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LC_{50} Determination of Barleria Prionitis L. by Brine Shrimp Lethality Assay

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ABSTRACT

Brine shrimp assay is very useful tool for the isolation, of bioactive compounds from plant extracts. The method is attractive because it is very simple, inexpensive, and low toxin amounts are sufficient to perform the test in micro well scale. The present studies widen the scope of the brine shrimp model that may prove quite helpful as a preliminary screen to determine toxic properties. In Brine shrimp lethality bioassay, compounds produced dose dependent cytotoxicity effect to brine shrimp nauplii. The result showed that LC_{50} value of methanolic extract was found to be 1.05 µg/ml possess significant cytotoxicity activity.

Keywords: Artemia salina; brine shrimp lethality test; medicinal plants; cytotoxicity

I. INTRODUCTION

The importance of medicinal plants and traditional health systems in solving the heath care problems of the word is gaining increasing attention[1]. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at international level, often to the detriment of natural habitats and mother population in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal application in various cultures. There are well known drugs that are directly developed from plant species[2-3]. *Barleria prionitis* also known as the porcupine flower, which belongs to the family Acanthaceae and genus Barleria. It is native to India, also distributed widely throughout Asia including Malaysia, Pakistan, Philippines, Sri Lanka, Bangladesh, Yemen and tropical Africa, Sri Lanka and Eastern Southern and Central Africa. It is an erect, perennial, prickly, and evergreen shrub, usually single-stemmed, growing to about 1.5 m in height from a single taproot[4-6]. The soxhlet extraction method has proved stronger and more efficient than its traditional counterpart. The popular technique of Soxhlet extraction is a continuous method of removal of solvents. Routine solvent extractions are performed using extraction systems for soils, sediments, sludges, polymers and fabrics, pulp and paper, biological tissues, textiles and foods[7-21]. Studies have shown that the microwaves use a lower solvent and sample

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volume and extract at a much faster rate than with Soxhlet extraction[22-41]. In view of ADME failure, docking studies should be carried out before pharmacological activity because receptors can be readily predicted with the assistance of a phytoconstituent structure for probable pharmacological activity. An outbreak of coronaviral disease (COVID-19) triggered by the novel severe acute respiratory syndrome (SARS-CoV-2) presents an unprecedenting impediment to the development of appropriate medicinal products for prevention and treatment. The context of the quick pace of scientific investigation and clinical data generated by the large number of people quickly infected with SRAS-CoV-2, clinicians need clear proof of good medical care for this infection, since in the first stage it is easy to use molecular docking software to conduct in-silico analysis with the help of a chemical structure of phytoconstituents of medicinal plant[42-57]. In the formulating system for the delivery of protein and peptide medicines, enzyme stability as well as membrane permeation must be improved[58-66]. Soon, one day, at home, you might take your own medicines. That's because researchers adapted the 3D printer to a drug delivery device from readily available medical active agents[67-68].

II. MATERIAL aND METHODS

Plant Material

The fresh matured leaves of the *B. prionitis* were collected randomly during the month of May-June, from Sangli region, Maharashtra, India. Department of Botony, Yashwantrao Chavan College of Science, Karad has identified the plant and authenticated it.

Preparation of Plant Extract

Shade drying was done for almost a month to prevent sunlight chemical degradation. The dried material was grinded and transformed in coarse powder with the aid of a grinder. The extraction of *B. prionitis* with solvent methanol was carried out by microwave extraction, and excess solvent present was evaporated.

Brine Shrimp Lethality Assay

Brine shrimp lethality test has been used as a bioassay for a variety of toxic substances. A general bioassay that appears capable of detecting a broad spectrum of bioactivity, present in synthetic compounds, rather than more tedious and expensive *in-vitro* and *in-vivo* antitumor assays. Furthermore, it does not require animal serum as is needed for cytotoxicities.

Procedure:

Preparation of seawater

38 gm sea salt (without iodine) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

Hatching of brine shrimp

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 moved to one side of the tank, and sealed on this side. The shrimp was allowed to hatch for two days and be matured like nauplii. Constant supply of oxygen was rendered during the process of hatching. The hatched shrimps are drawn to the light (phototaxis) and so egg shell-free nauplii from the illuminated portion of the tank was collected. The nauplii was taken by a pipette from the fish tank and filtered to improve visibility in fresh clear sea water and 10 nauplii was taken carefully by micropipette.

Preparation of test samples

In each experiment, 0.5mL of test compound of different concentration i.e (50, 100 and 150 µg/mL) was added to brine solution and maintained at room temperature for 24hr under the light and surviving larvae were counted. Vehicle treated used as control for the test. Test solutions were used in sets of three tubes per dose. Replicas should be maintained to get accurate results. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a (IC_{50})[69-71].

III. RESULT AND DISCUSSION

The lethality of a test sample in a simple zoological organism such as the shrimp (*Artemia salina*) has

been utilized in the Brine shrimp cytotoxicity test (BSCT). It is a very useful tool to screen a wide range of chemical compounds for their various bioactivities. It has been demonstrated that BSCT correlates reasonably well with cytotoxic and other biological properties. The brine shrimp bioassay has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound as well as plant products. The brine shrimp lethality bioassay also indicates antifungal effects, pesticidal effects, teratogenic effects, toxicity to environment and many more. Table 1 shows the lethality of different test sample to the brine shrimp nauplii. All the extractss were tested for cytotoxic activity by the brine shrimp lethality assay. Among them methanolic extract showed a dose dependent cytotoxic activity at concentrations of 1.05 µg/ml. The degree of lethality is directly proportional to the concentration of the extracts.

Table 1. Brine shrimp lethality assay data of *Barleria prionitis*

Sr. no	Test subs.	LC_{50} (µg/ml)
1	Methanolic extract	1.05
2	Ethanolic extract	2.56
3	Ethyl acetate extract	1.12
4	Chloroform extract	1.34

III. CONCLUSION

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities of several plant species that are used in the traditional medicine. Out of the several plants screened for toxicity against the brine shrimp, some species showed LC_{50} values less than 100 ppm. In addition, these interesting results lend further support to their traditional use. Even though, the present study on these crude extracts is an addition to the scientific literature, detailed investigations on individual plants for pharmacological activities and active ingredients could provide leads to interesting pharmaceuticals of plant origin.

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