generally, the low efficacy of the extract compared to the standard antibiotic used in the study may be due to the crude nature of the drug. So, it may require further purification.

The brine shrimp lethality bioassay was used in the primary screening of the ethanol extract to evaluate the cytotoxicity on brine shrimp which could indicate the possibility of toxicity of the plant extract (Meyer *et al*., 1982). The results showed moderate cytotoxic properties of the ethanol extract of the plant extract. The lethality of the crude extract (LC_{50} value less than 100 μg mL^{-1}) to brine shrimp indicates the presence of potent cytotoxic compounds which necessitate further investigation (Alam *et al*., 2011). Finally, the present study strongly suggests that *Elaeocarpus serratus* possesses cytotoxic and antibacterial properties.

CONCLUSION

In conclusion, it can be claimed that the plant extract possesses cytotoxic and antibacterial activities which provides a scientific basis for further extensive research to isolate and characterize the chemical compounds responsible for antibacterial and cytotoxic activities of the plant.

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