IN VIVO EVALUATION OF *MITRAGYNA PARVIFOLIA* (ROXB.) KORTH LEAF AND BARK EXTRACTS FOR TOXICITY USING *CAENORHABDITIS ELEGANS*

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Abstract

The toxicological assessment of medicinal plants is essential to ensure their safe use in therapeutic applications. *Mitragyna parvifolia* (Roxb.) Korth is a traditionally used plant known for its diverse pharmacological properties. The present study aimed to evaluate the in vivo toxicity of aqueous extracts derived from the leaf and bark of *Mitragyna parvifolia* using the nematode *Caenorhabditis elegans* as a model organism. Extracts were prepared by aqueous maceration, and worms were exposed to a series of concentrations over a defined time period. Toxicity was assessed based on mortality rates. The findings provide preliminary safety data for *Mitragyna parvifolia* and support the use of *C. elegans* as a rapid and sensitive platform for toxicological screening of plant-based extracts.

Keywords: *C. elegans*, *Mitragyna parvifolia*, aqueous extract, toxicity assay, medicinal plant.

Introduction

Medicinal plants, also known as medicinal herbs, have been an integral part of traditional healthcare systems for centuries. These plants synthesize a wide variety of bioactive compounds that serve critical ecological functions, including defense against pests, fungi, and pathogens (Chaudhari, 2022). Among the numerous plant families with medicinal value, Rubiaceae stands out for its therapeutic and economic importance and is widely distributed across various regions. Members of this family possess significant medicinal potential due to their rich phytochemical profiles (Das *et al*, 2020).One such valuable species is *Mitragyna parvifolia,* commonly known as Kadamb, which belongs to the Rubiaceae family. Native to the deciduous and evergreen forests of India, this tree holds deep cultural, medicinal, and economic relevance (Gupta *et al*, 2009). Phytochemical investigations of the *Mitragyna* genus have revealed the presence of various bioactive constituents, predominantly indole alkaloids, triterpenoids and saponins(Gong, 2012). The plant has a long-standing reputation in traditional medicine and is frequently used by tribal

communities and Ayurvedic practitioners for its diverse pharmacological benefits (Kaushik *et al*, 2009).Scientific investigations have confirmed that *Mitragyna parvifolia* exhibits a broad spectrum of immunopharmacological activities, including analgesic, antipyretic, anti-inflammatory, anti-arthritic, anthelmintic and antioxidant properties (Gupta ,2016). The bark and roots are employed in the treatment of fever, colic, muscle pain, burning sensations, poisoning, gynecological ailments, cough, and edema. Moreover, fruit juice is traditionally used to promote lactation in breastfeeding mothers (Kotval *et al*, 2018). *Caenorhabditis elegans* is a widely recognized non-mammalian model organism extensively used in biomedical and toxicological research. This free-living soil nematode provides an effective in vivo system for assessing the toxicity of both synthetic and natural compounds. Its application in the study of traditional medicines and plant-based bioactives has grown significantly (Leung *et al*, 2008). The model is valued for its short life cycle, small body size and ease of propagation, along with its high sensitivity to a wide range of toxins (Jiang *et al*, 2017). In addition, *C. elegans* has a compact, fully sequenced genome with many conserved molecular and cellular pathways shared with mammals, including humans. This evolutionary conservation allows for meaningful extrapolation of toxicity data to higher organisms, making *C. elegans* a more cost-effective alternative to traditional vertebrate models (Miao *et al*, 2020). Its established utility in various fields such as genomics, developmental biology, neurobiology and aging research further highlights its value in early-phase toxicological screening of medicinal plants and natural products (Silverman *et al*, 2009). Therefore, the present study aims to evaluate the in vivo toxicity of aqueous leaf and bark extracts of *Mitragyna parvifolia* using *C. elegans* as a model system.

Material and Methods:

Plant Material

Fresh samples of *Mitragyna parvifolia*, (leaf and bark) were collected from the Vile Parle, Mumbai. The plant material was authenticated at the Blatter Herbarium, St. Xavier's College. The collected samples were thoroughly washed. The cleaned material was dried and then ground into fine powder using a blender. The powdered plant material was stored in airtight containers for further use.

Preparation of aqueous extract

A known quantity of dried leaf and bark powders was subjected to cold maceration using distilled water. After the extraction period, the mixtures were filtered to obtain clear aqueous extracts.

Maintenance of *Caenorhabditis elegans* (Stiernagle ,2006):

Preparation of NGM Agar Plates

Caenorhabditis elegans was maintained on Nematode Growth Medium (NGM) agar plates prepared using standard protocols. The NGM medium was prepared by mixing NaCl (3 g), agar (17 g), and peptone (2.5 g) in 975 mL of distilled water. The mixture was autoclaved, cooled to 55°C, and supplemented with 1 ml each of 1M $CaCl_2$, 1M $MgSO_4$, and 5 mg/mL cholesterol (in ethanol), along with 25 mL of 1M potassium phosphate buffer (pH 6.0). The sterile medium was poured into sterile Petri dishes and allowed to solidify under aseptic conditions. Plates were left at room temperature for 2–3 days to check for contamination and remove excess moisture. Prepared plates were stored in airtight containers until use.

Seeding with *E. coli* OP50

NGM plates were seeded with *Escherichia coli* OP50, the standard food source for *C. elegans*. Approximately 50 µL of *E. coli* OP50 culture was added to each small or medium plate and spread gently to form a central lawn. Plates were incubated at room temperature overnight to allow bacterial growth.

Toxicity assay Using *Caenorhabditis elegans*

The toxicity assay was carried out using *C. elegans* worms cultured on NGM agar plates seeded with *E. coli* OP50. Aqueous extracts of *Mitragyna parvifolia* leaf and bark were prepared at concentrations of 25, 150 and 350 µg/ml. For the assay, worms were transferred onto sterile petri plates containing the respective plant extracts. After 24 hours of exposure at room temperature, the number of live and dead worms was recorded. Worms that failed to respond to gentle touch were considered dead. All treatments were performed in triplicates for accuracy and reliability.

Results and Discussions:

The toxicity of aqueous extracts of *Mitragyna parvifolia* leaf and bark was assessed in *Caenorhabditis elegans* at concentrations of 25, 150 and 350 µg/mL. The mortality percentages observed for the leaf extract were 8.93 ± 7.56%, 21.83 ± 10.38% and 31.98 ± 13.02% respectively, while the bark extract caused 2.56 ± 4.44%, 16.01 ± 4.20% and 23.81 ± 8.38% mortality. A clear increase in nematode mortality was observed with rising extract concentrations for both leaf and bark. Notably, the leaf extract consistently showed higher toxicity than the bark extract across all concentrations. The increased mortality at higher concentrations may be attributed to the cumulative presence of phytochemicals such as flavonoids, alkaloids and phenolics, which are known to influence cellular metabolism and stress pathways in nematodes (Shahlehi *et al*, 2024). *Caenorhabditis elegans*, a well-established invertebrate model, is widely utilized in toxicity

studies due to its short life cycle, rapid reproduction and fully mapped genome (Leung *et al*, 2008). It has proven effective in evaluating the toxic effects of various substances, including heavy metals, environmental pollutants and bioactive components from plant extracts (Jiang *et al*, 2017). This study underscores the significance of evaluating traditional medicinal plants for their potential biological effects using simple yet effective model organisms like *C. elegans.*

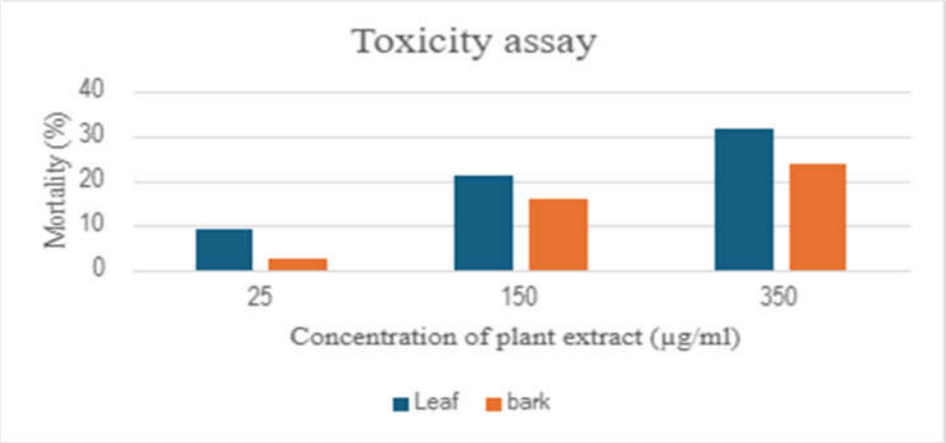

Fig.1 Graphical representation of the effect of aqueous extracts of *Mitragyna parvifolia* on the survival of *C. elegans* at different concentrations.

Conclusion:

The present study demonstrated that aqueous extracts of *Mitragyna parvifolia* leaf and bark exhibit notable toxic effects on *Caenorhabditis elegans*. These findings suggest the presence of active phytoconstituents in the plant, particularly in the leaves, which may possess biological potency. The use of *C. elegans* as a model organism proved to be a sensitive and reliable system for preliminary toxicity screening. Overall, the study provides a step towards understanding the biological safety of *Mitragyna parvifolia* and supports further investigations into its phytochemical components and potential therapeutic or toxicological applications.

Acknowledgements:

I sincerely thank the Department of Botany, Mithibai College (Empowered Autonomous), Mumbai, for providing the necessary facilities and resources that enabled the successful execution of this research work.

Conflict of Interest:

Authors declare no conflict of interest.

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