

**Cytotoxicity and Antifungal Activities of Root Bark of  
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**ABSTRACT**

In this study, methanol extract from the root bark of *Calotropis gigantea* L. and its petroleum ether (40°C-60°C), chloroform and ethyl acetate soluble fractions were tested for their cytotoxic activity against brine shrimp nauplii (*Artemia salina*, Leach) and for antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp and *Trichoderma harzianum*. Thin layer chromatography (TLC) screening showed that methanol extract and its different fractions contained different type compounds such as steroid, terpene, glycoside, heterocyclic and flavonoid. In brine shrimp lethality bioassay, it was found that chloroform fraction was highly cytotoxic (LD<sub>50</sub> 14.72 µg/ml) among the tested samples. Though methanol extract and ethyl acetate fraction have no activity against all the tested fungi but petroleum ether and chloroform fractions showed potent activity against *Aspergillus niger*, *Penicillium* sp, *Trichoderma harzianum* and *Aspergillus niger*, *Trichoderma harzianum*, respectively, in antifungal activity test.

**Key words:** *Calotropis gigantea*, Methanol extract, Antifungal activity, Cytotoxicity.**INTRODUCTION**

Vast natural resources of medicinal plants are being used for thousands of years for the cure of many diseases in all over the world. If we could use medicinal plants properly we could get medicines at low cost and then it might be possible to fulfill the demand of our medication. This will supply low cost medicine to our poor people and we could establish a better health care system (Ghani, 1998). *Calotropis gigantea* L. (Family: Asclepiadaceae), a wildy growing plant, has been reported to possess number of medicinal properties and other purposes (Chitme et al., 2004). Roots of *Calotropis gigantea* have been used in leprosy, eczema, syphilis, elephantiasis, ulceration and cough in the Indian system of traditional medicine (Chitme et al., 2005). Some biologically active compounds such as calotropin, frugoside, 4/-O-beta-D-glucopyranosylfrugoside, calotroposide A and B, gigantincine, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside, taraxasteryl acetate, 19-Nor- and 18, 20-epoxy-cardenolides etc. have been isolated from different parts of *Calotropis gigantea* (Kiuchi et al., 1998; Kitagawa et al., 1992; Pari et al., 1998; Sen et al., 1992; Lhinhatrakool and Sutthivaiyakit, 2006). In previous study extract of *Calotropis gigantea* showed poor fungitoxicity on the pineapple fruit-rotting fungus *Ceratocystis paradoxa* (Damayanti et al., 1996). So our present attention was concentrated on the root bark of *Calotropis gigantea* to evaluate their bioactivity against brine shrimp nauplii and some fungi other than *Ceratocystis paradoxa*. This will ultimately lead to proper use of this important medicinal plant without having side effects and toxicity for various diseases.

**MATERIALS AND METHODS****Plant material**

The root bark of *Calotropis gigantea* (Linn) (Family: Asclepiadaceae) were selected for the biological investigations. Roots of *Calotropis gigantea* (Linn) were collected from the relevant areas (Meherchandi) of Rajshahi University campus. The plant was taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, University of Rajshahi. Voucher specimen (No. 1A. Alam, collection date 15.08.2004) was kept in the Department of Botany, University of Rajshahi.

**Extraction**

The powdered plant materials (900gm) were extracted with methanol in Soxhlet extractor. The extraction process was performed repeating 8 cycles. The extract was then filtered through Whatman No.1 filter paper. The filtrate was concentrated with a rotary evaporator under reduced pressure at 60° C to afford crude methanol extract (40 gm). This crude methanol extract (30 gm) was then fractioned into petroleum ether (3 g), chloroform (10 g) and ethyl acetate soluble fraction (2 g) by solvent-solvent partitioning (Bahl and Bahl, 1992).

**Thin Layer chromatography (TLC) screening**

A small portion of methanol extract and its petroleum ether, chloroform and ethyl acetate fractions were dissolved in respective solvents and the solutions were spotted on TLC plates. Then the TLC plates were run by specific solvent system and viewed individually under UV light and vanillin-H<sub>2</sub>SO<sub>4</sub> reagent was used as spray reagent (Bobblt, 1963). Different colored spots on TLC plate indicated the presence of different type of compound in each fractions.

**Brine Shrimp Lethality Bioassay**

The experiment was carried out using the method described by Mclaughlin et al. (1982). In brief, *Artemia salina* Leach (brine shrimp eggs) was allowed to hatch and mature as nauplii (Larvae) in seawater for 48 h at 25°C. Serially diluted test solutions (80 µL in DMSO from a stock solution of 5 mg/mL DMSO) were added to the seawater (5 mL), containing 10 nauplii. After incubation for 24 h at 25°C, the number of survivors was counted. The LC<sub>50</sub> (50% lethal concentration, µg/ml) was determined from triplicate experiments using probit analysis as described by Finney (1971). Ampicillin trihydrate was used as positive control.

**Antifungal Activity Test**

*In vitro* antifungal screening was performed by disc diffusion assay method (Bear et al., 1966; Rios et al., 1988). The methanol extract and its petroleum ether, chloroform and ethyl acetate fractions were tested against four fungi (*Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp and *Trichoderma harzianum*) at a concentrations of 100 µg/disc, 200 µg/disc and 300 µg/disc for each and the results were compared with flugal (200 µg/disc). The activity was determined after 72 hours of incubation at 37.5°C. The fungal strains were collected from pathology laboratory, Department of Botany, Rajshahi university, Bangladesh.

**RESULTS AND DISCUSSION**

On TLC screening methanol extract and its petroleum ether, chloroform and ethyl acetate soluble fractions showed mixture of coloured compounds such as steroid, triterpene, heterocyclic, glycoside and flavonoid when sprayed with vanillin-H<sub>2</sub>SO<sub>4</sub> spray reagent. In brine shrimp lethality bioassay, the crude methanol extract and its petroleum ether, chloroform and ethyl acetate soluble fractions showed positive results indicating that all testing samples were biologically active. The mortality was not shown in the solvent control batch. The results obtained from this experiment are shown in Table 1. Among the tested samples, chloroform fraction showed lowest LD<sub>50</sub> value (14.72 µg/ml), whereas positive control ampicillin trihydrate showed LD<sub>50</sub> value 12.27 µg/ml. Rasid et al. (2004) showed that the LD<sub>50</sub> for petroleum ether, chloroform and methanol extracts of *Wedelia calendulacea* against the brine shrimp nauplii were found to be 4.59 µg/ml, 7.99 µg/ml and 14.88 µg/ml respectively, whereas the positive control, vincristine sulfate showed an LD<sub>50</sub> of 0.58 µg/ml. So cytotoxicity of chloroform fraction was remarkable in respect to ampicillin trihydrate. Chloroform fraction showed activity against *Aspergillus niger* (16, 24 and 28 mm) and *Tricoderma harzianum* (14, 16 and 18 mm) at a concentration of 100, 200 and 300 µg/disc, respectively and petroleum ether fraction showed activity against *Aspergillus niger* (20, 24, and 28 mm), *Penicillium* sp (20, 22 and 33 mm) and *Tricoderma harzianum* (20, 26 and 27 mm) at 100, 200 and 300µg/disc, respectively.

Methanol extract and ethyl acetate fraction showed no antifungal activity against all the tested fungi. It might be due to the presence of low concentration of active phytochemicals in methanol extract or due to absence of these phytochemicals in ethyl acetate fraction. The results of antifungal activity (zone of inhibition) of test materials against respective fungi were given in the Table 2.

**Table 1. Cytotoxic effect of the methanol extract and its petroleum ether, chloroform and ethyl acetate fractions.**

Treatment	LD <sub>50</sub> (in ppm)
Ampicillin trihydrate	12.27
Methanol extract	66.86
Petroleum ether fraction	39.34
Chloroform fraction	14.72
Ethyl acetate fraction	18.57

**Table 2. In-vitro antifungal activity of methanol extract and its petroleum ether, chloroform and ethyl acetate fractions.**

Tested fungi	Treatment												
	Methanol extract			Petroleum ether fraction			Chloroform fraction			Ethyl acetate fraction			Flugal
	A	B	C	A	B	C	A	B	C	A	B	C	B
	Diameter of zone of inhibition (mm)												
<i>Aspergillus flavus</i>	R	R	R	R	R	R	R	R	R	R	R	R	24
<i>Aspergillus niger</i>	R	R	R	20	24	28	16	24	28	R	R	R	33
<i>Penicillium sp</i>	R	R	R	20	22	23	R	R	R	R	R	R	30
<i>Trichoderma harzianum</i>	R	R	R	20	26	27	14	16	18	R	R	R	31

## CONCLUSION

The findings of this study indicate that some chemical principles with potent cytotoxic and antifungal activity present in chloroform and petroleum ether fractions. If we can isolate and characterize these bioactive principles from petroleum ether and chloroform fractions, we can use them in various medicinal purposes and our future research plan is going on this direction.

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