Erythrocyte Toxicity Assessment in Oreochromis Niloticus Exposed to Avicennia Marina Extract

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**Abstract:** This study investigates the genotoxic potential and antibacterial properties of crude extract from *Avicennia marina*. Using Fourier Transform Infrared (FT-IR) spectroscopy, key functional groups were identified, revealing the presence of bioactive compounds such as phenolics, fatty acids, alkaloids, terpenoids, and flavonoids. The antibacterial activity of the extract was evaluated against *Klebsiella sp.* and *Streptococcus mutans* using the disc diffusion method, while erythrocyte abnormalities was assessed through micronucleus and nuclear abnormality tests in *Oreochromis niloticus* erythrocytes. FT-IR analysis confirmed the presence of bioactive compounds with known antimicrobial properties. The extract demonstrated significant antibacterial activity, particularly against *S. mutans*, with inhibition zones increasing at higher concentrations, indicating a dose-dependent response. However, an increase in nuclear abnormalities in *O. niloticus* erythrocytes suggested potential erythrocytic effects, emphasizing the extract's impact at the cellular level. This study highlights the pharmacological potential of *A. marina* crude extract and underscores the need for further research to optimize its efficacy and safety for therapeutic applications.

**Keywords: *Avicennia marina*; FT-IR; Bioactive compounds; Antibacterial activity; Nile tilapia; nuclear abnormalities**

# Introduction

India's inland aquaculture industry has grown rapidly over the last three decades, positioning it as a significant player in the production of aquatic food for both local and international markets [(Alam, 2024; Sidiq et al., 2024)](https://paperpile.com/c/3E1xYb/WJAUz+nZB2h). Indian giant carps have been the most often farmed species, except for shrimp. But the nation's farmed fish business has also benefited greatly from smaller carps and catfish like Pangasius[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/3E1xYb/oS5ul+momFG+vWweL). India's fish consumption is rising steadily; by 2024–2025, the government wants per capita consumption to reach 12 kg, up from an anticipated 5.6 kg in 2018–19[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/3E1xYb/GnByR+w2S8S+LpgdK). In order to meet the request of an expanding population and raise per capita fish consumption, the Indian government has set a target of producing 22 million tons of fish by 2024–2025, up from 13.75 million metric tons in 2018–19 – an increase of roughly 8 million metric tons[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/3E1xYb/UnYKL+dfLAw+lbRtl). An important portion of the productivity increase is anticipated to come from inland aquaculture[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/3E1xYb/HXWeD+Tw7fG+6ipC5). The Nile tilapia (*Oreochromis niloticus*), one of the most extensively farmed fish in the world, greatly raises local incomes, especially in developing countries. The skin of the Nile tilapia has drawn interest in the field of biomaterials science due to its abundance, low cost, and capacity to recover from fish industry waste products[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/3E1xYb/6AiU0+4vDtN+wtkwC)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/3E1xYb/6AiU0+4vDtN+wtkwC+JtmPI). About 40% dry weight of type I, highly biocompatible collagen may be extracted from Nile tilapia skin [(Sridhar et al., 2024)](https://paperpile.com/c/3E1xYb/TYxSH). Recent days Nile Tilapia is one of the most common species in aquaculture because it is very easy to maintain and observable in an experimental setting also it involves toxicological research [(Hamed et al., 2021)](https://paperpile.com/c/3E1xYb/yIMrV). According to Arulkumar et al., increasing research on mangrove plant extracts for use in aquaculture is suitable and eco-friendly[(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi & Department of Public Health Dentistry, 2021)](https://paperpile.com/c/3E1xYb/3Nafb+Vdqsf+HVJJl). These plant extracts may help reduce the pressure to generate antibiotic resistance through selection [(San Chitta raj & Arthanari, 2023)](https://paperpile.com/c/3E1xYb/xsD3x). The cytotoxic effects of natural extracts on erythrocytes must be investigated in order to better understand their possible toxicological consequences and therapeutic benefits [(Macrì et al., 2020)](https://paperpile.com/c/3E1xYb/hBq89); [(Fimognari et al., 2012)](https://paperpile.com/c/3E1xYb/qRuSR)). *A. marina*, often known as the grey mangrove, is a species known for its ecological importance and diverse range of bioactive chemicals with antibacterial, anti-inflammatory and antioxidant activities [(Al-Mur, 2021)](https://paperpile.com/c/3E1xYb/mug9m)[(Yassien et al., 2021)](https://paperpile.com/c/3E1xYb/Dr2Y) [(Yassien et al., 2021)](https://paperpile.com/c/3E1xYb/Dr2Y). *A. marina* contains several bioactive compounds, including phenolics, flavonoids, alkaloids, terpenoids, tannins, and sulfur-containing compounds, which can act as toxicants [(Samal et al., 2024)](https://paperpile.com/c/3E1xYb/eoFuP)[(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/3E1xYb/pW3su+G4ftv)). This study investigates the cytotoxicity of *A. marina* crude extract on the erythrocytes of *Oreochromis niloticus*, a freshwater fish species important in aquaculture.

# Materials and Methods

*Avicennia marina* leaves were collected from the Kalpakkam mangrove area in Chennai, Tamil Nadu. The samples were identified by Dr. Pitchiah Sivaperumal and verified through manual identification. Voucher specimens were deposited at the Centre for Marine and Aquatic Research, under Specimen ID No. SIMATS/CMAR/MG/AM-01. The collected leaves were first washed with running tap water, followed by rinsing with distilled water. After thorough cleansing, they were shade-dried at room temperature for two weeks. The dried leaves were then ground into a fine powder. For extract preparation, 10 g of powdered leaves were mixed with 100 ml of 70% ethanol, kept in an orbital shaker for 2 days. After that, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4°C in a sealed bottle for further analysis [(Sobuj et al., 2021)](https://paperpile.com/c/3E1xYb/9drYY).The ethanol extract of the mangrove species *Avicennia marina* was screened for preliminary phytochemical analysis using standard procedures with minor modifications. The screening focused on detecting the presence of saponins, alkaloids, steroids, flavonoids, tannins, terpenoids, and phenolic compounds [(Gomes & Mostowy, 2020)](https://paperpile.com/c/3E1xYb/Vx0lJ); [(Indriaty et al., 2023)](https://paperpile.com/c/3E1xYb/YzQk1).

## Characterization of crude extract

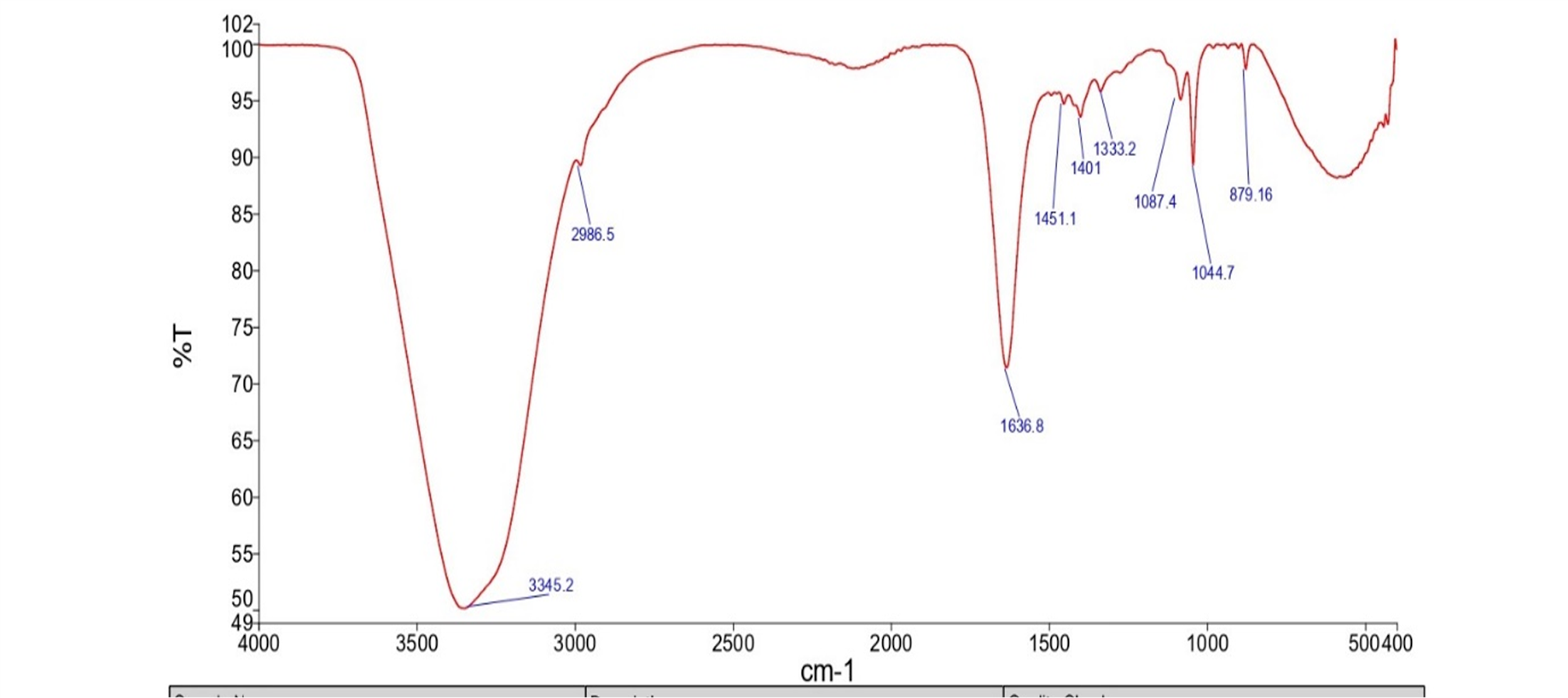
FT-IR analysis was used to describe the samples of extracted mangrove plants. An IR spectrophotometer (PerkinElmer model 297IR) was used to record the crude extract's IR spectrum. A portion of the material was blended with 99 components of dehydrated potassium bromide and examined at a rate of 1 μm/min over a 600–4000 cm⁻¹ range.

## Biological activity

The disc diffusion method was used to evaluate the mangrove crude extract's antibacterial activity. Tetracycline was used as the positive control in the assay, and discs with a diameter of 6 mm were used. Samples of plant extract at different doses (50 and 100 μg/mL) were also evaluated. After that, the agar plates were incubated for a day at 37ºC. The zone of inhibition against the pathogens was used to evaluate the results; a larger zone indicated considerable antibacterial activity. By measuring the diameter of the suppressed pathogenic growth zone, the antibacterial activity against the oral pathogens was calculated [(Reddy et al., 2023)](https://paperpile.com/c/3E1xYb/NwLOr).Sixty Nile tilapia healthy seeds were obtained from the local fish farm, Chennai. The fish were acclimatized for 2 weeks before randomly distributing 60 Nile tilapia with the length of 1-3cm stocked in a glass aquarium tank (30 x 16 x 14 cm). Throughout the trial, the water quality was monitored, and the physico-chemical properties were maintained within the capable ranges of Nile tilapia (temperature 26.1± 0.39ºC; dissolved oxygen 5.64 ± 0.22 mg/L; pH 6.9 ±0.39). During both the acclimation and trial periods, the fish were exposed to a 12-h light and 12-h dark photoperiod. A total of 30% of water was exchanged with freshwater, and uneaten food was removed by siphoning daily. Fish were fed with the experimental diets at 0.5% of their body weight twice a day for a period of 2 weeks. Fish were divided into four groups: a control group (no extract) and two treatment groups exposed to 250 and 100 mg/L of the extract for 96 hours. Behavioral changes, survival rates, and stress responses were monitored daily. Data were statistically analyzed using ANOVA, with significance set at *p* < 0.05.After 96 hours of exposure to *A. marina* extract, blood samples were collected from five randomly selected fish per treatment group for hematological and cytotoxic analysis. Fish were first anesthetized using clove oil (50 mg/L) to minimize stress and ensure safe handling. Once immobilized, blood was drawn from the caudal vein using a sterile 1 mL syringe with a 23G needle. Approximately 0.5 mL of blood was collected per fish. The collected blood was immediately transferred into two different tubes: EDTA-coated tubes for hematological analysis and heparinized tubes for further biochemical studies. For cytotoxicity assessment, blood smears were prepared on clean glass slides, air-dried, and stained with Giemsa stain for erythrocyte morphology and nuclear abnormality examination under a light microscope. All procedures were performed under aseptic conditions to prevent contamination. Micronuclei were defined as non-refractive, circular or ovoid chromatin structures that were less than one-third of the major nucleus and had the same staining and focusing pattern as the main nucleus. Five categories were created for nuclear abnormalities (NAs) in erythrocytes that weren't micronuclei. In brief, cells having two nuclei were referred to as binucleated. BL contained chromatin and had a comparatively modest nuclear membrane evagination. Nuclei having several lobes and evaginations greater than those in the BL were categorized as LB. NT was assigned to nuclei that had voids or vacuoles that extended a significant distance into the nucleus [(Cavaş & Ergene-Gözükara, 2005)](https://paperpile.com/c/3E1xYb/eeMeY).

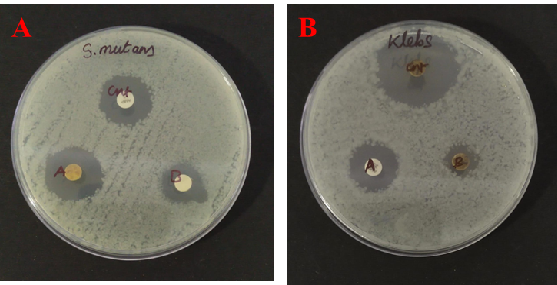
# Results

Phytochemical analysis of *A. marina* ethanol crude extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, steroids, tannins, anthraquinones, and terpenoids. However, proteins and saponins were absent. These bioactive compounds contribute to the extract's potential antioxidant, antimicrobial, and medicinal properties. Phytochemical analysis of *A. marina* leaves reveals a diverse array of bioactive compounds with potential medicinal applications. Overall, the results corroborate existing literature while highlighting slight compositional differences that could be attributed to extraction conditions or regional variations in plant chemistry. The FTIR spectrum of *Avicennia marina* crude extract reveals a wide range of functional groups, indicating the presence of various bioactive compounds (Figure 1). The medium intensity absorption at 3345.2 cm⁻¹ corresponds to N-H stretching vibrations of secondary amines, indicating the presence of alkaloids. The peak at 2986.5 cm⁻¹ is indicative of C-H stretching in alkanes, indicating the presence of saturated hydrocarbons. A significant peak at 1636.8 cm⁻¹ signifies C=C stretching in conjugated alkenes, referring to the presence of unsaturated compounds such as flavonoids. The strong absorption at 1401 cm⁻¹ is characteristic of S=O stretching in sulfonyl chlorides; however, this could also indicate other sulfur-containing compounds. The medium intensity band at 1333.2 cm⁻¹ corresponds to O-H bending vibrations in alcohols. The strong peak at 1087.4 cm⁻¹ is due to C-O stretching in secondary alcohols. The broad and strong absorption at 1044.7 cm⁻¹ suggests CO-O-CO stretching in anhydrides. Finally, the strong peak at 879.16 cm⁻¹ reflects C-H bending in 1,3-disubstituted aromatic compounds, typical of phenolic compounds. The FTIR analysis demonstrates that *Avicennia marina* crude extract contains a rich mixture of chemical components, including alkaloids, terpenoids, flavonoids, phenolics, fatty acids, and sulfur-containing compounds, highlighting its potential for various pharmacological applications.



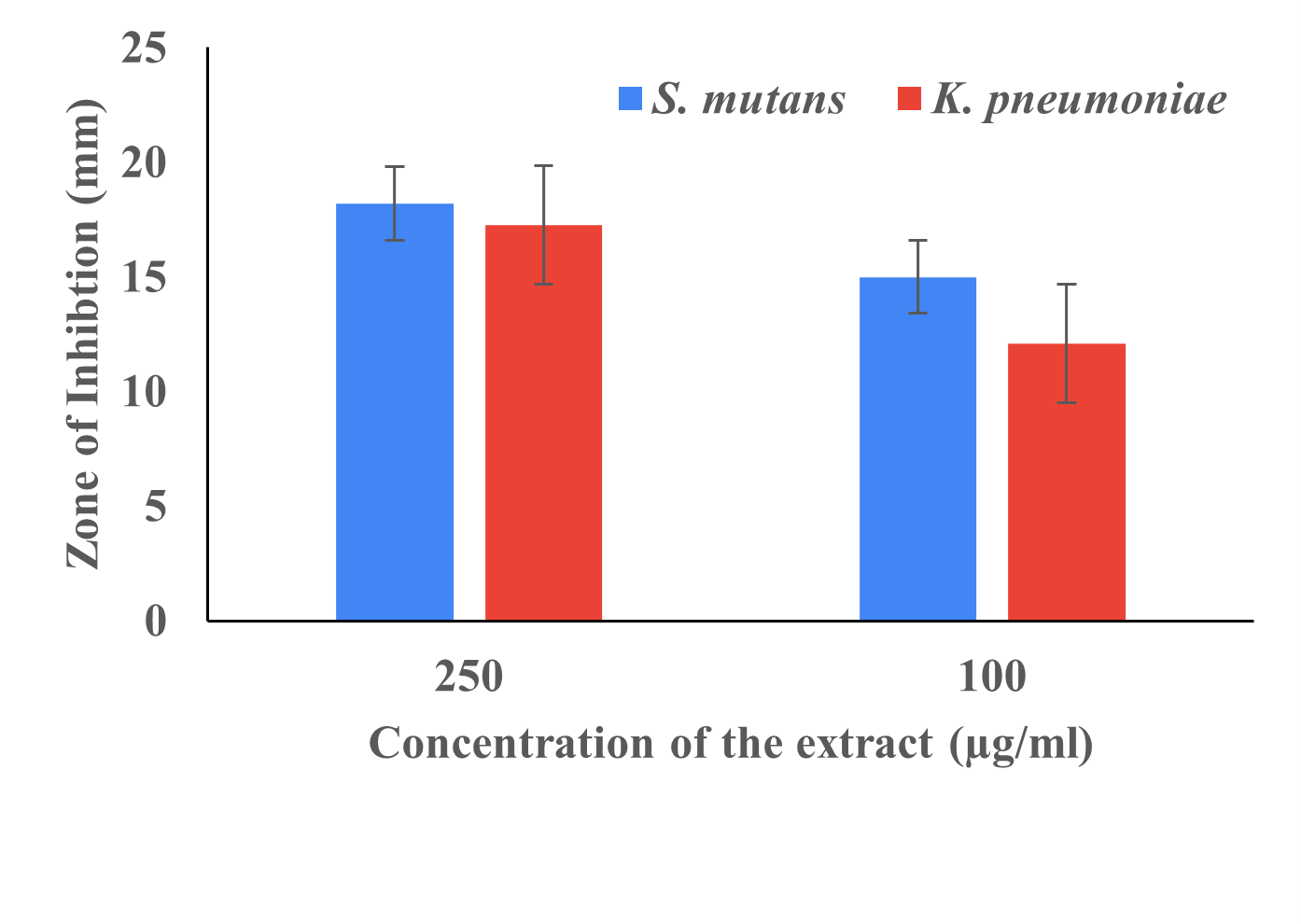
**Figure 1.** shows the FT-IR spectrum of *Avicennia marina* crude extract

The antibacterial activity of *Avicennia marina* crude extracts against *Streptococcus mutans* and *Klebsiella sp*. was determined using the disc diffusion method. For *Streptococcus mutans*, the control exhibited a zone 19 ± 0.12 mm, although Concentration A (250 µg/ml) showed a zone of inhibition of 18 ± 0.44 mm, and Concentration B (100 µg/ml) exhibited a zone of 15 ± 0.45 mm. For *Klebsiella sp*., the control exhibited a zone of 26 ± 0.25 mm, while Concentration A (250 µg/ml) showed a zone of 17 ± 0.25 mm, and concentration B (100 µg/ml) exhibited a zone of inhibition of 12 ± 0.06 mm. The results indicate that *Avicennia marina* crude extracts possess antibacterial activity against both *S. mutans* and *Klebsiella sp.,* with effectiveness increasing at higher concentrations (Figure 2 and 3). However, the extracts are more effective against *S. mutans* than *Klebsiella sp*.



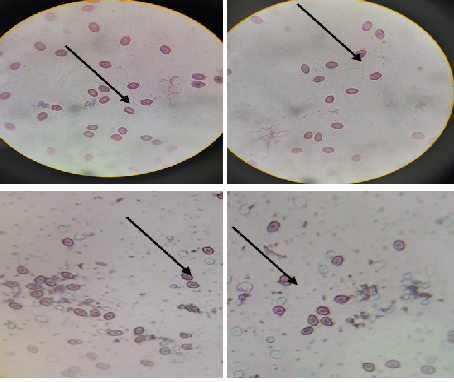
**Figure 2.** The zone of inhibition against oral pathogens: A. *Streptococcus mutans*; B. *Klebsiella pneumoniae*

These findings suggest that while *A. marina* could be a potential source of antibacterial agents, further purification and concentration may be necessary to enhance its efficacy. Antibacterial activity was analyzed using ANOVA with Tukey’s post hoc test. Results were expressed as mean ± SD, and a p-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS.



**Figure 3.** The graph represents the zone of inhibition

In this study, we assessed the micronucleus frequencies in the erythrocytes of *O. niloticus* (Nile tilapia) following exposure to mangrove crude extract, along with negative and positive controls (Figure 4). After 12 hours of post-treatment, no mortality or visible macroscopic lesions were observed in any of the fish across all treatment groups, including controls. We performed a detailed examination of blood smears from *O. niloticus* exposed to the mangrove crude extract, specifically at 72-hour intervals.



**Figure 4. The figure represents the micronuclei and nuclear abnormalities in *O. niloticus* fish exposed to mangrove crude extract**

Our observations revealed the presence of micronuclei and various other nuclear abnormalities in the erythrocytes of fish subjected to the mangrove extract (Nikalje et al., 2024) (Chehelgerdi et al., 2023). These findings suggest that exposure to the mangrove crude extract induces genotoxic effects in *O. niloticus*, as evidenced by the increase in nuclear abnormalities over the exposure period. Fish micronuclei (MN) and nuclear abnormalities (NA) assessment has become a useful method for determining the genotoxicity of environmental contaminants.

# Discussion

The phytochemical analysis of *A. marina* ethanol crude extract in this study aligns with previous research, confirming the presence of key bioactive compounds such as alkaloids, flavonoids, terpenoids, steroids, and tannins, which have been widely reported for their medicinal properties. However, while prior studies have also highlighted the presence of phenols and saponins, the current findings indicate the absence of saponins and proteins, suggesting possible variations due to extraction methods, environmental factors, or plant maturity [(Rozirwan et al., 2022)](https://paperpile.com/c/3E1xYb/M4dkE). Additionally, GC-MS analysis in other studies has identified compounds with anticancer, antimicrobial, anti-inflammatory, and antioxidant properties, supporting the therapeutic potential of these phytochemicals. FT-IR is an effective method for analyzing mangrove crude extracts by revealing functional groups and phytochemical substances. Functional groups such as phenols, alkanes, amides, and carboxylic acids have been detected in studies of many mangrove species, including *Rhizophora apiculata* and *Rhizophora mucronata* [(Othman et al., 2020)](https://paperpile.com/c/3E1xYb/ThbfD); [(Duraipandian et al., 2022)](https://paperpile.com/c/3E1xYb/vY6Ak). These chemicals help plants retain their therapeutic characteristics and adapt to their surroundings. The FT-IR study of mangrove extracts revealed the presence of hydroxyl, aromatic C=C, and carboxylic acid groups. Furthermore, UV-Vis spectroscopy revealed the presence of phenolics and flavonoids in mangrove extracts [(Saragih et al., 2020)](https://paperpile.com/c/3E1xYb/yiGi0). The identification of these functional groups and molecules contributes to understanding the therapeutic potential of mangrove extracts and their prospective applications in different industries, including medicine and food [(Verma et al., 2023)](https://paperpile.com/c/3E1xYb/q2pQg). These chemicals contribute to the antibacterial activities of mangrove extracts, which have demonstrated activity against pathogens such as Proteus mirabilis. In cementitious materials, FTIR may detect important bonds such as Si-O, -OH, H-O-H, C-O, and S-O, with significant bands signifying carbonation, calcium carbonate, hydroxylation, and silicate organization [(Yusuf, 2023)](https://paperpile.com/c/3E1xYb/ShlCw). Elemental displacement, temperature, pH, and activator concentration all have an effect on FTIR spectra. This technique may be used for both destructive and non-destructive testing, making it useful for a variety of research applications such as material characterization and bioactive component identification from mangrove ecosystems [(Lin et al., 2023)](https://paperpile.com/c/3E1xYb/K3GTh). Mangrove plants have demonstrated promising antibacterial action against a variety of diseases. Aqueous extracts of Indian Sundarban mangroves showed inhibitory zones ranging from 13 to 20 mm against human and plant diseases [(Sett et al., 2025)](https://paperpile.com/c/3E1xYb/YIKyL). Root extracts of *Rhizophora apiculata* from Bali showed high efficacy against Gram-positive bacteria, with chloroform extracts producing a 19.83mm inhibitory zone against *Streptococcus mutans* [(Wijaya & Indraningrat, 2021)](https://paperpile.com/c/3E1xYb/Qog1i). Strong antibacterial activity was shown by the natural products found in *A. officinalis* against a range of bacteria, including the gram-negative strains of *Klebsiella pneumonia* (22 mm) and *Enterobacter aerohenes* (14 mm), as well as the gram-positive strains of *Streptococcus mutans* (19 mm), *Bacillus subtilis* (17 mm), and *Pseudomonas aeruginosa* (16 mm) [(Lalitha et al., 2021)](https://paperpile.com/c/3E1xYb/VR00O). According to the study [(I et al., 2023)](https://paperpile.com/c/3E1xYb/vgO0I) reported the antibacterial efficacy against *S. mutans*, *S. aureus*, and *Klebsiella sp*., and the inhibition zones of *S. aureus*, *S. mutans*, and *Klebsiella sp*. at 100 µg/mL of ZnO NPs were 7.5 ± 0.2, 9.5 ± 0.5, and 9.5 ± 1.2 mm, respectively. Similarly, for each of the different bacterial strains, the inhibition zones were determined at 75 µg/mL and found to be 7 ± 0.25, 9 ± 1, and 7.5 ± 0.5 mm. Research has indicated that exposure to a range of pollutants can cause MN and NA in different fish species. These contaminants include pesticides, heavy metals [(de Mendonça Francisco et al., 2023)](https://paperpile.com/c/3E1xYb/z2PQR), and microplastics [(Canedo et al., 2021)](https://paperpile.com/c/3E1xYb/jirYr). Among these anomalies are binucleated cells, blebbed nuclei, and notched nuclei [(Chakraborty et al., 2024)](https://paperpile.com/c/3E1xYb/moBic). According to the results, [(Hamed et al., 2021)](https://paperpile.com/c/3E1xYb/yIMrV) reported the MPs-exposed groups exhibited a variety of nuclear abnormalities, such as hemolyzed, notched, lobed, blebbed, and micronucleated erythrocytes. After the recovery period, there was still a discernible increase in the percentage of eryptosis, poikilocytotic cells, and nuclear abnormalities in RBCs between the MPs-exposed groups and the control group. The findings reveal alarming information on MP toxicity in tilapia. In 2020, [(Santana et al., 2020)](https://paperpile.com/c/3E1xYb/9Ccw7) reported the minimal frequencies of micronuclei and other nuclear anomalies are seen in catfish in Brazilian tropical estuaries, which may indicate minimal levels of pollution exposure and adaptive responses to contamination. Overall, the study demonstrates that while the mangrove crude extract does not cause immediate mortality or macroscopic lesions in *O. niloticus*, it does induce significant genotoxic effects, as seen in the increased frequency of micronuclei and other nuclear abnormalities in erythrocytes over time.

# Conclusion

In the current findings, *Avicennia marina* crude extract displays a diverse array of bioactive compounds as identified by FT-IR spectroscopy, indicating its potential for various pharmacological applications. The extract demonstrates notable antibacterial activity, especially against *Streptococcus mutans*, though its effectiveness against *Klebsiella sp*. is relatively lower. This suggests that while the extract holds promise as an antibacterial agent, further optimization may be needed to enhance its potency. Additionally, the observed genotoxic effects in *Oreochromis niloticus* highlight potential risks associated with the extract, necessitating further research to evaluate its safety profile. Overall, *Avicennia marina* presents valuable antimicrobial potential but requires careful consideration of its genotoxicity for safe therapeutic use.

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