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Comparative Brine Shrimp Lethality Bioassay of Different Plant Parts of *Bauhinia Purpurea* L.

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Abstract: The brine shrimp lethality bioassay is extensively used for the isolation of anitumour and cytotoxic agents from medicinal plants. The present study was designed to assess as well as to compare the cytotoxic activity of different plant parts of *Bauhinia purpurea* L using brine shrimp lethality bioassay. All the extracts of both leaves and bark were found to have promising cytotoxic activity. The highest activity was found for mehanol extract of bark and *n*-hexane fractions of leaves with LC50 value of 0.357µg/ml which is followed by *n*-hexane fractions of bark and ethyl acetate fractions of leaves with LC50 values of 0.385 and 0.625 µg/ml respectively. These results suggest that the leaves and bark of the *B. purpurea* may be a promising source for novel anticancer agents.

Key words: Brine shrimp lethality, cytotoxic, *Bauhinia purpurea* L.

INTRODUCTION:

Brine shrimp lethality bioassay is a general assay and capable of detecting broad spectrum of bioactivity present in crude extracts of medicinal plants. The technique is cost effective, can be mastered easily and require small amount of sample [1]. Since its introduction by Meyer *et al.*, 1982, it has been successfully used for the bioassay guided isolation of active antitumour and cytotoxic agents trilobacin from the bark of *Asimina triloba* [2].

Bauhinia purpurea L. locally known as "kanchan", "raktokanchan", is a medium sized deciduous tree which belongs to the family Fabaceae [3]. The presence of glycosides, saponins, flavonoids, phenolic compounds, triterpenoids, oxepins, fatty acids and phytosterols has been confirmed by phytochemical screening [4]. Particularly, the aerial parts of the plant are reported to contain flavone glycosides, foliar flavonoids, 6-butyl-3- hydroxy flavanone, amino acids, phenyl fatty ester, lutine and ß-sitosterl [5].

This plant is traditionally used in dropsy, pain, rheumatism, convulsions, delirium and septicemia [6]. The bark of the plant is used as an astringent and its decoctions are recommended for ulcers as a useful wash solution [7]. The leaves and roots are used for the treatment of catarrh, infection of children, boil, glandular and swelling [6].

Previously, the brine shrimp lethality assay of the bark of this plant by Alluri *et al.*, 2005 [7]. But to the best of our knowledge no body compares the activity between bark and leaves of this plant. The purpose of this study was to both explore and compare the brine shrimp lethality bioassay of the bark and leaves of this plant.

MATERIALS AND METHODS:

Plant materials:

The fresh leaf and bark of the *B. purpurea* plant was collected from the area of Savar in Jahangirnagar University during the month of February, 2011. The *Bauhinia purpurea* plant was taxonomically identified by "The National Herberium" having the accession number of 35516.

Drying and Pulverization:

The fresh leaf and bark of the plant of *B. purpurea* was washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days and finally dried at 45ºC for 36 hours in an electric oven. After complete drying, the entire portions were pulverized into a coarse powder with help of a grinding machine and were stored in an airtight container for further use.

Extraction of plant material:

The powdered 200g of leaf and bark part of *B. purpurea* was extracted with three times methanol of their weight in a flat bottom glass container, through occasional shaking and stirring for 7 days. The extracts were then filtered through filter paper. The filtrates were concentrated at 40ºC under reduce pressure.

Solvent-solvent partitioning of methanolic extracts:

Partitioning with n-hexane:

The concentrated methanolic extract of *B. purpurea* was made slurry with water. The slurry was taken in a separating funnel and *n*-hexane (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The *n*-hexane fraction (upper layer) was collected. The process was repeated three times. The n-hexane fractions of different parts of the plants were evaporated using rotary evaporator at 40^0C.

Partitioning with ethyl acetate:

The concentrated methanolic extract of *B. purpurea* was made slurry with water. The slurry was taken in a separating funnel and few ml of ethyl acetate (100 ml) was added. The funnel was shaken vigorously and allowed to stand for a few minutes. The ethyl acetate fraction (lower layer) was collected. The process was repeated three times. The ethyl acetate fractions of different parts of the plants were evaporated using rotary evaporator at 40^0 C.

BRINE SHRIMP LETHALITY BIOASSAY:

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [2,8] (Meyer et al., 1982; Zhao et al., 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of Brine shrimp (*Artemia salina* Leach) were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in a tank at a temperature around 37 °C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii.

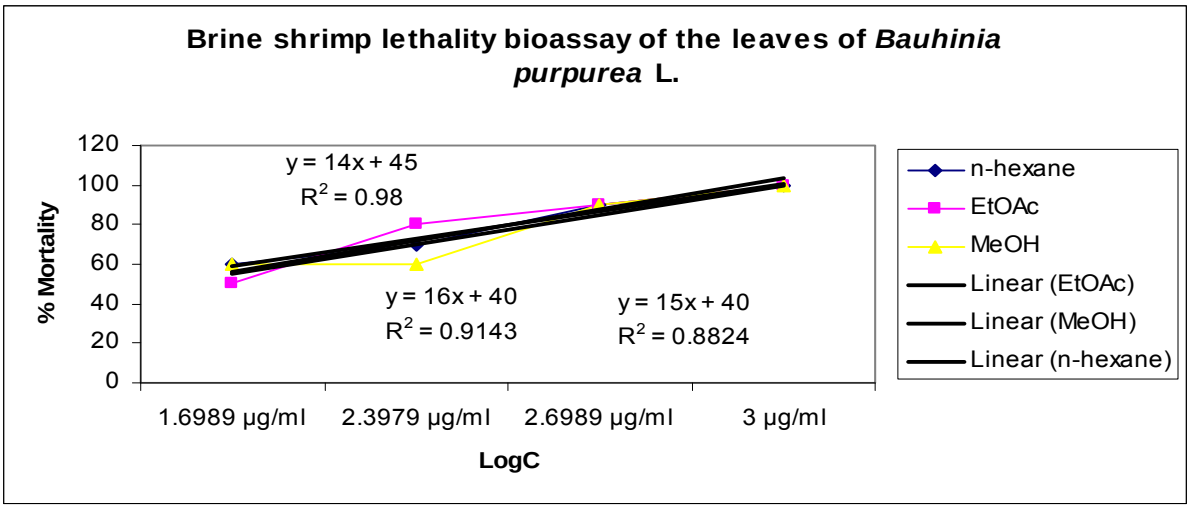
4.0 mg of each sample was dissolved in 200µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 100 µl were added to the pre marked glass test tubes containing 5 ml of sea water and 10 shrimp nauplii. So, the final concentration of samples in the test tubes was 50µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml respectively. With the help of a Pasteur pipette 20 living nauplii were put to each of the vials. After 24 h the vials were observed and the number of nauplii survived in each vial was counted with the help of magnifying glass. From this, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extract. Vincristine sulphate was used as standard cytotoxic agent.

RESULTS AND DISCUSSION:

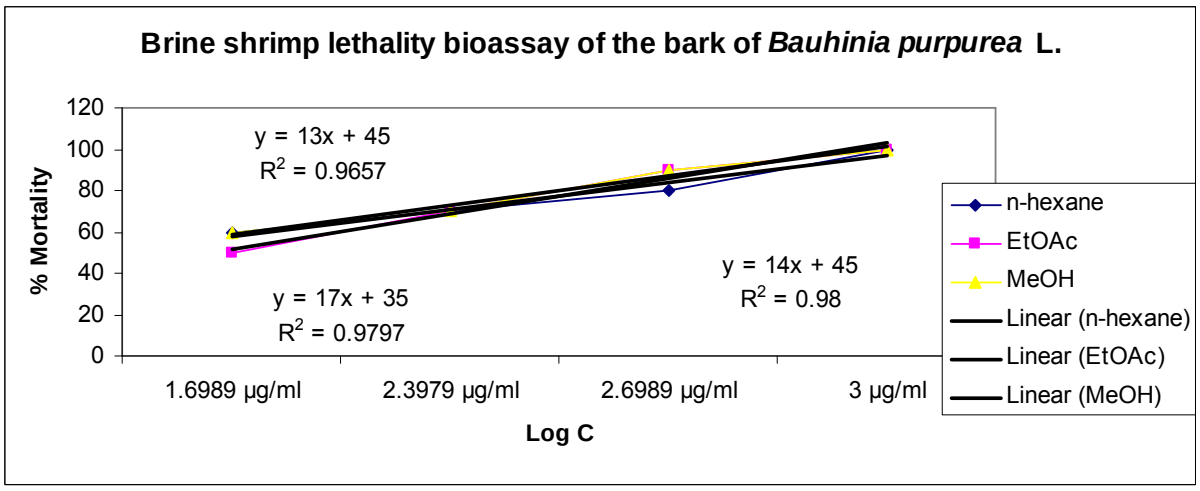
Methanol extract of bark and *n*-hexane fractions of leaves exhibited highest brine shrimp lethality with LC50 value of 0.357µg/ml. This is followed by followed by *n*-hexane fractions of bark and ethyl acetate fractions of leaves with LC50 values of 0.385 and 0.625 µg/ml respectively. The lowest lethal activity was found with the ethyl acetate extract of bark with LC50 value of 0. 882 µg/ml (Graph 1 and 2). The results of the present study, specifically the brine shrimp lethality of the bark is not congruent with the previous findings by Alluri *et al.*, 2005 [7].

The brine shrimp lethality bioassay is found to have a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity, and led to the discovery of new class of natural pesticides and active antitumoral agents [9]. Cytotoxic action of a drug is believed to be provided by disturbing the fundamental mechanisms associated with cell growth, mitotic activity, differentiation and function [10]. The observed cytotoxic activity for these extracts may be due one of these mechanisms.

Graph 1: Brine shrimp lethality bioassay of the leaves of *B. purpurea* L.



Graph 2: Brine shrimp lethality bioassay of the bark of *B. purpurea* L.



CONCLUSION:

The findings from this study is promising but preliminary in nature . Detailed investigation of each of the extracts for pharmacological activity may lead to the isolation of interesting pharmaceuticals of plant origin.

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