REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.1 (2024)
http://www.veterinaria.org
Article Received: Revised: Published:

Studies On Genotoxicity In Ornamental Fish Of *Poecilia Reticulata* Using Mangrove Crude Extract

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Abstract
Genotoxicity, the potential of substances to damage genetic material in cells, poses significant risks to aquatic organisms, which serve as crucial bioindicators of environmental health. This study investigates the genotoxic effects of *Avicennia marina* crude extract on *Poecilia reticulata* (guppy fish) using the comet assay. Phytochemical analysis of the extract revealed the presence of tannins, saponins, steroids, flavonoids, terpenoids, and alkaloids, indicating a diverse array of secondary metabolites with significant therapeutic potential. Fourier-transform infrared spectroscopy confirmed the presence of various bioactive compounds in the crude extract, such as aliphatic primary amines, alkanes, amine salts, alkenes, and halo compounds. Guppy fish exposed to 25 µg/ml of the extract showed no significant DNA damage, indicating non-genotoxicity at this concentration. However, exposure to higher concentrations (50, 75, and 100 µg/ml) resulted in a progressive increase in DNA fragmentation, with the highest concentration causing the most substantial damage (18.5% DNA fragmentation). These findings highlight the dose-dependent genotoxic potential of *A.marina* crude extract, likely mediated by oxidative stress and direct DNA interactions. This study underscores the importance of evaluating the environmental impact of natural bioactive compounds to ensure the safety and protection of aquatic ecosystems.

KEYWORDS: Genotoxicity, *Avicennia marina*, *Poecilia reticulata*, Comet assay, Phytochemical analysis, Oxidative stress, Environmental impact

1 INTRODUCTION
Genotoxicity, ability of certain substances to damage genetic information in cells, leading to mutations, which may result in cancer or other health disorders (Castro et al., 2020). Assessing genotoxicity in aquatic organisms is critical, as these organisms often serve as bioindicators of environmental health (Galhano et al., 2021). Ornamental fish like *Poecilia reticulata* are used in genotoxicity studies because they are sensitive to environmental changes and can reflect the effects of substances that may also impact other aquatic organisms. *Poecilia reticulata*, commonly known as the guppy, is a popular ornamental fish widely used in ecotoxicological studies due to its sensitivity to various pollutants and ease of maintenance in laboratory conditions (Lopes et al., 2023). Their small size, ease of breeding, and sensitivity to stressors make them suitable for studying the impacts of substances like mangrove crude extracts. Mangrove ecosystems are rich in biodiversity and produce a wide array of bioactive compounds, many of which exhibit significant pharmacological & toxicological properties (Mahmud et al., 2020, Arthanari et al., 2024). The crude extracts from mangrove plants have shown potential antibacterial, antifungal, and antitumor activities (Ragavan et al., 2022, Francis et al., 2021). *Avicennia marina* inhabits muddy shores, estuaries, and lagoons, playing a crucial ecological role by stabilizing coastal sediments, reducing erosion, and protecting shorelines from storm surges. It also provides habitat and nursery grounds for various marine and terrestrial species, including fish (Asari et al., 2020). This species is notable for its bioactive compounds with significant pharmacological properties, including antibacterial, antifungal, and anticancer activities due to flavonoids, alkaloids, and other compounds (Ullah et al., 2022). However, the genotoxic effects of these extracts on aquatic life, particularly ornamental fish like *Poecilia reticulata,* have not been extensively studied (Sharma & Shukla, 2021). Recent studies have highlighted the significance of evaluating the environmental impact of natural product extracts, as their use in various industries, including pharmaceuticals and cosmetics, is increasing.

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Understanding the genotoxic potential of mangrove crude extracts is essential to ensure their safe application and to protect aquatic ecosystems from possible adverse effects.

2 MATERIALS AND METHODS

2.1 Sample collection and preparation

The *Avicennia marina* mangrove samples were collected from the Parangipettai, Tamilnadu. The obtained sample was washed thoroughly with tap water and then dried in the shade for 48 hours. It was then further dried in an oven for 24 hours and finely powdered using a mortar and pestle (Figure 1a, b)

2.2 Preparation of crude extract

20 grams of dried powdered *Avicennia marina* sample was combined with 100 ml of 70% ethanol, and the mixture was kept in a shaker for 24 hours at 37°C. The mixture was then filtered through Whatman No. 1 filter paper. The filtrate was then heated in a water bath at 60°C to facilitate the extraction of the crude components (Figure 1c, d, e).

2.3 Phytochemical Screening

In this study, bioactive compounds were identified by conducting a qualitative phytochemical analysis of crude extracts from *Avicennia marina* plant samples. The extracts underwent preliminary phytochemical screening following standard procedures outlined by Gokul et al., 2019.

2.4 FTIR Characterization

The prepared crude extract was characterized using a FT-IR analysis. The IR spectrum of the crude was recorded with a Bruker Alpha II spectrophotometer. The IR spectrum of the crude was recorded over the range between 500 to 3500 cm^{-1} at a scanning speed of 1 μm/min.

2.5 Animals and experimental design

Poecilia reticulata (Guppy fish) were sourced from a local fish market and acclimated for 15 days at the Marine Biomedical Research Lab & Environmental Toxicology Unit, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai. Following acclimation, the fish were allocated into three groups, each group containing five individuals, & placed in separate 50-liter glass tanks for the duration of the experiment. Throughout the experiment, the fish were maintained in oxygenated water under natural lighting conditions of a 12:12 hr light-dark cycle.

2.6 Genotoxicity

The comet assay was performed following the protocol outlined by Vijitkul et al. (2022). Blood samples were collected through cardiac puncture, 3μl of sample was diluted in 1000μl of Phosphate Buffer Saline. Subsequently, 5μL of the diluted blood mixture was mixed with 15μL of Phosphate Buffer Saline and mixed with 0.7% agarose at 37°C. Then the samples were placed on slides coated with 1% agarose, covered with coverslips, cooled with an ice pack for 5 minutes. The coverslips were carefully removed, and the slides were immersed in lysis buffer (containing 100 mM Na EDTA, 2.5 M NaCl, 10 mM Tris, 1% Triton X-100, 10% DMSO, pH 10) for 24 hours.

Following lysis, the slides were subjected to electrophoresis in an electrophoresis buffer (0.3M NaOH, 1mM Na EDTA, pH 13) at 50 V for one hour. After electrophoresis, the slides were stored in the dark & neutralized in neutralization buffer (400 mM Tris, pH 13) for 10 minutes. Slides were then stained with propidium iodide & observed under fluorescence microscope.

3 RESULTS

3.1 Phytochemical Analysis of *Avicennia marina* crude extract

Phytochemical assays are commonly used to identify and characterize the presence of various bioactive compounds in plant extracts or natural products. In this study, phytochemical analysis of *Avicennia marina* crude extract revealed the presence of tannins, saponins, steroids, flavonoids, terpenoids, and alkaloids. These findings indicate that the plant contains a diverse array of secondary metabolites with significant therapeutic potential (Table 1).

3.2 FTIR characterization of crude extract

The Fourier-transform infrared spectrum of *A.marina* crude extract illustrates a diverse array of organic compounds, characterized by prominent absorption peaks indicative of various functional groups (Figure 2). The peak at 3349.54 cm^{-1} represents aliphatic primary amines. The absorption peak at 2947.25 cm^{-1} corresponds to the C-H stretching vibrations of alkanes, which are typically present in lipid components and organic substrates. A strong absorption peak at 2835.97 cm^{-1} reveals N-H stretching in amine salts, indicating the presence of complex nitrogen-containing chemicals such as alkaloids or other bioactive molecules. The peak at 1649.34 cm^{-1} indicates C=C stretching vibrations, confirming the presence of alkenes, an unsaturated hydrocarbon that may contribute to the extract's biological activity. Notable peaks at lower wavelengths are also present: 1408.88 cm^{-1} for sulfonyl chloride's S=O stretching, 1112.67 cm^{-1} for secondary alcohols C-O stretching, and 1015.56 cm^{-1} for a fluorinated compound's C-F stretching. The peaks at 615.54 cm^{-1}, 574.92 cm^{-1}, 549.31 cm^{-1}, & 529.11 cm^{-1} correspond to C-Br and C-I stretching vibrations, which are typical for halo compounds.

3.3 Genotoxicity

The genotoxicity assessment of guppy fish (*Poecilia reticulata*) exposed to varying concentrations of *Avicennia marina* crude extract was evaluated using the comet assay (Fig. 3). The control group displayed predominantly intact nuclei with minimal DNA migration, indicating no detectable DNA damage and serving as a baseline for genomic stability. At the lowest concentration of 25 µg/ml of *A. marina* crude extract, no DNA damage was detected, indicating that this exposure level does not induce genotoxic effects in guppy fish. This result reflects the natural state of the DNA without the influence of the extract, maintaining genomic stability similar to the control group. Increasing the concentration to 50 µg/ml resulted in a noticeable rise in DNA damage, with 8.2% of the DNA showing signs of fragmentation or strand breaks. This suggests that at this concentration, the bioactive compounds within the extract begin to interact with cellular DNA, leading to mild genotoxic effects. The extent of DNA damage further increased to 14.5% at a concentration of 75 µg/ml. This significant rise indicates a stronger genotoxic response, where higher levels of *A. marina* crude extract result in more extensive DNA damage, possibly due to increased oxidative stress or direct interactions with DNA molecules, overwhelming the fish cellular repair mechanisms. The highest concentration tested, 100 µg/ml, resulted in the most substantial DNA damage observed, with 18.5% of the DNA exhibiting fragmentation. This pronounced genotoxic effect highlights the potential of *A. marina* crude extract to induce significant DNA damage at high concentrations, likely through cumulative effects of its bioactive constituents (Fig. 4). The genotoxicity assessment using the comet assay distinctly illustrates the DNA integrity in guppy fish under different treatment conditions. The control group shows guppy fish not exposed to *A. marina* crude extract, with comet assay results indicating predominantly intact nuclei and minimal or no DNA migration, evidenced by the absence of comet tails. This suggests that under normal conditions, the DNA in the control group remains largely undamaged, reflecting baseline genomic stability. In contrast, guppy fish exposed to the highest concentration of *A. marina* crude extract exhibit pronounced comet tail formation, a hallmark of DNA fragmentation and strand breaks. The extended comet tails observed in this group indicate substantial DNA damage, suggesting that exposure to high concentrations of the crude extract leads to considerable erythrocytic abnormalities and genotoxicity effects.

4 DISCUSSION

Tannins in *Avicennia marina* could contribute to its traditional use in treating wounds and diarrhea, given their strong astringent and antimicrobial properties (Balasundram et al., 2006). Saponins may enhance the plant's antimicrobial efficacy, which aligns with their known ability to disrupt microbial cell membranes (Timilsena et al., 2023). The presence of steroids and terpenoids could explain the anti-inflammatory effects of *Avicennia marina*, making it a potential candidate for treating inflammatory diseases (Masyita et al., 2022). The identification of flavonoids supports the use of Avicennia marina in managing oxidative stress, which is a key factor in the pathogenesis of chronic diseases such as cancer and cardiovascular disorders (Ciumărnean et al., 2020). The presence of alkaloids further highlights the pharmacological potential of this plant, as these compounds form the basis of many therapeutic agents used in modern medicine (Kumar & Pandey, 2015).

FTIR spectrum of *Avicennia marina* crude extract demonstrates a diverse chemical profile, revealing compounds with significant biological and pharmacological potential. The detection of aliphatic primary amines at 3349.54 cm^{-1} points to the presence of nitrogen-containing compounds like amino acids or alkaloids, which are known for their bioactive properties in mangrove species (Chen et al., 2021). The C-H stretching vibrations of alkanes (2947.25 cm^{-1}) suggest the presence of lipids and hydrocarbons, aligning with previous studies on the chemical composition of *A. marina* (Senthilkumar et al., 2016). The strong peak at 2835.97 cm^{-1} for N-H stretching further supports the presence of bioactive alkaloids. Alkenes, identified by C=C stretching at 1649.34 cm^{-1}, and the presence of sulfonyl chlorides, secondary alcohols, and fluorinated compounds, contribute to the extract's complexity and potential therapeutic value. The identification of halo compounds through C-Br and C-I stretching suggests additional bioactivity, as these compounds have pharmacological applications (Huang et al., 2023).

Bioactive compounds in *A. marina* could cause oxidative stress or direct interactions with DNA, leading to increased DNA fragmentation and potential genetic instability. (Dos et al., 2019) studied BP-3 induced significant DNA damage in *Poecilia reticulata* at concentrations of 100 and 1000 ng L^{-1}, as evidenced by the comet assay results. This genotoxic effect underscores the environmental risk posed by BP-3 at environmentally relevant concentrations. (Rabelo et al., 2021) who found cylindrospermopsin promotes genotoxicity in the brain, liver, and blood cells of *Poecilia reticulata*, with mutagenicity in erythrocytes, highlighting its toxic effects for aquatic organisms and human health.

5 CONCLUSION

In conclusion, this study reveals that *Avicennia marina* crude extract has a dose-dependent genotoxic effect on *Poecilia reticulata*. The phytochemical analysis identified a range of bioactive compounds, including tannins, saponins, steroids, flavonoids, terpenoids, and alkaloids, suggesting significant therapeutic potential. FTIR characterization further supported the presence of various functional groups associated with these compounds. The findings demonstrate that while lower concentrations of the extract do not induce significant DNA damage, higher concentrations lead to increasing DNA fragmentation. This genotoxic potential, likely mediated by oxidative stress and direct interactions with DNA, emphasizes the importance of evaluating the safety and environmental impact of natural products to protect aquatic ecosystems and inform their use in various applications.

ACKNOWLEDGEMENT
The authors would like to thank Saveetha Institute of Medical and Technical Sciences, Chennai for moral support. The corresponding author is thankful to University Grant Commission-BSR Research Start-Up Grant, Government of India for financial support (No. F.30-603/2021 (BSR)-Dr. P. Sivaperumal).

CONFLICT OF INTEREST STATEMENT
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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