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RESEARCH ARTICLE

CYTOTOXIC EFFECT OF *LAGENARIA SICERARIA* CRUDE EXTRACTS OBTAINED FROM ITS FLOWERS.

Chinmoy Kumar Sen *[1] , Binita Paul [1], Bishyajit Kumar Biswas [2], A.F.M Shahid-Ud-Daula [1]

[1]Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3802, Bangladesh

[2]Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh

Abstract:
The plant *Lagenaria siceraria*, a plant belonging to the family Cucurbitaceae, has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. The n-hexane extract of flowers of Lagenaria *siceraria* was screened for antitumor properties using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate was used as a positive control. The LC_{50} values of n-hexane extract found to be 99.167 µg/ml. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.563 µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the n-hexane extract possess cytotoxic activity. Comparison with positive control vincristine signifies that cytotoxicity exhibited by the n-hexane extract might has mild antitumor and pesticidal activity.

KEYWORDS: *Lagenaria siceraria*, cytotoxic effect, flowers, n-hexane, extract.

INTRODUCTION

Plants have been used for mankind as remedies from the very beginning of civilization. The presence of diverse bioactive metabolites like carbohydrates, saponins, flavonoids, tannins etc in plant has formed the therapeutic basis of herbal medication. The uses of medicinal plants as traditional medicine are well known in rural areas of Bangladesh.Our present study was designed to determine the cytotoxic activity and antimicrobial activity of *Lagenaria siceraria* with n-hexane extract. The plant *Lagenaria siceraria belongs to* Cucurbitaceae family. Cucurbitaceae family

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is commonly mentioned as the bottle gourd, melon or pumpkin family, is medium sized generally a climbing plants family composing 118 genera and 825 species having wide distribution in the warmer regions of the world[1,2]. The bottle gourd is one of humankind's first domesticated plants, providing food, medicine and a wide variety of utensils and musical instruments. It is used as medicine in Bangladesh, India, China, European Countries, Brazil, Hawaiian island etc. for its cardiotonic, general tonic and diuretic[3] properties. Further the antidiabetic[4], antihyperlipidemic[5], antihepatotoxic[6], analgesic[7], CNS activity[8], anticancer[9], cardioprotective[10], anti-inflammatory[11], immunomodulatory[12] and antioxidant[13] activities of its fruit extract have been evaluated. In many countries, this plant has been used traditionally as a single treatment for diabetes mellitus[14].The usable parts are pulp, fruit, shoots, leaves, seeds. For this study flowers were investigated.

MATERIAL AND METHODS

Collection and Identification

The plant *Lagenaria siceraria* was collected from Noakhali Science and Technology Campus, Sonapur, Noakhali, Bangladesh.. The flowers of the plant were collected followed by thorough washing with water several times. During collection any type of adulteration was strictly prohibited. The plant sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. After identifying they gave an accession no. of 35,399.

Drying and grinding

The collected plant parts (flowers) were washed with water, separated from undesirable materials or plants or plant parts. They were air-dried under shade to protect from sunlight for one week after cutting into small pieces. The plant parts were ground into a fine powder with the help of a Hammer Mill. Fine powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Cold extraction (Methanol extraction)

About 284 gm of powered material was taken in a clean, flat bottomed glass container (4litres) and soaked in 1300 ml of 90% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper and the filtrate thus obtained was concentrated by using traditional spontaneous natural vaporization method at room temperature.

Preparation of mother solution

5 gm of methanol extract was triturated with 100 ml methanol containing 10%of distilled water (90ml ethanol+10 ml water). The crude extract went to the solution completely. This is the mother solution, which was partitioned off successively by three solvents of different polarity.

n-Hexane extraction

The mother solution was taken in a separatory funnel.100 ml of n-hexane was added and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice. n-hexane fractions were collected together and evaporated at room temperature. The aqueous fraction was preserved for the next step.

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CYTOTOXICITY TEST

Pharmacology is simply toxicology at a lower dose, and toxicology is simply pharmacology at a higher dose. Bioactive compounds are almost always toxic in high doses. The *in vivo* lethality in a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products. Meyer *et al*., 1982 focused on *Artemia salina* as a test organism and developed a protocol for Brine shrimp lethality bioassay to monitor cytotoxicty of a compound[15].

Brine Shrimp Lethality Bioassay

Principle (Meyer *et al*., 1982)

Brine shrimp eggs are hatched in simulated sea water to get nauplii. Sample solutions are prepared by dissolving the test materials in pre-calculated amount of DMSO. Ten nauplii are taken in vials containing 5 ml of simulated sea water. The samples of different concentrations are added to the premarked vials with a micropipette. The assay is performed using three replicates. Survivors are counted after 24 hours. These data are processed in a simple program for probit analysis to estimate LC_{50} values with 95% confidence intervals for statistically significant comparisons of potencies.

Materials

- *Artemia salina* leach (brine shrimp eggs)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes (5, 25ml) and Micropipette (5-40µl)
- Glass vials and Magnifying glass
- Test samples (n-hexane extract of flowers *Lagenaria siceraria*)

Procedure

Preparation of seawater: 38 gm sea salt (pure NaCl) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

Hatching of brine shrimps: *Artemia salina* leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to the vial containing 5 ml of seawater.

Preparation of test solutions with samples of experimental plants

Clean test tubes were taken. These test tubes were used for eight different concentrations (one test tube for each concentration) of test samples and eight test tubes were taken for standard drug Vincristin for eight concentration of it and another one test tubes for control test. Then 100 µl of solution was taken in test tube each containing 5ml of simulated seawater and 10 shrimp nauplii. Thus, 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. Thus the concentrations of the obtained solution in each test tube were as 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, 1.5625 µg/ml, 0.7815 µg/ml.

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Preparation of 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, such as (i) Positive control and (ii) Negative control

Preparation of the positive control group

Positive control in a cytotoxicty study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study vincristine sulphate is used as the positive control. Measured amount of the vincristine sulphate is dissolved in DMSO to get an initial concentration of 20 μg/ml from which serial dilutions are made using DMSO to get 10 μg/ml, 5 μg/ml, 2.5μg/ml, 1.25 μg/ml, 0.625 μg/ml, 0.3125 μg/ml and 0.15625 μg/ml. Then the positive control solutions are added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Preparation of the negative control group

100 μl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups. 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 were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

RESULTS AND DISCUSSION

Brine shrimp lethality bioassay

Bioactive compounds are almost always toxic at higher dose. Thus, *in vivo* lethality in a simple zoological organism can be used as a convenient informant for screening and fractionation in the discovery of new bioactive natural products. In the present bioactivity study all the crude extracts, VLC fractions and pure compounds showed positive results indicating that the test samples are biologically active. Each of the test samples showed different mortality rates at different concentrations. Plotting of log of concentration versus percent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) was determined for the samples. The positive control groups showed non linear mortality rates at lower concentrations and linear rates at higher concentrations. There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due to the activity of the test agents.

Test samples of *Lagenaria siceraria*

The brine shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties (McLaughlin,1991). Following the procedure of Meyer (Meyer *et al.*, 1982) the lethality of the n-hexane extract was determined and the summary of the result is expressed in Table 2.

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Table 1: Results of the test samples of *Lagenaria siceraria*

Sample	**LC_{50} (µg/ml)**	**Regression equation**	**R^2**
Vincristine sulphate (positive control)	0.563	y = 30.056x + 56.016	0.9168
n-hexane extract	99.167	y =0.264x +23.82	0.545

The LC_{50} values of n-hexane extract found to be 99.167 µg/ml. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.563 µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the n-hexane extract possess cytotoxic activity.

Comparison with positive control vincristine signifies that cytotoxicity exhibited by the n-hexane extract might has mild antitumor and pesticidal activity. However this cannot be confirmed without further higher and specific tests.

Table 2: Effect of n-hexane extract on shrimp nauplii

Conc. (C) (µg/ml)	Log C	n-hexane extract
400	2.602	100
200	2.301	100
100	2	100
50	1.699	90
25	1.398	30
12.5	1.097	20
6.25	0.796	10
3.125	0.495	0
1.5625	0.1938	0
0.78125	-0.107	0

Table 3: : N-Hexane soluble fraction

Log C	% of mortality
2.602	100
2.301	100
2	100
1.699	90
1.398	30
1.097	20
0.796	10
0.495	0
0.1933	0
-0.107	0

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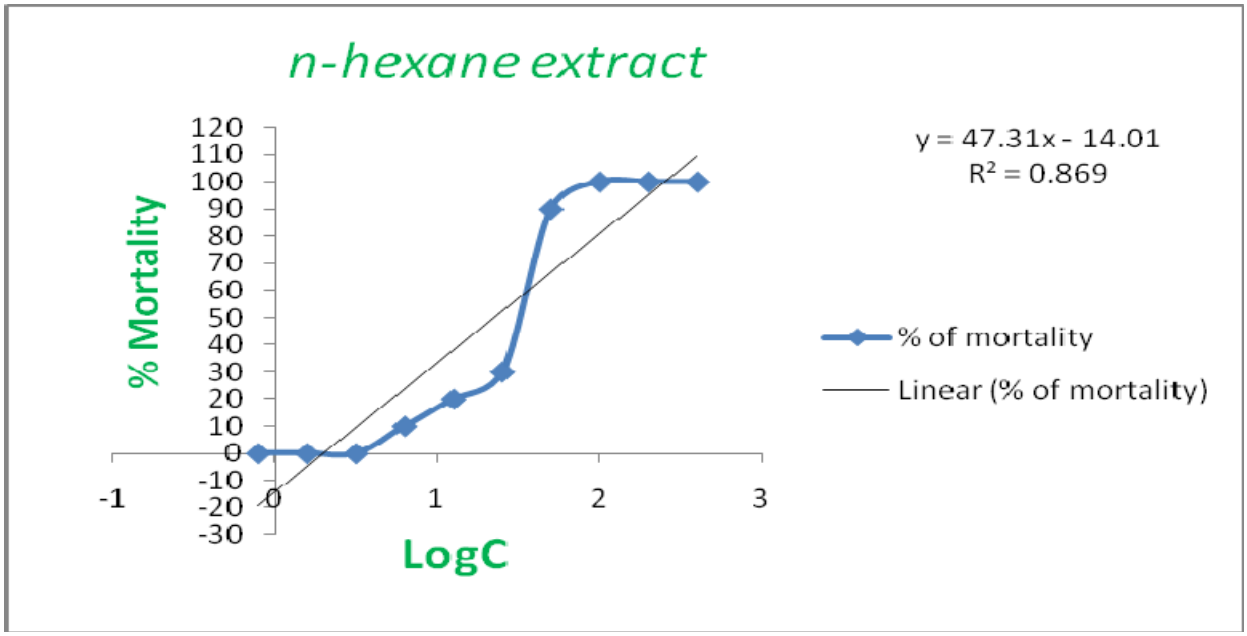

Fig 1: Cytotoxic activity of n-hexane extract against log C

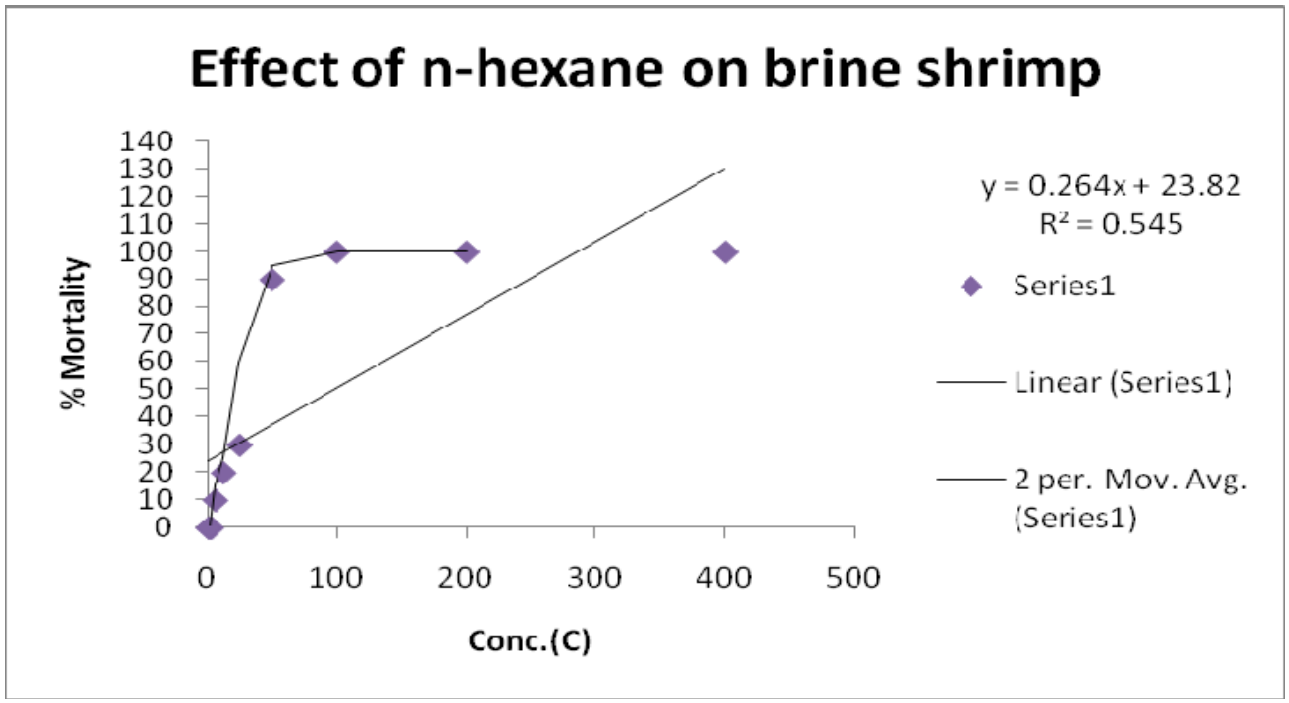

Fig 2: Cytotoxic activity of n-hexane extract against Con.(C)

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