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PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND CYTOTOXICITY STUDY OF THE ETHANOLIC EXTRACTS OF *PHYLLANTHUS ACIDUS* L. ROOT BARK

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ABSTRACT: Traditional medicine plays a very important role in Bangladesh, particularly at the primary health care level. The modern types of traditional medicine, namely, the Ayurvedic and Unani medicines are now officially recognized in Bangladesh .The objective of the present study was to evaluate antioxidant potential and cytotoxicity of ethanolic extract of root bark of *Phyllanthus acidus* L. The phytochemical screening demonstrated the presence of carbohydrates, saponins, and tannins. Antioxidant activity was evaluated by using 1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radical assay. In DPPH assay, free radical scavenging activity of the extracts was evaluated comparing with ascorbic acid at the wavelength of 517 nm and the IC_{50} value of *Phyllanthus acidus* extracts was found to be 26 µg/ml, whereas ascorbic acid showed the value of 17.49 µg/ml. In case of brine shrimp cytotoxicity assay, LC_{50} value was obtained to be 35.48 µg/ml. This is the first research report regarding *in vitro* evaluation of DPPH scavenging activity of ethanolic extracts of the root barks of *Phyllanthus acidus* L. The present study might be extended for the formulation and evaluation of different antioxidant herbal dosage forms.

INTRODUCTION: There has been a rising attention in traditional medicine and their application to public health both in developed and developing countries in the current past [1]. For drug development, Plants are the tremendous source for the discovery of new products with medicinal importance. At the present time several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world.

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Secondary metabolites are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives [2].It is now well recognized that the plants synthesize secondary metabolites (like alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinones, volatile oil etc.) as well as possess minerals and vitamins that bear medicinal properties [3]. Plants containing antioxidant compounds play an important role in the treatment of large number of major diseases such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [4].

Phyllanthus acidus L. plant belonging to Phyllanthaceae family, commonly known as

Arboroi or Harbori in Bangladesh. Various parts of the plant are used for food. The plant is used medicinally in the treatment of various diseases like coughs, hypertension, asthma, skin diseases etc [5-6]. The peppered leaves of *Phyllanthus acidus* are used in the treatment of sciatica, lumbago and rheumatism. The root is significantly purgative and it is used to treat psoriasis of the soles of feet externally [7-8]. It has also hepatoprotective [9], anti-diabetic activity [10]. The juice of the root bark contains saponin, gallic acid, tannin and a crystalline substance, lupeol. The root bark is used as tanning agent in India [11]. In Bangladesh, previously phytochemical screening, antioxidant activity ,cytotoxicity and antibacterial activity of bark and fruit part of *Phyllanthus acidus* was investigated [6,12]. But as per our knowledge, root bark of *Phyllanthus acidus* has not investigated earlier for its medicinal value.

Therefore, our aim of the present study was to determine the cytotoxicity and antioxidant activity of ethanolic extracts of the root barks of *Phyllanthus acidus*.

MATERIALS AND METHODS:

Plant material: The fully mature, fresh root bark of *Phyllanthus acidus* was cut by knife after digging the surrounding area of the few selected plants of Jahangirnagar University campus, Dhaka, Bangladesh. The plant was identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka (Accession no.35623).

Extraction: Collected plant materials were sliced into small pieces and dried in an oven at reduced temperature ($<50^0$c) to make suitable for grinding. The powdered plant materials were submerged in sufficient volume of ethanol in an air-tight flat bottomed container for seven days, with occasional shaking and stirring. The extracts were then filtered and the filtrate was dried on electrical water bath.

Drugs and Chemicals used:1, 1-diphenyl-2-picryl-hydrazil (DPPH), ascorbic acid was obtained from sigma Chemical Co (MO, USA). All the chemicals and solvents were of analytical grade.

Phytochemical tests: Various phytochemical tests that include Molisch's test for carbohydrates, test for glycosides, Borntrager's test for anthraquinone glycosides, Mayer's reagent; Hager's reagent and Dragendroff's reagent for alkaloids, Frothing test for saponins, Hydrochloric acid test for flavonoids, Salkowski's test for steroids and Ferric chloride test for tannins.

DPPH scavenging activity: DPPH scavenging activity of the *Phyllanthus acidus* root bark was measured by the method developed by Manzocco *et al* [13]. The sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated.

Cytotoxic activity: Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts (Meyer et.al) [14]. It was carried out to investigate the cytotoxicity of the extract. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared by using sea salt 38 g/L and adjusted pH 8.5) under constant aeration for 48h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay.

Ten nauplii were drawn through a micropipette and placed in each test tube containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the extract was added to 4.5 mL of brine solution and maintained at the ambient room temperature for 24 h and surviving nauplii were counted. For the investigations test solution of the extract was prepared by dissolving 20 mg of the extract in 1 mL of pure dimethyl sulfoxide (DMSO). 500µl of solution was taken in test tubes each containing 500 µl of simulated seawater. Stock solution having the concentration 1mg/mL was obtained by adding 9mL of simulated sea water in the test tube. A series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. Tests were conducted along with negative control (DMSO treated), and different concentrations (1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 500 µg/mL) of the root-bark extract of *Phyllanthus acidus* in a set of two tubes per dose.

RESULTS AND DISCUSSION: Phytochemical tests: The results of the various qualitative chemical tests for the detection of chemical constituents of the extract are placed in the (**Table 1).**

TABLE 1: QUALITATIVE RESULTS OF PHYTOCHEMICAL SCREENING

Test for Phytochemicals	*Phyllanthus acidus*
Carbohydrates	+
Glycosides	-
Anthraquinone glycosides	-
Alkaloids	-
Saponins	+
Flavonoids	-
Steroids	-
Tannins	+

'+' Indicates presence, '-' Indicates absence.

As evident from the Table 1 the extract gave positive reaction for carbohydrates, saponins and tannins while gave negative reaction for glycosides, anthraquinone glycosides, alkaloids, flavonoids and steroids. In a previous study, Phytochemical screening of methanol extract of fruit part of *Phyllanthus acidus* showed evidence of glycoside, tannin and resin [12].In another study, the crude ethanol extract of *Phyllanthus acidus* L. bark showed presence of alkaloids, glycosides and steroids [8]. Both root bark and fruit part of *Phyllanthus acidus* L. contains tannin.

DPPH scavenging activity: DPPH easily accepts an electron or hydride radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up. **Fig. 1** shows the amount of each extract needed for 50% inhibition (IC_{50}).The IC_{50} value of *Phyllanthus acidus* extracts was found to be 26 µg/ml, whereas for ascorbic acid showed the value of 17.49 µg/ml. In another study, IC_{50} value of fruit part of *Phyllanthus acidus* was 1192.263 µg/ml, compared to 13.37 µg/ml which was the IC_{50} value for the reference ascorbic acid[12]. Root bark part of ethanolic extract of *Phyllanthus acidus* L. showed better DPPH scavenging activity compared to fruit part.

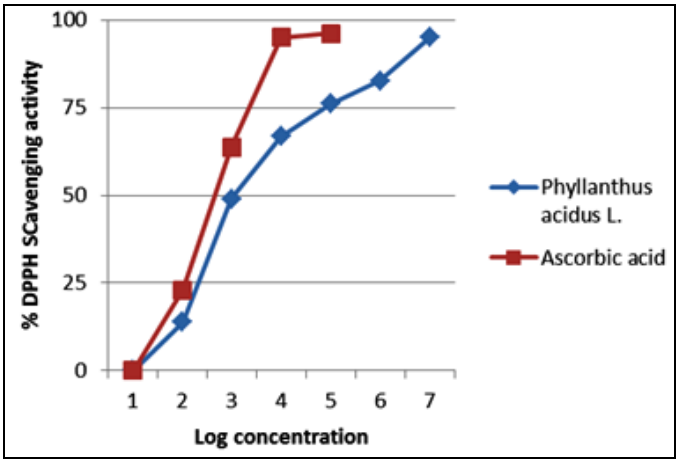

FIGURE 1: COMPARATIVE DPPH SCAVENGING ACTIVITY

Cytotoxic study: The ethanolic extract of *P. acidus* L.root bark was tested for Brine shrimp lethality bioassay using brine shrimp nauplii and DMSO as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. For the extract, the number of nauplii died and percent mortality was counted. The result is shown in the following (**Table 2)**

TABLE 2: CYTOTOXICITY OF ETHANOLIC EXTRACT OF *PHYLLANTHUS ACIDUS* L.

Extract	Concentration of samples (µg/ml)	Log C	% mortality	LC_{50} (µg/ml)
Root bark part of ethanolic extract of *Phyllanthus acidus* L.	500	2.698	100	35.48
	200	2.301	100	
	100	2	100	
	50	1.698	60	
	20	1.301	10	
	10	1	0	
	5	0.698	0	
	1	0	0	

The LC_{50} value of ethanolic extract of *P. acidus root bark* was 35.48 µg/ml. In another study the ethanol extracts of *Phyllanthus acidus* L. bark showed cytotoxicity with LC_{50} and LC_{90} values of 501.19 µg/mL and 794.33µg/mL, respectively [8]. As apparent from our results it can be revealed that the plant extract exhibited mild cytotoxic effect.

CONCLUSION: The ethanolic extracts of *Phyllanthus acidus* L. root bark exhibited significant antioxidant activity. In the present study, the observed DPPH scavenging activity of the ethanolic extracts of root bark might be useful for the development of newer and more potent natural antioxidants. The plant extract exhibited mild cytotoxic effect. Further phytochemical and pharmacological studies are also required to use their medicinal and pharmaceutical potentialities.

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