

Assessment of cytotoxic activities of *Phyllanthus amarus* and *Monstera deliciosa*

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ABSTRACT

Medicinal plants constitute an important component of flora and are widely distributed in Bangladesh. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which shows the way to the development of novel and safe medicinal agents. Based on the ethnopharmacological literature two widely used medicinal plants *Phyllanthus amarus* and *Monstera deliciosa* were chosen to investigate their cytotoxicity through brine shrimp lethality bioassay which is simple, reliable and convenient method for assessment of bioactivity of medicinal plants. The plants were collected from their natural habitat, dried under shade and extracted with ethyl acetate. In this study, ethyl acetate extract of *Phyllanthus amarus* exhibited potent cytotoxicity with LC₅₀ values of 9.15µg/ml and 20.16µg/ml of leaves and the whole plant respectively. *Monstera deliciosa* exhibited cytotoxicity with LC₅₀ values of 36.60µg/ml and 300.4 µg/ml of leaves and branches respectively. From the result, it can be predicted that extractives of *Phyllanthus amarus* possess cytotoxic principles and showed significantly more potency than in leaves rather than whole plant. In case of *Monstera deliciosa* the extractives of leaves exhibited very mild mortality while the extractives of branches did not show considerable cytotoxicity.

INTRODUCTION

In recent years, there has been a great deal of attention and discoveries in exploiting plant kingdom for pharmaceutical application and the interest in plants as a source of potential therapeutic agents, particularly as anticancer agents is growing tremendously (Sujatha *et al.*, 2011). Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures. The present study was concentrated on the cytotoxic activity of folklore claimed plant *Phyllanthus amarus* and *Monstera deliciosa* using brine shrimp lethality bioassay. This bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, antiviral, pesticidal and anti-tumor etc. of the compounds (Meyer *et al.*, 1982; McLaughlin, 1988). *Phyllanthus amarus* (Family: Euphorbiaceae) is an annual, glabrous herb widely spread

throughout the tropics and subtropics as a weed in cultivated and waste land (Oyewole *et al.*, 2013; Gill, 1992) with much availability in Bangladesh and India.

Various parts of the plants were reported to be have anti-inflammatory (Mahat and Patil, 2007), antidiabetic (Bensky and Gamble, 1993), antimicrobial (Ojo *et al.*, 2010), antihyperlipidemic (Umbare *et al.*, 2009), antioxidant (Antara and Amla, 2013), anticancer, antimalarial (Cimanga *et al.*, 2010), antifertility, antibacterial (Uchehi, 2006), antidiarrhoeal (Calixto *et al.*, 1998), antiallodynic, chemoprotective, antiviral, hepatoprotective (Calixto, 2000), antispasmodic and diuretic (Srividya and Perival, 1995) properties.

Monstera deliciosa (Family-Araceae) is a species of flowering plant native to tropical rainforests of southern Mexico, south to Colombia. In Mexico, a leaf or root infusion is taken daily to relieve arthritis. In Martinique the root is used to make a remedy for snakebite. However, both the plants have a long history of use in traditional medicine. The study was aimed at bringing the cytotoxic activities of these plants into lime light.

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MATERIALS AND METHODS

Collection of plant material

The matured whole plants were collected from their natural habitat around Dhaka University campus under the supervision of expert taxonomist. The plant of *Phyllanthus amarus* was collected on November, 2013. For investigation, the whole plants were divided into two groups of sample: sample 1-whole plant and Sample 2-Leaves and branches. The plant of *Monstera deliciosa* was collected on May-June, 2013 and was divided into two groups of sample: sample 3-Leaves and sample 4-Branched and roots. The plant parts were dried under shade for several days avoiding scorching sunlight and were then pulverized into coarse powder using high capacity grinding machine (Obianime and Uche, 2008; Ghosh *et al.*, 2006).

Preparation of extract

350 gm of the powdered plant material of each sample was taken in a separate clean, round bottomed flask (5 liters) and soaked in 3.5 liter of ethyl acetate. The containers with its content were sealed by cotton plug and aluminum foil and kept for a period of 2-3 days accompanying occasional shaking and stirring. The whole mixture of each container was then filtered through cotton followed by Whatman No. 1 filter paper and the filtrate thus obtained was concentrated under reduced pressure with a vacuum evaporator at a temperature not exceeding 40° C. The concentrated extracts were then air dried to solid residues. The weight of the extract obtained from powdered sample of 1, 2, 3 and 4 were 5.5, 6.0, 4.0 and 5.0 gm respectively.

BRINE SHRIMP LETHALITY BIOASSAY

The brine shrimp, *Artemia salina*, is an invertebrate component of the fauna of saline aquatic and marine ecosystem. It plays an important role in the energy flow of the food chain. And it can be used in a laboratory bioassay in order to determine the toxicity by the estimation of the medium lethality concentration LC₅₀ which have been reported for a series of toxins and plant extracts. From a pharmacological point of view, a good relationship has been found with the brine shrimp lethality test to detect antitumor compounds in terrestrial plant extracts (Aseer *et al.*, 2009; Ramachandran *et al.*, 2011).

Preparation of the test sample

Ten test tubes were taken each containing 5 ml of simulated seawater and 10 living shrimp nauplii and numbered accordingly. 4 mg of extracted solid residue was taken in a clean and dry vial, and 100 µl of pure dimethyl sulfoxide (DMSO-d₆) was added to get the stock solution. 50 µl of solution was then added to a test tube. Thus, the final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In each case 50 µl solution was added to test tube and fresh 50 µl DMSO-d₆ was added to vial. Thus different concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml,

50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.563 µg/ml, 0.781 µg/ml were found in the different test tubes.

Preparation of the control group

Control groups are used in cytotoxicity study to validate the test method and ensure that the results obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. Usually two types of control groups are used-

- i) Positive control
- ii) Negative control

Preparation of the positive control group

Vincristine Sulphate (VS) was used as the positive control. Measured amount of VS was dissolved in DMSO-d₆ to prepare the stock solution in a clean and dry vial. 50 µl of the solution was then added to one of ten pre-marked test tube containing ten living brine shrimp nauplii in 5 ml of simulated sea water to get a solution with concentration of 40 µg/ml. A serial dilutions technique was applied using DMSO-d₆ to prepare the solutions of varying concentrations (20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.156 µg/ml and 0.0781 µg/ml) from the stock solution in each remaining test tube. In each case 50 µl solution was added to test tube and fresh 50µl DMSO-d₆ was added to vial.

Preparation of the negative control group

50 µl of DMSO-d₆ was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 living shrimp nauplii. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

Counting of nauplii

After 24 hours, the vials and test tubes were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by linear regression using a simple IBM-PC program. The effectiveness or the concentration-mortality relationship of plant product was expressed as a median lethal concentration (LC₅₀) value.

RESULT

The lethal concentration (LC₅₀) of the test sample was obtained by a plot of average percentage of the shrimps died after 24 hours from triplicate data against the logarithm of the sample concentration (toxicant concentration) and the standard deviations of data were exhibited in the plot with error bars. The best-fit line was obtained from the curve data by means of regression analysis. The LC₅₀ for positive control VS was found as 0.544 µg/ml indicating significant mortality compared to negative control and the LC₅₀ values of different extractives were compared with the positive control (Fig. 1).

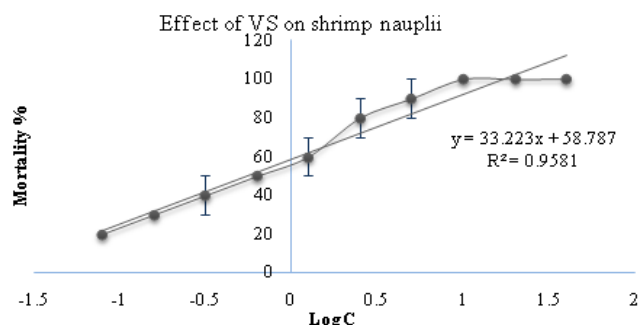


Fig. 1: Plot of % mortality and predicted regression line of VS with error bar (Standard deviation).

Cytotoxic activity evaluation of *Phyllanthus amarus*

The effects of ethyl acetate crude extracts of *Phyllanthus amarus* (sample-1 and sample-2) on shrimp nauplii were observed to evaluate cytotoxic activity. The crude extract exhibited cytotoxicity with the LC_{50} values of 9.15 $\mu\text{g/ml}$ and 20.16 $\mu\text{g/ml}$ of only leaves and the whole plant respectively (Fig.2, 3).

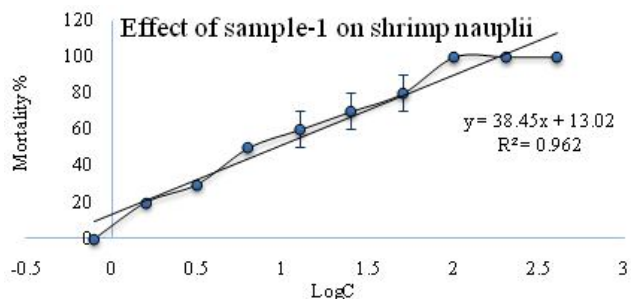


Fig. 2: Plot of % mortality and predicted regression line of sample-1 with error bar (Standard deviation).

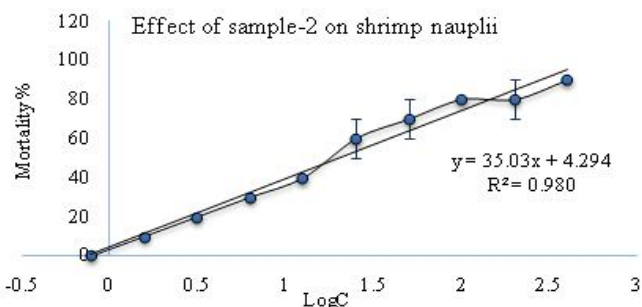


Fig. 3: Plot of % mortality and predicted regression line of sample-2 with error bar (Standard deviation).

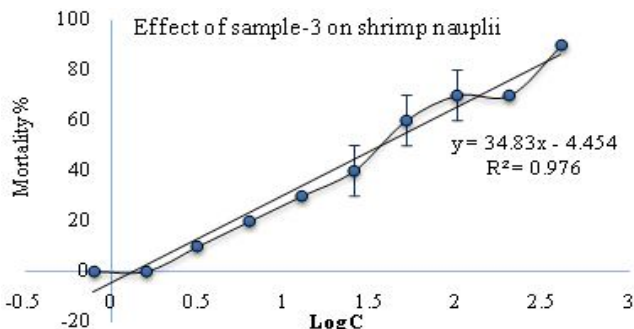


Fig. 4: Plot of % mortality and predicted regression line of sample-3 with error bar (Standard deviation).

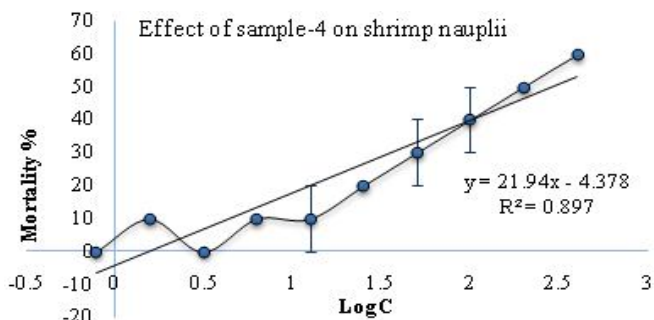


Fig. 5: Plot of % mortality and predicted regression line of sample-4 with error bar (Standard deviation).

Cytotoxic activity evaluation of *Monstera deliciosa*

The ethyl acetate crude extracts of *Monstera deliciosa* (sample-3 and sample-4) exhibited cytotoxicity with the LC_{50} values of 36.60 $\mu\text{g/ml}$ and 300.4 $\mu\text{g/ml}$ of leaves and branches respectively (Fig. 4, 5).

CONCLUSION

In brine shrimp lethality bioassay (BSLB), varying degree of lethality to *Artemia salina* was observed following the exposure to different dose levels of test samples. The ethyl acetate crude extracts of *Phyllanthus amarus* exhibited considerable cytotoxicity compared to VS (LC_{50} value of 0.544 $\mu\text{g/ml}$) where extractive of only leaves was more cytotoxic (LC_{50} value of 9.15 $\mu\text{g/ml}$) than that of whole plant (LC_{50} value of 20.16 $\mu\text{g/ml}$). Compared to VS, the ethyl acetate crude extract from leaves of *Monstera deliciosa* exhibited little cytotoxic potency (LC_{50} value of 36.60 $\mu\text{g/ml}$) whereas the extractive of branches did not show considerable cytotoxicity (LC_{50} value 300.4 $\mu\text{g/ml}$). From this assay, it can be predicted that extractives of *Phyllanthus amarus* possess cytotoxic principles with considerable cytotoxic potency. If this study is extended for assessment of cytotoxic activity after isolation of pure compounds some useful drugs of plant origin may develop out of the research.

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