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Assessment of cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (family: Euphorbiaceae) bark

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ABSTRACT

In the present work, the ethanol extract of the barks of *Phyllanthus acidus* L. (Family: Euphorbiaceae) were investigated for preliminary phytochemical screening, cytotoxicity and antibacterial activities. The presence of phytochemical constituents was identified by characteristic changes using standard procedure. Brine shrimp lethality (BSL) bioassay was used to investigate the cytotoxic effects and the agar disc diffusion method was used for antimicrobial assay of the plant extract. The phytochemical screening of ethanol extract of the plant showed the presence of alkaloids, glycosides and steroids. The ethanol extracts of *Phyllanthus acidus* L. bark showed cytotoxicity with LC₅₀ and LC₉₀ values of 501.19µg/mL and LC₉₀: 794.33µg/mL, respectively. The extract of *Phyllanthus acidus* L. bark showed significant antibacterial activity against gram negative bacteria only such as such as *E. coli* (19.25 ± 0.54mm), *S. typhi* (32.08 ± 0.51mm) and *Vibrio cholerae* (16.42 ± 0.42mm). The obtained results showed a potential source of biologically active compounds which can be used as antibacterial agents.

Key words: BSL bioassay, cytotoxicity, antibacterial, *Phyllanthus acidus*.

INTRODUCTION

Phyllanthus has a remarkable diversity of growth forms including annual and perennial herbaceous, arborescent, climbing, floating, aquatic, pachcaulous, and phyllocladous. *Phyllanthus acidus* L. (Family: Euphorbiaceae) is an annual erect little branched herb, 10-50 cm high. Leaves are simple, oblong, acute, or obtuse, slightly oblique to 14 mm long and 6 mm broad and bear the inconspicuous flowers in pairs in their axils. Each pair of flowers comprises one male and one female. The capsule is flattened globose about 2 mm in diameter (Jagessar et al., 2008). *Phyllanthus acidus* L. is commonly known as Arboroi or Harbori in Bangladesh and gooseberry or star gooseberry in India. The medicinal activities of *Phyllanthus* species are antipyretic, analgesic, anti-inflammatory, antihepatotoxic and antiviral (Unander et al., 1995; Chang et al., 2003; Zhang et al., 2004; Sousa et al., 2007). The present study has been investigated for cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (Family: Euphorbiaceae) bark.

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MATERIALS AND METHODS

Plant materials and preparation of the extract

The fresh barks of *Phyllanthus acidus* were collected from Agrabad under Chittagong division, Bangladesh in the month of December in 2010 at day time. After collection, the barks were cut into very small pieces and dried at room temperature under shade. The barks were ground into a coarse powder with the help of a suitable grinder and the powder was then stored in an airtight container and stored in a cool, dark and dry place till the use. About 350 gm of powdered materials was taken in a clean, flat bottomed plastic container and soaked in 1400 ml of 99.8% ethanol (MW: 46.07 g/mol, MERCK). The container with its contents was sealed and kept for a period of 21 days accompanied by continuous shaking with the shaker. The whole mixture was then filtered by a piece of sterilized white cotton filter and the obtained filtrate was evaporated and concentrated by a rotary evaporator.

Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents using the following reagents and chemicals: alkaloids with Dragendroff's reagent and Mayer's reagent, glycosides with sodium hydroxide, tannins with ferric chloride, flavonoids with concentrated hydrochloric acid and steroids with sulphuric acid (Trease and Evans, 1989). Carbohydrate with Fehling solution, resin with acetic acid and sulphuric acid were also tested (Wallis, 1985).

Test microorganisms

The test microorganisms included both gram positive (*Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi* and *Vibrio cholerae*) were collected from the Department of Pharmacy, Southern University Bangladesh and stock cultures of bacteria were maintained on nutrient agar media for their growth.

Standard drug

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

Cytotoxic activity

The cytotoxic activity of the crude ethanol extract of *Phyllanthus acidus* bark was tested on brine shrimp nauplii according to brine shrimp lethality bioassay (Meyer et al., 1982). The eggs of brine shrimp used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29 °C for 48 h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. The matured nauplii were then used in the experiment.

Ten (10) mature larvae (nauplii) were kept in glass vials containing 10 ml of seawater. The test bacteria dissolved in DMSO (10 mg/ml) was applied to the nauplii in each vial. However, not more than 50 µl of DMSO was added to the vials containing the shrimps. For each concentration, vials containing the same volume of DMSO plus seawater and shrimps were used as controls. After

24 h, the vials were observed for mortality. The number of survived nauplii in each vial was counted and from this data the percentage of lethality of the brine shrimp nauplii was calculated (Goldstein et al., 1974) and the lethal concentration (LC₅₀) and (LC₉₀) of the ethanolic extract was determined.

In vitro antimicrobial assay

The antibacterial activity was determined by the agar disc diffusion method (Bauer et al., 1966). The concentrations of the crude ethanol extract of the plant and the standard drug Amoxicillin under this research were 500 µg/disc and 10 µg/disc, respectively.

RESULTS AND DISCUSSION

Phytochemical screening

The crude ethanol extract of *Phyllanthus acidus* L. bark was qualitatively tested for the presence of alkaloids, glycosides, tannins, flavonoids, steroids, gums, carbohydrate and resin and the results were given in **Table 1**. The qualitative studies indicated the presence of alkaloids, glycosides and steroids.

Table 1: Results of phytochemical analysis of ethanolic extracts of *Phyllanthus acidus* L.

Alkaloids	Glycosides	Tannins	Flavonoids	Steroids	Carbohydrate	Resin
+	+	-	-	+	-	-

(+) = present & (-) = absent.

Cytotoxicity studies

In brine shrimp lethality bioassay (**Table 2**), the ethanolic extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration, LC₅₀ (µg/ml) and LC₉₀ (µg/ml) were deduced (LC₅₀: 501.19 µg/mL and LC₉₀: 794.33 µg/mL).

Table 2: Brine shrimp lethality bioassay of ethanol extract of *Phyllanthus acidus* L.

Conc. of samples (Conc) (µg/ml)	Log	No. of alive shrimp				Average	% mortality	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
		Test -1	Test -2	Test -3	Average				
100	2.00	9	10	9	9.3	7			
200	2.30	6	7	8	7.0	30			
300	2.48	4	5	7	5.3	47			
400	2.60	5	5	6	5.3	47			
500	2.70	4	6	5	5.0	50	501.19	794.33	
600	2.78	4	4	4	4.0	60			
700	2.85	2	3	2	2.3	77			
800	2.90	1	2	0	1	90			
900	2.95	0	0	0	0	100			
1000	3.00	0	0	0	0	100			

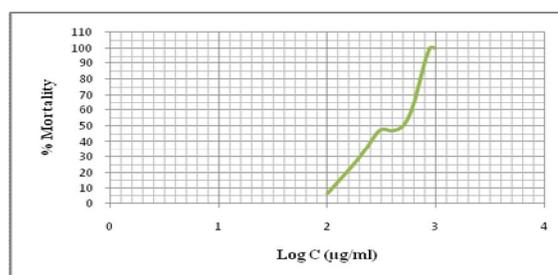


Fig 1: Graphical Presentation of LC₅₀ (µg/ml) and LC₉₀ (µg/ml) of ethanol extracts of *Phyllanthus acidus* L.

Antimicrobial activities

Table 3 showed the results of antibacterial test. The antibacterial potential of the extract was assessed against eight bacterial strains (both gram positive and gram negative) at the dose of 500µg/disc and the results (diameter of zone of inhibition) were compared with the activity of the standard drug, Amoxicillin (10µg/disc). The ethanol extract of *Phyllanthus acidus* L. bark exhibited significant zone of inhibition against only the gram negative bacteria such as *E. coli* (19.25 ± 0.54mm), *S. typhi* (32.08 ± 0.51mm) and *Vibrio cholerae* (16.42 ± 0.42mm).

Table 3: Antimicrobial activities of ethanol extracts of *Phyllanthus acidus* L.

Test Organisms	Blank	Diameter of Zone of Inhibition (mm)	
		Ethanol extracts of <i>Phyllanthus acidus</i> (500µg/disc)	Standard drug, Amoxicillin (10µg/disc)
Gram positive			
<i>B. megaterium</i>	-	-	32.80 ± 0.55
<i>B. subtilis</i>	-	-	31.79 ± 1.99
<i>S. aureus</i>	-	-	33.75 ± 0.78
Gram negative			
<i>E. coli</i>	-	19.25 ± 0.54	30.90 ± 1.66
<i>P. aeruginosa</i>	-	-	34.05 ± 0.73
<i>S. dysenteriae</i>	-	-	32.10 ± 0.90
<i>S. typhi</i>	-	32.08 ± 0.51	31.35 ± 1.71
<i>Vibrio cholerae</i>	-	16.42 ± 0.42	33.32 ± 0.93

Data were represented as Mean ± SD of triplicate determination and (-): no inhibition

CONCLUSION

Finally, it can be suggested that the ethanol extract of *Phyllanthus acidus* L. bark showed significant zone of inhibition against gram negative bacteria and also revealed the cytotoxic activity. Further phytochemical and pharmacological studies are also required to use their medicinal and pharmaceutical potentialities.

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