

## *In vitro* Brine Shrimp Lethality Bioassay of Aqueous Extract of Bark of *Mangifera indica*

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### ABSTRACT

Mangos belong to the genus *Mangifera* of the family *Anacardiaceae*. The genus *Mangifera* contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mangos belong to the species *Mangifera indica*. It has also been used for its medicinal value. In Samoa, a bark infusion has been a traditional remedy for mouth infections in children (*pala gutu*), and in Tonga, infusions of leaves of mango, the orange (*Citrus sinensis*), and other species are used to make a potion to treat relapse sickness (*kita*). In India, a drink made from unripe mango fruit is used as a remedy for exhaustion and heat stroke. Half-ripe fruit eaten with salt and honey is used for a treatment of gastro-intestinal disorders, bilious disorders, blood disorders, and scurvy. Diabetes has been treated with a drink made from the infusion of fresh mango leaves. Dried mango seed ground into flour is used to treat diarrhea. Diarrhea and throat disorders are treated by gargling bark extracts mixed with water. Fruit sap has been used to treat the pain of bee and scorpion stings<sup>1</sup>. Many of the traditional Indian medicinal uses of mango involve eating unripe fruit. The present work was accomplished to explore the cytotoxic potential of aqueous extract of *Mangifera indica* using brine shrimp lethality bioassay method. In this method, *Mangifera indica* showed a significant activity. The cytotoxic activity of the extract was moderate having LC<sub>50</sub> value of 5.05 µg/ml.

**Key words:** Brine shrimp, Cytotoxicity, Bark of *Mangifera indica*, *Anacardiaceae*.

### INTRODUCTION

In developing countries, especially in rural contexts, people usually turn to traditional healers when in diseased conditions, and plants of ethnobotanical origin are often presented for use. Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal

chemistry as a major route for the discovery of novel and more effective therapeutic agents<sup>2</sup>. Thus, plants are considered as one of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates. Plants in *Anacardiaceae* are being used traditionally in a wide variety of ethnomedical remedies<sup>1</sup> & is widely distributed all over India.

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It is used widely in Indian folk medicine for the treatment of various diseases, including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions<sup>3</sup>. The present work was accomplished to explore the cytotoxic potential of aqueous extract of *Mangifera indica* using brine shrimp lethality bioassay method. In this method, *Mangifera indica* showed a significant activity.

## MATERIALS AND METHODS

### Collection and Identification of the plant:

The fresh Bark of *Mangifera indica* was collected during June 2015 from the area of Kasegaon, Sangli, Maharashtra. The plant was identified by the Dr. Potdar Yashwantrao Chavan College of Science, Karad. where a voucher specimen was deposited having the accession number of RCP/21.

### Extraction of Plant Material

Ten grams of powdered bark were mixed with 1000 ml distilled water, boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2mm) to remove particulate matter. The filtrate was then dried by using Spray-dried LU222 (Labultima).

**Phytochemical screening:** The powdered bark extracts of *M.indica* were evaluated for the presence of phytochemical compounds using standard methods. For performing phytochemical tests the extracts were allowed to convert in the powdered form.

**Detection of Steroids (Salvoskis Test):** The 100mg of dry extracts were dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were added to form a lower layer. A reddish brown colour at the interface was indicative of the presence of steroidal ring.

**Detection of Cardiac Glycosides (Keller Killian's Test) :** The 100mg of dry extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar characteristic of cardenolides.

**Detection of Saponins (Froth Test):** The 100mg extracts were diluted with distilled water to 20ml which was shaken in a graduated

cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

**Detection of Resins:** The 100mg of dry extracts were dissolved in ethanol then 5 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of concentrated sulphuric acid was added. Bright purple colour produced indicated the presence of resins.

### Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds<sup>4,5</sup>. The brine shrimp, *Artemia salina*, was used as a convenient monitor for the screening. The eggs of the brine shrimp, hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. The test samples (extract) were prepared by dissolving in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain log concentrations of – 0.11 µg/ml, 0.19 µg/ml, 0.49 µg/ml, 0.89 µg/ml, 1.09 µg/ml, 1.40 µg/ml, 1.70 µg/ml, 2.00 µg/ml, 2.30 µg/ml and 2.60 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard vincristine sulfate was used as positive control<sup>6,7,8</sup>. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial were counted. The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC50) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version); the LC50 was derived from the best-fit line obtained.

## RESULTS

The lethality of the aqueous extracts of Bark of *Mangifera indica* to brine shrimp was determined after 24 hours of exposure to the test solutions and the positive control, vincristine sulfate by following the procedure of Meyer *et al.*, 1982. The aqueous extract of Bark of *Mangifera indica* showed potential cytotoxic activity having an LC50 value of 5.05 µg/ml in contrast to the LC50 value of standard vincristine sulfate of 0.397 µg/ml.

### DISCUSSION

A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in crude extracts is the brine shrimp lethality bioassay (BSLT). The technique is easily mastered, of little cost, and utilizes small amount of test material. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays once the active compounds have been isolated. It appears that BSLT is predictive of cytotoxicity and pesticidal activity (Ghisalberti, 1993). The result obtained from the brine shrimp lethality bioassay of Bark of *Mangifera indica* can be used as a guide for the isolation of cytotoxic compounds from the aqueous extract of the barks of this plant.

### REFERENCES

1. Species Profiles for Pacific Island Agroforestry *Mangifera indica* (mango), **3(1)**: 17 (2006).
2. www.traditionaltree.org.
3. Indian materia Medica , A. K. Nadkarni, Vol I, Popular Publication, Delhi.
4. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, J.B., Nicholsand, D.E., Mclaughlin, J.L., Brine shrimp; a convenient general bioassay for active plant constituents, *Planta Medica* **45**: 31-34, (1982).
5. Zhao, G.X., Hui, Y.H., Rupprecht, J.K., McLaughlin, J.L., Wood ,K.V., Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*," *Journal of Natural Products*, **55**: 347-356 (1992).
6. Hossain, M.S., Hossain, M.A., Islam, R., Alam, A.H., Kudrat-e- Zahan, Sarkar, S., Farooque, M.A., Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2- aminophenol and their coordination complexes with Magnesium (Mg- II), *Pakistan Journal of Biological Sciences*, **7**: 25-27 (2004).
7. Khan, A., Rahman, M., Islam, M.S., Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*, *Indian J. Pharmacology*, **40**: 41-44 (2008).
8. Nikkon, F.Z., Saud, A., Rahman, H., Haque, M.E., *In vitro* antibacterial activity of the compounds isolated from chloroform extract of *Moringa oleifera* Lam” *Pakistan Journal of Biological Sciences*, **6**: 1888 – 1890 (2003).