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Cytotoxicity Analysis of Selected Plants Using *Artemia salina*

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ABSTRACT

Validation and evaluation of food and medicinal potential, including toxicity and cytotoxicity level analysis, is an important task in a contemporary situation where the whole world is facing antimicrobial resistance and lethal diseases like cancer, corona, etc. Considering this, an effort was undertaken to evaluate the degree of cytotoxicity of specific food and medicinal plants, including *Blumea flava, Ludwigia adscendens, Oenanthe javanica, Phragmites karka* and *Polygonum barbatum*, consumed by the native population of Loktak Lake, Manipur, India. Tests on Brine shrimp (*Artemia salina*) lethality and plant collection were done in accordance with ethnobotanical data. The results showed that *O. javanica* had the highest level of cytotoxic capability against Brine Shrimp nauplii followed by *L. adscendens, P. karka, B. flava* and *P. barbatum.* The findings showed that the plant parts could be employed as anticancer medicines and should be used in low quantities. The present study offers fundamental concept for potential medication formulations.

Key words: Anticancer, *Artemia salina,* Loktak Lake, safe use, toxicity

INTRODUCTION

Wetlands are one of the most potentially rich ecosystems. This ecosystem serves an enormously beneficial ecological function (Mahanti and Kumar, 2017). Some of the important functions include groundwater recharge and discharge; protection from storms and mitigation of floods; water purification; stabilising local temperature and rainfall; drainage of waste chemicals, etc. Such ecosystems show the highest carbon sequestration rates. The carbon pool is found in the soil, followed by its vegetation and water (Were *et al.*, 2019). Besides its ecological importance, it has the potential to generate huge economic value in terms of supplying water for fisheries, agriculture, production of timber, wildlife resources, recreational and tourism opportunities, etc. A wetland is a home that supports a huge diversity of flora and fauna, especially avifauna, fishes, reptiles, mammals, etc. A vast diversity of hydrophytes and moisture loving flora, phytoplanktons and zooplanktons flourish storing good carbon content (Shah, 2021). India possesses a diverse topography, due to which its harbours have different types of wetlands distributed in the vast geographical regions of the country (Singh and Chaturvedi, 2017). It has been estimated that about 15.26 MHA are wetlands in the country, out of which about 10.564 MHA account for inland wetlands. About 75 wetlands in India have been listed as Ramsar sites and about 1,200 plant species have been reported from wetlands in India. Loktak Lake is an important wetland and one of the Ramsar sites that was added to the Montreux Record in 1993. Ramsar sites are wetlands that are designated to be of international importance under the Ramsar convention. Loktak Lake is a home of several food and medicinal plants which could

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be used as a source of nutraceutical agents against contemporary serious health problems like corona, cancer, antimicrobial resistance, drug toxicity, etc. In this aspect, the plant wealth of Loktak Lake is a suitable source and interest of research. Keeping this in view, an attempt has been made to evaluate the cytotoxicity level of some selected plant wealth of Loktak Lake as per ethnomedicinal uses.

METHODOLOGY

A survey was made during 2021-23 in and around Loktak Lake, Manipur, India. Random interview with the local communities was made and five plants were selected for cytotoxicity test and safe use as per local use (Plate 1). The plants were identified by Dr. Sanjeet Kumar and extracts were prepared using standard protocol (Sarah *et al.*, 2017; Kumar *et al.*, 2021). DMSO (dimethyl sulfoxide) was taken as the standard solvent for dissolving three extracts (Ethanol, methanol and aqueous) to obtain the stock solution. One per cent DMSO was taken for the brine shrimp assay to prevent the toxicity of DMSO. One per cent DMSO and artificial sea water were used as negative controls. The hatching of eggs was done using artificial sea water made by dissolving commercial marine salt (3.6 g) in distilled water (200 ml). The nauplii hatched within 18-24 hrs at 30-35°C. Ten larvae of brine shrimps were transferred to each prepared test tube containing extracts of different concentrations (200, 300, 400, 500, and 1000 µg/ml) as per standard methods (Sarah *et al.*, 2017). Survivors were counted every hour up to 24 hrs and then up to 48 hrs. The total death and percentage mortality at each concentration level and control were determined and recorded (Sarah *et al.*, 2017).

Plate 1. Toxicity activity of selected plants using *A. salina*.

RESULTS AND DISCUSSION

To know about the primary cytotoxic potential of plants used in the present work, cytotoxicity was evaluated using *Artemia salina*. The *in vitro* cytotoxicity activity of selected plant extracts was carried out against brine shrimp (Nauplii) at various concentrations (200, 300, 400, 500 and 1000 µg/ml). The cytotoxicity test revealed that the 1000 µg/ml of methanol and 1000 µg/ml of ethanol extract of *B. flava* caused the death of 4 larvae at the end of 24 hrs and 10 larvae at the end of 48 hrs (Figs. 1 and 2), whereas 1000 µg/ml of aqueous extract of the same plant caused the death of 5 larvae at the end of 24 hrs and 10 nauplii at the end of 48 hrs (Figs. 1 & 2). 300, 400, 500 and 1000 µg/ml

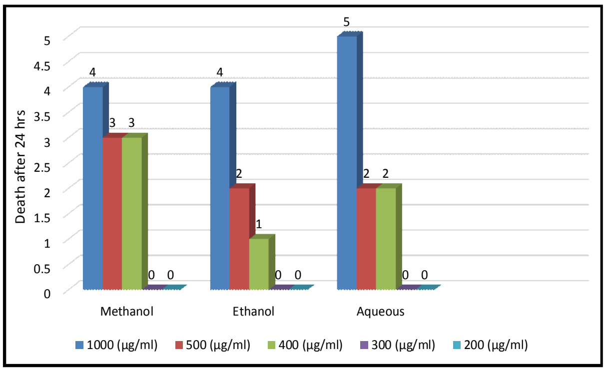

Fig. 1. Death of *Artemia salina* after 24 hrs in methanol, ethanol and aqueous extract of *Blumea flava*.

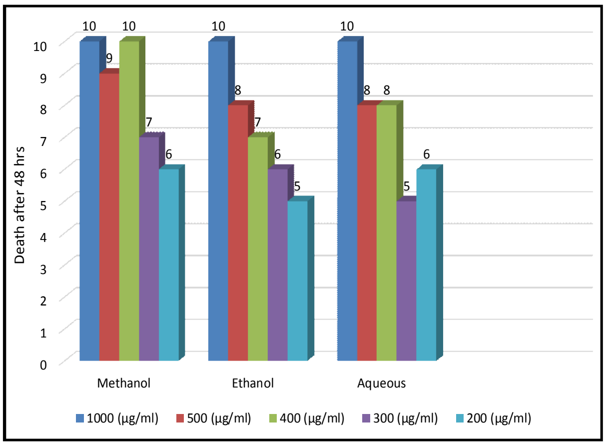

Fig. 2. Death of *Artemia salina* after 48 hrs in methanol, ethanol and aqueous extract of *Blumea flava*.

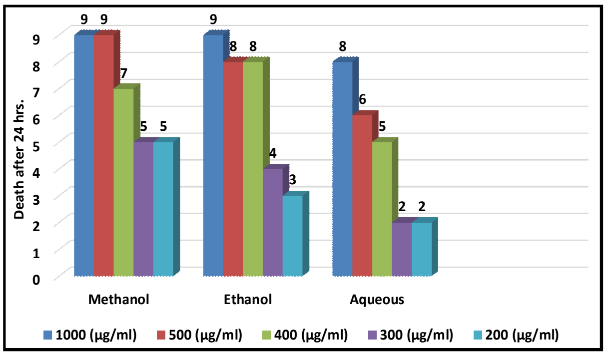

Fig. 3. Death of *Artemia salina* after 24 hrs in methanol, ethanol and aqueous extract of *Ludwigia adscendens*.

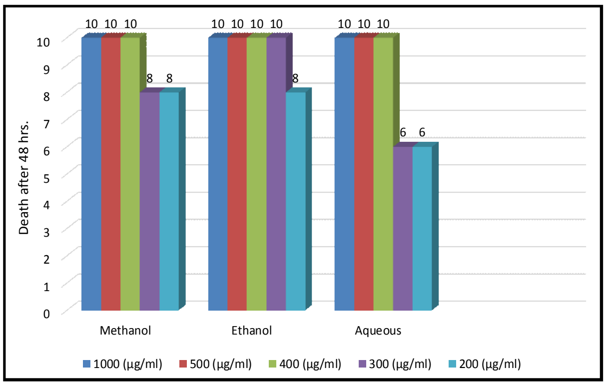

Fig. 4. Death of *Artemia salina* after 48 hrs in methanol, ethanol and aqueous extract of *Ludwigia adscendens*.

of ethanolic extract of *L. adscendens* caused the death of 10 nauplii at the end of 48 hrs, whereas the same concentration caused the death of 4, 8, 8 and 9 nauplii at the end of 24 hrs (Figs. 3 and 4). Methanolic and aqueous extracts of the same plant at concentrations of 400, 500 and 1000 µg/ml caused the death of 10 nauplii at the end of 48 hrs, whereas the same concentration of methanolic extract caused the death of 7, 9 and 9 nauplii, respectively, at the end of 24 hrs, and the same concentration of aqueous extract caused the death of 5, 6 and 8 nauplii, respectively, at the end of 24 hrs (Figs 3 & 4). 400, 500 and 1000 µg/ml of ethanolic extract of *O. javanica* were very effective and caused the death of 10 nauplii at the end of 24 hrs (Fig. 5). However, 200, 300, 400, 500 and 1000 µg/ml of ethanol, methanol, and an aqueous extract of the same plant caused the death of 10 nauplii at the end of 48 hrs (Fig. 6). 400, 500 and 1000 µg/ml of methanolic extract, and 500 and 1000 µg/ml of ethanolic extract of *P. karka* caused the death of 10 nauplii at the end of 48 hrs

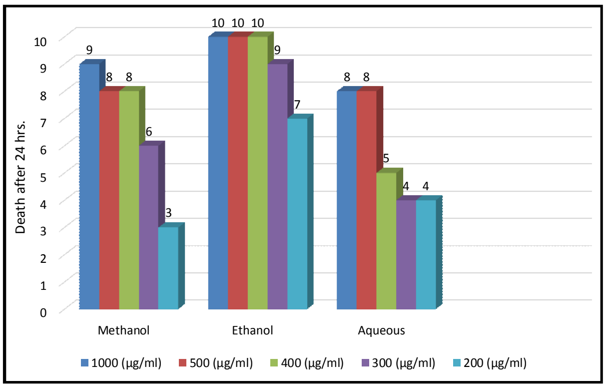

Fig. 5. Death of *Artemia salina* after 24 hrs in methanol, ethanol and aqueous extract of *Oenanthe javanica*.

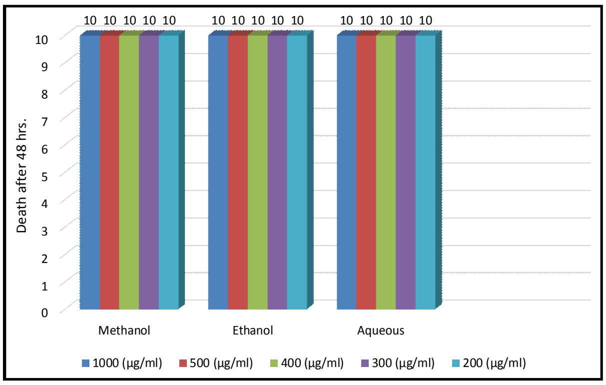

Fig. 6. Death of *Artemia salina* after 48 hrs in methanol, ethanol and aqueous extract of *Oenanthe javanica*.

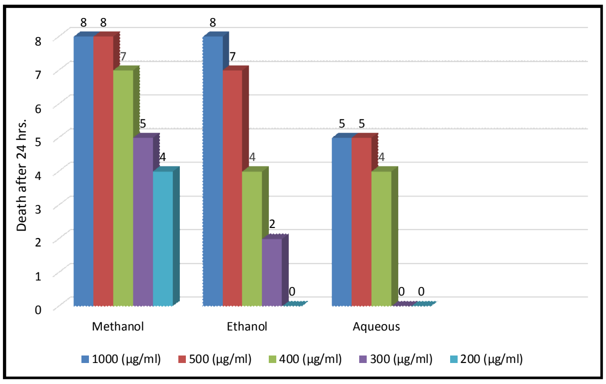

Fig. 7. Death of *Artemia salina* after 24 hrs in methanol, ethanol and aqueous extract of *Phragmites karka*.

(Fig. 8). 400, 500 and 1000 µg/ml of methanolic extract, and 500 and 1000 µg/ml of ethanolic extract of *P. barbatun* caused the death of all 10 nauplii treated at the end of 48 hrs (Fig. 10). Whereas, the same concentration of methanolic extract caused the death of 7, 8, and 8 nauplii respectively, at the end of 24 hrs, and the same concentration of ethanolic

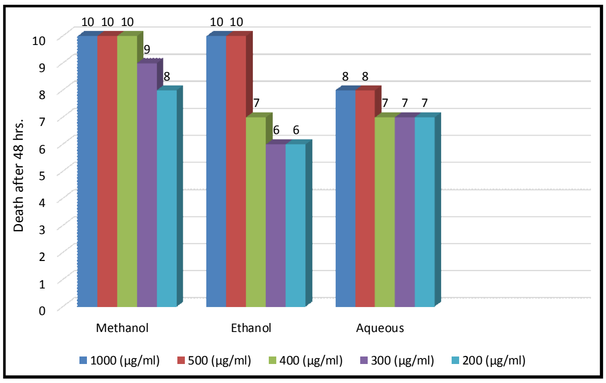

Fig. 8. Death of *Artemia salina* after 48 hrs in methanol, ethanol and aqueous extract of *Phragmites karka*.

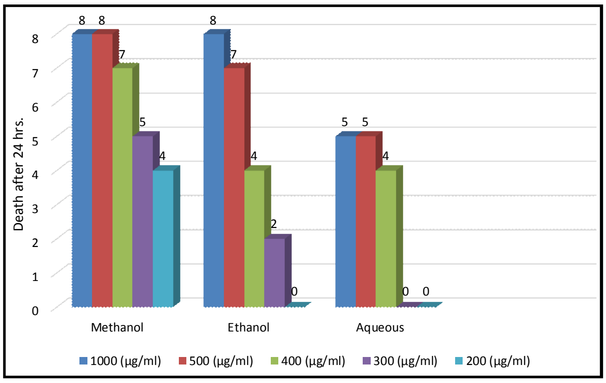

Fig. 9. Death of *Artemia salina* after 24 hrs in methanol, ethanol and aqueous extract of *Polygonum barbatum*.

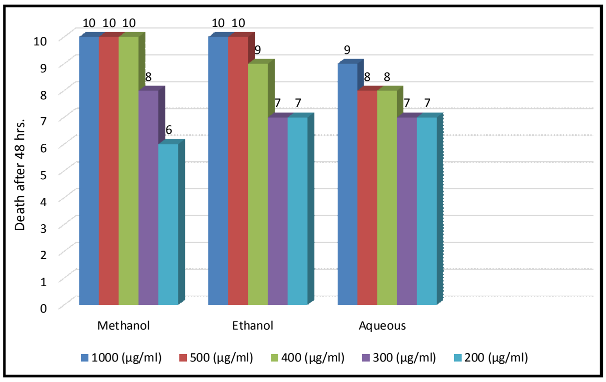

Fig. 10. Death of *Artemia salina* after 48 hrs in methanol, ethanol and aqueous extract of *Polygonum barbatum*.

extract caused the death of 4, 7 and 8 at the end of 24 hrs (Fig. 9). The study on toxicity analysis of selected plants revealed that *O. javanica* showed high toxic potential against Brine Shrimp nauplii, followed by *L. adscendens*, *P. karka*, *B. flava* and *P. barbatum*. Many researchers have reported the cytotoxicity levels of different medicinal plants. Selvi (2018) demonstrated the aqueous, methanol, hexane and chloroform extracts of 16 medicinal plants often used in the Sidha and Ayurveda systems of traditional medicine against brine shrimp and found that the hexane extracts of these plants were the most poisonous to Brine Shrimp nauplii, followed by methanol extract, chloroform extract and aqueous extract. *Pancratium verecundum* leaf and bulb extracts were also found to have cytotoxic properties (Tripathy and Bhatnagar, 2021). Leaf extracts of *Clerodendrum siphonanthus* also showed cytotoxic activity (Mohapatra and Bhatnagar, 2022). Ethyl acetate leaf extract of *Antigonon leptopus* exhibited mild cytotoxic activity (Pradhan and Bhatnagar, 2016). The methanol extract of *Turnera ulmifolia* showed maximum cytotoxicity i.e. 80.94% at 200 µg/ml followed by ethyl acetate extract which showed 75% cytotoxic activity (Patra and Bhatnagar, 2016). Methanol leaf extracts of *Wedelia chinensis* extract showed 100% cytotoxic activity at the higher dose 400 µg/ml in brine shrimp mortality assay (Kumari and Bhatnagar, 2016). Continuous evaluation of food and medicinal potential of common and unexplored plants is an important task for researchers for the sustainable development and to provide fundamental data for making strategies against several health problems like cancer, corona, microbial infections and many disorders. In this aspect, present study on selected plants of Loktak Lake highlights the importance of traditional knowledge in general and plant wealth consumed by the local communities of Loktak Lake against contemporary health problems. It also brings attention towards the value addition of mentioned plants in this study for the sustainable uses and bio-wealth conservation of Loktak Lake, Manipur, India.

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