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# CYTOTOXIC POTENTIALITY EVALUATION OF METHANOLIC, ETHYL ACETATE AND N-HEXANE EXTRACTS OF LEAVES OF *LITSEA SALICIFOLIA (ROXB. EX NEES) HOOK.* THROUGH BRINE SHRIMP LEATHALITY BIOASSAY.

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## ABSTRACT

Medicinal plants are the richest source of natural compounds for drug discovery. The present study was designed to investigate the cytotoxic potentiality of *Litsea salicifolia (Roxb. Ex Nees) Hook.* leaf using n-hexane, ethyl acetate and methanol extracts by brine shrimp lethality bioassay (BSLA). Different extracts of *Litsea salicifolia (Roxb. Ex Nees) Hook.* leaf using n-hexane, ethyl acetate and methanol solvents possess various phytochemical constituents demonstrated by phytochemical screening. All of the extracts of *Litsea salicifolia (Roxb. Ex Nees) Hook.* leaf have cytotoxic potentiality in term of brine shrimp lethality. Methanol extract of leaf was found to be the most toxic to brine shrimp nauplii, with $LC_{50}$ of 244.599 µg/ml, whereas anticancer drug vincristine sulphate showed $LC_{50}$ value 0.699 µg/ml. On the other hand, ethyl acetate and n-hexane extract showed 371.550 µg/ml and 539.842 µg/ml respectively which are also cytotoxic. Further studies are needed to isolate bioactive compound that is responsible for biological activities.

**KEYWORDS:** Cytotoxicity, Brine Shrimp Lethality Bioassay, vincristine sulphate.

## INTRODUCTION

Almost 87% of all types of human diseases including bacterial infection, cancer and immunological disorders are being treated with natural products and related drugs.[1] In developing countries about 80 % of people depend on plants based medicines for their ailment[2] and more than 3000 plant species have been found to have anticancer property.[3] *Litsea salicifolia (Roxb. Ex Nees) Hook.* is a plant belongs to Lauraceae family, the leaf of which is traditionally used in the treatment of dysentery, menstrual problem and also as an insecticidal.[4, 5, 6]

The in vivo lethality bioassay in a simple zoological organism (eggs of *Artemia salina*) that is known as the brine shrimp lethality bioassay (BSLA), developed by Meyer et al.[7] This bioassay is a rapid (24 h), simple (e.g., no aseptic techniques are required), easily mastered, inexpensive, which requires small amounts of test material (2 -20 mg or less).[8] There is a positive correlation between the result found from the bioassay and the cytotoxic activity in some human solidtumors.[8] Since the introduction of this brine shrimp lethality bioassay (BSLA), this in vivo test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated. The aim of the work was to investigate the cytotoxic potentiality of methanolic, ethyl acetate and n-hexane extracts of leaves of *Litsea salicifolia (Roxb. Ex Nees. ) Hook*. through brine shrimp leathality bioassay.

## MATERIALS AND METHODS

### Chemicals

Methanol, Dimethylsulfoxide (DMSO) and Folin-Ciocalteu reagent were purchased from Merck, Germany. Vincristine sulphate was collected from Techno Drugs Ltd., Bangladesh. All chemicals and reagents used were of analytical grade.

### Collection of plant material

The fruit of the plant was collected from Gazipur, Bangladesh and identified by the taxonomist of the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

### Preparation of plant material & extraction procedure

Leaf of the plant were first washed with water to remove adhering dirt and then cut into small pieces and sun-dried for few days and then dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C). Dried leaf was grinded into coarse powder using high capacity grinding mill. The powdered leaf was used for serial extraction by Soxhlet apparatus at elevated temperature (65°C) using n-Hexane, Ethyl Acetate and

Methanol consecutively (500 mL of each solvent). After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be completed when the leaf materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. The filtrates obtained were dried at temperature of 40±2°C to have gummy concentrate of the crude extract. The extract was kept in a suitable container with proper labeling and then stored in cold and dry place for further use.[9]

**Phytochemical screening**

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents i.e. carbohydrates through molisch's test and fehling's test, flavonoids, glucosides through general test for glycoside and glucoside, steroids through liebermann-burchard's test, saponins through frothing test, tannins through Ferric chloride and Potassium dichromate test, alkaloids through mayer's test, hager's test, wagner test and dragendorff's test. These phytochemicals were identified from their respective characteristic color changes as stated in the standard procedures.[10]

**Brine shrimp lethality bioassay**

Lethal activity of the leaf extracts of *Litsea salicifolia (Roxb. Ex Nees ) Hook*. was determined by Brine shrimp lethality bioassay described by Meyer *et al*.[7] Brine shrimp eggs (*Artemia salina* leach) were hatched in simulated seawater (3.8% NaCl) with continuous oxygen supply for two days and got the nauplii. Stock solution of the sample was prepared by dissolving 20 mg of extract in 400 µL of pure dimethylsulfoxide (DMSO) and adding sea water to make the total volume 20 mL. Thus the stock solution gained concentration of the extract as 1µg/µL. Then specific volumes of stock solution was transferred into different test tubes so that the final concentration of the extract becomes 6.25, 12.5, 25, 50, 100, 200, 400 and 800µg/mL in the respective test tubes after volume adjustment to 5 mL with sea water. In the control tubes 75µL and 150µL DMSO were taken and volume was adjusted to 5mL with sea water (as in the sample tubes). Vincristine sulfate was used as positive control and evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06µg/mL). Using a Pasteur pipette 10 living nauplii were put to each of the test tubes. After 24 hours the test tubes were observed and the number of nauplii survived in each test tube were counted.The mortality was corrected using Abott's formula.[11]

$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$

Where, $P_t$ = Corrected mortality, $P_o$= Observed mortality and $P_c$= Control mortality.

$LC_{50}$ values of the test samples after 24 hours are obtained by regression analysis.

## RESULTS

**Phytochemical screening**

The preliminary phytochemical screening was done to detect the presence of different phytochemical compounds in the methanolic, ethyl acetate and n-hexane extracts of the leaf of *Litsea salicifolia (Roxb. Ex Nees) Hook*. The results of the phytochemical testing are given in Table 1.

**Table 1: Results of Phytochemical Screening of the extracts.**

| Test | | Extracts | | |
|---|---|---|---|---|
| | | **Methanol Extract** | **Ethyl Acetate Extract** | **n-Hexane Extract** |
| Cabrohydrate | Molisch's Test | + | ++ | + |
| | Barfoed's Test | - | - | - |
| | Fehling's test | + | + | + |
| Glycoside | | - | - | - |
| Glucoside | | - | - | - |
| Alkaloids | Mayer's Test | + | - | - |
| | Hager's Test | + | - | + |
| | Dragendorff's Test | + | - | + |
| | Wagner's Test | + | - | + |
| Steroids | | - | - | - |
| Tannin | $FeCl_3$ (Ferric Chloride) Test | + | - | + |
| | Lead Aceatate | - | - | + |
| Flavonoids | | + | - | - |
| Saponin | | + | - | - |

[*LSH= n-Hexane extract of leaves of L. salicifolia, LSE= Ethyl acetate extract of leaves of L. salicifolia, LSM= n-Methanol extract of leaves of L. salicifolia; '++' sign indicates strongly presence & '+' sign indicates presence of phytochemical group of compounds while the '-' sign indicates absence of phytochemical group of compounds tested for]*

**Brine shrimp lethality bioassay (BSLA)**

All the extracts of leaf were subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol extract of leaf was found to be the most toxic to Brine Shrimp nauplii, with LC50 of 244.599 µg/ml, whereas anticancer drug vincristine sulphate showed LC50 value 0.699µg/ml. On the other hand, ethyl acetate and n-hexane extract showed 371.550 µg/ml and 539.842 µg/ml respectively (table 2).

**Table 2: $LC_{50}$ values of the different extracts in brine shrimp lethality bioassay.**

| Test Sample | Concentration (µg/ml) | % Mortality | $LC_{50}$ (µg/ml) |
|---|---|---|---|
| LSH | 6.25 | 10 | 539.842 |
| | 12.5 | 20 | |
| | 25 | 20 | |
| | 50 | 30 | |
| | 100 | 50 | |
| | 200 | 60 | |
| | 400 | 50 | |
| | 800 | 60 | |
| LSE | 6.25 | 30 | 371.550 |
| | 12.5 | 30 | |
| | 25 | 40 | |
| | 50 | 50 | |
| | 100 | 50 | |
| | 200 | 50 | |
| | 400 | 60 | |
| | 800 | 70 | |
| LSM | 6.25 | 40 | 244.599 |
| | 12.5 | 30 | |
| | 25 | 40 | |
| | 50 | 50 | |
| | 100 | 50 | |
| | 200 | 60 | |
| | 400 | 70 | |
| | 800 | 80 | |
| VS | 0.06 | 10 | 0.699 |
| | 0.125 | 20 | |
| | 0.25 | 30 | |
| | 0.5 | 40 | |
| | 1 | 50 | |
| | 5 | 90 | |
| | 10 | 100 | |

**DISCUSSION**

Different crude extracts of leaf of *L. salicifolia* have been shown to possess various phytoconstituents including carbohydrates (monosaccharides, reducing sugars), alkaloids, tannins, flavonoids and saponin. There is absence of glycosides, glucosides, steroids among all the extracts. Ethyl Acetate Extract of leaf of *L. salicifolia* possesses least phytochemical constituents. These phytoconstituents present in the extracts may account for their various pharmacological activities shown in other investigations.[12]

This is a very useful tool to screen a wide range of chemical constituents for their various bioactivities. It has been well utilized to screen and fractionation of physiologically active plant extracts as well. Table 2 shows the lethality of different extracts of leaf of *L. salicifolia* to the Brine Shrimp nauplii. The degree of lethality demonastrated by the extracts was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (6.25 µg/mL) to the highest concentration (800 µg/mL). This concentration dependent increment in percent mortality of Brine Shrimp nauplii produced by the leaf extracts of *L. salicifolia* indicates the presence of cytotoxic principles in these extractives.

The significant correlation between in vitro growth inhibition of human solid tumor cell lines and the Brine shrimp assay demonstrated by the national Cancer Institute (NCI, USA) is significant as it shows the value of this bioassay as a pre-screening tool for antitumor drug research.[13] To evaluate the toxicity of plant extracts by Brine shrimp lethality bioassay $LC_{50}$ values

lower than 1000 µg/mL are considered bioactive.[7] Principle of brine shrimp toxicity for plant extracts or compounds above 1000µg/ml is non-toxic, between 500 & 1000 µg/ml is weakly toxic, and bellow 500 µg/ml is toxic which were established as $LC_{50}$ values.[14] So, methanol and ethyl acetate extracts of leaf of *L. salicifolia* is said to be toxic to Brine Shrimp nauplii with $LC_{50}$ of 244.599 µg/ml and 371.550 µg/ml respectively and n-hexane extracts can be said to weakly toxic with 539.842 µg/ml (table 2). Preliminary phytochemical screening revealed the presence of alkaloids. So the observed cytotoxic potentiality may be due to the presence of such compounds. Again, reports exist on the role of alkaloids and steroids in cytotoxic activity of plant extract. [15, 16, 17] In addition, Hoechst 33258 fluorescence assay by inhibiting cellular DNA the phenolics and flavonoids are also known to show cytotoxicity in a concentration-dependent manner.[18]

## CONCLUSION

The methanol and ethyl acetate extracts of leaf of *L. salicifolia* can be said to have cytotoxic potentiality due to presence of various phytoconstituents in these extracts. The present research has opened a new scope for more focused studies on isolation and biological screening of isolated compounds.

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