Antibacterial Potential of Rhizophora Apiculata Ethanolic Extract: In Vitro Screening and Zebrafish (Danio rerio) Larvae Toxicity Evaluation

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**Abstract:** The present study investigated the antimicrobial potential and larval toxicity of the ethanol extract of *Rhizophora apiculata* leaves. The crude extract (yield: 2.47 g from 30 g of leaf powder) was subjected to phytochemical screening, FT-IR analysis, antibacterial assessment, and toxicity evaluation on *Danio rerio* (zebrafish) larvae. Phytochemical analysis confirmed the presence of secondary metabolites, suggesting significant bioactive potential. FT-IR spectroscopy revealed characteristic functional groups, including polyphenols, glycosidic bonds, and sulfated polysaccharides, supporting the medicinal properties. Antibacterial activity was assessed, with the highest concentration (100 µg/ml) demonstrating the greatest efficacy, producing inhibition zones of 13 mm against *Vibrio alginolyticus* and 11 mm against *Vibrio vulnificus*. Toxicity assessment revealed a dose-dependent effect, with 20–40 µg/mL showing minimal toxicity 90–100% survival, while 80–100 µg/mL caused significant mortality 70–80% survival and abnormalities like pericardial edema and erratic swimming. These findings highlight the bioactive potential of *R. apiculata* extract while emphasizing its toxicity at elevated concentrations, warranting further investigation for therapeutic applications.

**Keywords:** *Rhizophora apiculata*; Phytochemicals; FT-IR; antimicrobial activity; zebrafish toxicity; bioactive compounds

# Introduction

Mangrove plants, particularly Rhizophora apiculata, play a crucial role in coastal ecosystems by stabilizing shorelines, providing habitats for marine life, and contributing to nutrient cycling [(Akhrianti et al., 2025)](https://paperpile.com/c/PTD8xP/HXg1)[(Padarthi et al. 2023; Ajay et al. 2023; Chokkattu et al. 2023)](https://paperpile.com/c/MudIdv/Biyp+pveV+SIDp)[(Sindhu et al. 2023; Dharman et al. 2023; Sreenivasagan et al. 2023)](https://paperpile.com/c/MudIdv/2bDT+Tn0g+xMG4)[(Shenoy and Maiti 2023; Ramakrishnan et al. 2023; Sindhu et al. 2023)](https://paperpile.com/c/MudIdv/ugZg+feI9+5Pxr). Beyond their ecological importance, mangroves have been traditionally used in folk medicine due to their rich bioactive secondary metabolites, including flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds [(Sadeer et al., 2023)](https://paperpile.com/c/PTD8xP/3Uef)[(Varghese et al. 2023; Kasabwala et al. 2021; Rajeshkumar and Lakshmi 2021)](https://paperpile.com/c/MudIdv/O40P+qWHi+HQhQ)[(Tiwari and Jain 2021; Murugesan 2021](https://paperpile.com/c/MudIdv/kqjT+mPKw+wpEm)[; Keerthana and Ramesh 2021; Subramanian et al. 2021)](https://paperpile.com/c/MudIdv/kqjT+mPKw+wpEm+wj20)[(Pranati et al. 2021; Sakthi and Department of Public Healt..](https://paperpile.com/c/MudIdv/RMQw+uqPK+s04I)[(Kumar and Ramesh 2021; G. and Ganapathy 2022](https://paperpile.com/c/MudIdv/PV5r+krfm)). These phytochemicals exhibit a broad spectrum of biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and even anticancer properties, making mangrove plants a valuable resource for drug discovery and natural product research ([(Sinha et al., 2024)](https://paperpile.com/c/PTD8xP/lZKZ); [(Karunakaran et al., 2023)](https://paperpile.com/c/PTD8xP/70ZO)). Among the various extraction methods, ethanolic extraction is widely employed due to their ability to dissolve both polar and moderately non-polar compounds, thereby maximizing the yield of diverse phytochemicals ([(Goti & Dasgupta, 2023)](https://paperpile.com/c/PTD8xP/n5dY); [(Pattapulavar et al., 2025)](https://paperpile.com/c/PTD8xP/TBGm). Previous studies have demonstrated that ethanolic crude extracts from R. apiculata possess significant bioactive potential, including antibacterial activity against pathogenic strains and free radical scavenging capabilities [(Acharya et al., 2023)](https://paperpile.com/c/PTD8xP/2him). However, despite their therapeutic potential, the toxicity of mangrove extract, particularly in aquatic organisms, remains poorly understood [(Wang et al., 2025)](https://paperpile.com/c/PTD8xP/x00z). Since mangrove ecosystems serve as nurseries for numerous aquatic species [(Arceo-Carranza et al., 2021)](https://paperpile.com/c/PTD8xP/ne4j), evaluating the ecotoxicological effects of mangrove-derived compounds is crucial to ensuring environmental safety.The zebrafish (Danio rerio) has emerged as a premier model for toxicity testing due to its genetic similarity to humans, rapid larval development, and physiological transparency, making it an ideal model for assessing the ecotoxicological impact of bioactive compounds [(Chowdhury & Saikia, 2022)](https://paperpile.com/c/PTD8xP/zWpn); [(Wimalagunarathna & Gunathilake, 2025)](https://paperpile.com/c/PTD8xP/KuCZ). Zebrafish larvae toxicity studies provide valuable insights into the developmental, physiological, and behavioral effects of these extracts [(Rosa et al., 2022)](https://paperpile.com/c/PTD8xP/XdZ3); [(Licitra et al., 2021)](https://paperpile.com/c/PTD8xP/6eJp). Exposure to varying concentrations can result in developmental abnormalities such as delayed growth, pericardial edema, and spinal deformities [(Chandra et al., 2024)](https://paperpile.com/c/PTD8xP/kNEQ), while acute toxicity assessments help determine lethal concentrations (LC50) and sub-lethal effects on survival and metabolic function [(Xiong et al., 2022)](https://paperpile.com/c/PTD8xP/9bna). Behavioral changes, including altered locomotion and response to stimuli, indicate potential neurotoxicity, while oxidative stress markers and gene expression studies reveal underlying molecular mechanisms [(Licitra et al., 2021)](https://paperpile.com/c/PTD8xP/6eJp). Additionally, histological analysis of zebrafish gill and muscle tissues can provide further evidence of toxicity, as exposure to mangrove crude extracts may cause gill lamellar fusion, epithelial hyperplasia, and mucus cell proliferation, which can impair respiration and osmoregulation [(Chahardehi et al., 2020)](https://paperpile.com/c/PTD8xP/nDOM); [(Azadikhah et al., 2023)](https://paperpile.com/c/PTD8xP/PoEh). This research aims to evaluate the importance of establishing safe thresholds, promoting environmental sustainability, and ensuring the responsible utilization of mangrove bioresources in pharmaceuticals and aquaculture.

# Materials and methods

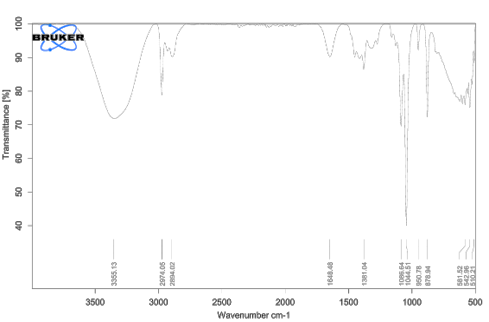
The leaves of *Rhizophora apiculata* were collected and thoroughly washed with tap water to remove surface contaminants, and air-dried in the shade. The specimen was documented under the reference code 

**Figure 1.** Preparation of *R. apiculata* crude extract

The dried leaves were then finely ground into a powder, and 30 g of the powdered sample was mixed with 300 mL of 70% ethanol. The mixture was heated to a temperature range of 60–80°C using a heating mantle to facilitate extraction, followed by filtration through Whatman No. 1 filter paper (Figure 1). The resulting filtrate was collected and stored in a refrigerator for further experimental analysis [(Kamala et al., 2024)](https://paperpile.com/c/PTD8xP/Jhnd). The ethanolic leaf extract of Rhizophora apiculata was analyzed for various phytoconstituents using standardized phytochemical screening methods as described by [(Muthulingam & Chaithanya, 2018)](https://paperpile.com/c/PTD8xP/SC7m). The FTIR spectrum was utilized to analyze the chemical bonds and functional groups present in the components of the Rhizophora apiculata crude extract, using peak value ratios for identification. A total of 10 mg of dried extract powder, obtained from leaf samples extracted with ethanol solvent, was mixed with 100 mg of KBr to form a pellet for preparing translucent sample discs. The FTIR analysis was performed using a Bruker Alpha II spectrometer to identify potential functional groups in the R. apiculata crude extract [(Sonbol et al., 2021)](https://paperpile.com/c/PTD8xP/AbTg).The antibacterial activity of Rhizophora apiculata extract against the selected fish pathogen was evaluated using the disc diffusion method. Nutrient agar was poured into sterile Petri plates and allowed to solidify, followed by inoculation with the fish pathogen using a sterile cotton swab to ensure uniform distribution. Sterile discs saturated with the R. apiculata extract and then placed onto the inoculated plates. The plates were incubated at room temperature for 12–24 hours, and antibacterial activity was assessed by measuring the diameter of the clear inhibition zones surrounding the discs. The experiment was performed with slight modifications to the method described by [(Thiraviyam et al., 2025)](https://paperpile.com/c/PTD8xP/Tj17).Adult wild-type zebrafish (Danio rerio) will be maintained in a glass fish tank at 28 ± 1°C with a 14:10-hour light-dark cycle. For breeding, males and females will be placed in spawning tanks in a 1:2 ratio overnight. Eggs will be collected within one-hour post-fertilization (hpf) and rinsed with embryo medium (E3 medium: 5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄). Fertilized eggs will be examined under a stereomicroscope, and only viable embryos will be selected for the experiment, while unfertilized or dead eggs will be discarded.The ethanolic crude extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution, ensuring the final DMSO concentration in exposure media does not exceed 0.1% to avoid solvent toxicity. Serial dilutions will then be prepared in E3 medium to achieve desired test concentrations of (20, 40, 60, 80 and 100µg/mL). A negative control (E3 medium only) and a solvent control (0.1% DMSO in E3) will be included to assess toxicity effects. Healthy 24 hpf (hours post fertilization) zebrafish larvae will be selected and distributed into 24-well plates (10 larvae per well in 2 mL of exposure medium). Each concentration group will be tested in triplicate to ensure reproducibility. Larvae will be exposed to the extract for 96 hours at 28°C, with daily renewal of the test solution to maintain consistent exposure conditions. Observations will be recorded at 24-hour intervals using Magnus Binocular Microscope. The toxicity of the ethanolic Rhizophora apiculata extract on zebrafish larvae will be evaluated through multiple endpoints over a 96-hour exposure period. Mortality will be recorded every 24 hours by counting larvae lacking heartbeat or movement. Hatching success will be assessed at 48, 72, and 96 hpf to determine developmental delays. Surviving larvae will be examined for morphological abnormalities, including yolk sac edema, spinal deformities, fin malformations, and swim bladder defects. For this study, ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) The experimental results were expressed as Mean ± SD and further ANOVA test at 5% was performed to determine its significance (SPSS 16.0).

# Results

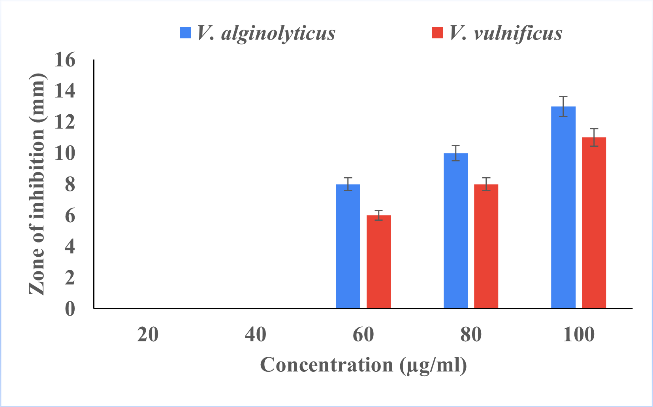
In the present study, the ethanol extract of *Rhizophora apiculata* was prepared and evaluated for its antimicrobial potential and larval toxicity. A total of 30 g of *R. apiculata* leaf powder was soaked in 300 mL of 70% ethanol for 48 hours. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was subjected to solvent evaporation in a water bath at 55°C. The resulting crude extract was carefully scraped, yielding 2.47 g from the initial 30 g of leaf sample. The crude extract was stored at 4°C for further biological analysis.The phytochemical analysis of *Rhizophora apiculata* ethanolic extract confirmed the presence of alkaloids, tannins, saponins, phenols, terpenoids, and glycosides, steroids, while flavonoids were absent. aliphatic C-H stretching vibrations at 2974 cm⁻¹ suggests lipid-derived functional groups [(Seaton et al., 2024)](https://paperpile.com/c/PTD8xP/BAr1).



**Figure 2.** shows the FT-IR Spectrum of *R. apiculata* crude extract

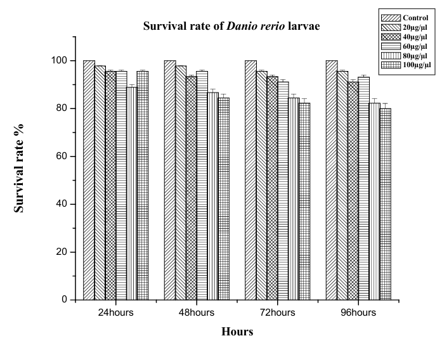
These bioactive compounds exhibit antimicrobial, antioxidant, and anti-inflammatory properties, enhancing the extract’s therapeutic potential. Phenols and terpenoids contribute to its antioxidant and anticancer activities, while saponins and glycosides suggest possible haemolytic effects. The Fourier-transform infrared (FT-IR) spectrum of Rhizophora apiculata revealed characteristic absorption bands corresponding to its diverse bioactive constituents. A broad absorption peak at 3355 cm⁻¹ is attributed to O-H and N-H stretching vibrations, indicative of polyphenols and potential amine-containing compounds with reference to [(Faris et al., 2024)](https://paperpile.com/c/PTD8xP/Odww). The presence of

A distinct C=C stretching band at 1648 cm⁻¹ is characteristic of flavonoid structures, confirming the presence of aromatic compounds [(Arceo-Gómez et al., 2024)](https://paperpile.com/c/PTD8xP/L80x). A strong absorption at 1381 cm⁻¹, corresponding to S=O stretching, suggests the occurrence of sulfated polysaccharides. The distinct bands at 1086 and 1044 cm⁻¹ correspond to C-O stretching vibrations, likely associated with glycosidic bonds in carbohydrates or ether linkages (Figure 2). In 2023,[(Mitra et al., 2023)](https://paperpile.com/c/PTD8xP/epo1) reported the antibacterial activity of plant-derived phenolic compounds is largely attributed to the presence of hydroxyl groups. These functional groups play a crucial role in microbial inhibition, as increased hydroxylation enhances their antibacterial potency while also influencing their toxicity. Additionally, the absorption peak at 878 cm⁻¹, assigned to C-H bending, indicates the presence of 1,3-disubstituted aromatic systems, which may be derived from lignin or tannins. Additionally, an absorption band at 2894 cm⁻¹, attributed to amine salt vibrations, suggests the presence of nitrogen-containing compounds, potentially alkaloids. These spectrum findings are consistent with the known phytochemical composition of *R. apiculata*, which is rich in polyphenols, polysaccharides, and lignin, further supporting its medicinal and structural applications.The antibacterial activity of *R. apiculata* extract showed a significant variation (*P < 0.07*) between bacterial pathogens with remarkably pronounced antagonistic activity against various fish pathogens.The antibacterial activity of the crude extract was evaluated against *Vibrio alginolyticus* and *Vibrio vulnificus* at varying concentrations (20, 40, 60, 80, and 100 µg/ml). The results indicate a concentration-dependent inhibitory effect on both bacterial strains. At lower concentrations (20 and 40 µg/ml), no inhibition zones were observed, suggesting that these concentrations were insufficient to use antibacterial effects.



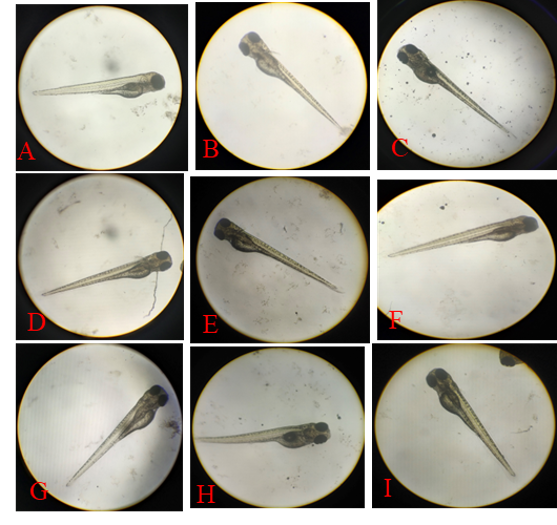
**Figure 3**. shows the Zone of inhibition against fish pathogens

However, at 60 µg/ml, inhibition zones of 8 mm for *V. alginolyticus* and 6 mm for *V. vulnificus* were recorded, indicating the onset of antibacterial activity (Figure 3). With increasing concentrations, the inhibitory effect became more pronounced. At 80 µg/ml, inhibition zones increased to 10 mm and 8 mm for *V. alginolyticus* and *V. vulnificus*, respectively. The highest concentration (100 µg/ml) exhibited the strongest antibacterial activity, with inhibition zones of 13 mm for *V. alginolyticus* and 11 mm for *V. vulnificus*. These findings underscore the potential of mangrove-derived compounds in addressing antibiotic resistance in aquaculture and improving fish health. These findings suggest that the test compound exhibits dose-dependent antibacterial activity, with *V. alginolyticus* being slightly more susceptible than *V. vulnificus*. The zebrafish larvae acute toxicity (FLT) test is a more convenient and efficient alternative to the fish acute toxicity test, with comparable sensitivity and easier implementation (Xiong et al. 2022). The survival rate of *Danio rerio* larvae exposed to different concentrations of the test compound (20, 40, 60, 80, and 100 µg/ml) was monitored over 96 hours (Figure 4). The control group consistently maintained a high survival rate 100% throughout the experiment, indicating no external environmental stressors(Saadh et al., 2024). In contrast, larvae exposed to increasing concentrations of the compound exhibited a dose-dependent decline in survival(Almatrafi et al., 2024). At lower concentrations (20 and 40 µg/ml), survival remained relatively high 90–100% over 96 hours, suggesting minimal toxicity. However, at 60 µg/ml, a slight reduction in survival was observed, particularly after 72 and 96 hours. A more pronounced decrease in survival was evident at 80 µg/ml and 100 µg/ml, with mortality increasing over time. By 96 hours, the highest concentration (100 µg/ml) showed a significant drop in survival 70–80%, suggesting increased toxicity with prolonged exposure.



**Figure 4.** indicates the survival rate of zebrafish larvae exposed to crude extract

Additionally, larvae exposed to 100 µg/ml exhibited distinct signs of physiological stress, including pericardial edema, inflammation, abnormal swimming, fin damage, and increased mortality, indicating severe toxic effects at this concentration (Figure 5).



**Figure 5.** (a)-(f) shows the microscopic images of Zebrafish larvae exposed to mangrove extract

Overall, these findings suggest that while lower concentrations exhibit minimal toxicity, exposure to 100 µg/ml induces severe developmental abnormalities and mortality, highlighting potential risks at elevated concentrations.

# Discussion

Consistent with previous studies [(Laith, 2021)](https://paperpile.com/c/PTD8xP/7Ymx), phytochemical analysis of *R. apiculata* confirmed the presence of terpenoids, saponins, alkaloids, tannins, and steroids, although flavonoids were not detected among the mangrove-derived bioactive compounds. The ethanolic leaf extract of *R. apiculata* revealed abundant flavonoids and tannins, aligning with [(Muthulingam & Chaithanya, 2018)](https://paperpile.com/c/PTD8xP/SC7m), suggesting strong antioxidant/antimicrobial potential.*Sonneratia alba* exhibited strong antimicrobial effects against *Salmonella arizonae* in both in vitro and in vivo studies [(Limbago et al., 2021)](https://paperpile.com/c/PTD8xP/mYd5), while *Bruguiera gymnorrhiza* root extract showed notable efficacy against *Escherichia coli* and *Staphylococcus aureus* [(“Antibacterial Screening of Mangrove Extract Library Showed Potential Activity against Escherichia Coli and Staphylococcus Aureus,” 2020)](https://paperpile.com/c/PTD8xP/EZrj). *Excoecaria agallocha* extracts effectively inhibited *Aeromonas hydrophila*, a common freshwater fish pathogen [(Mulia et al., 2023)](https://paperpile.com/c/PTD8xP/aMik), and Rhizophora apiculata leaf extracts displayed antibacterial activity against *Streptococcus agalactiae* and *E. coli* [(Laith, 2021)](https://paperpile.com/c/PTD8xP/7Ymx). The antimicrobial properties of these extracts are attributed to bioactive compounds such as flavonoids, saponins, tannins, and terpenoids [(Limbago et al., 2021)](https://paperpile.com/c/PTD8xP/mYd5); [(Laith, 2021)](https://paperpile.com/c/PTD8xP/7Ymx). In 2023, [(Yoswaty et al., 2023)](https://paperpile.com/c/PTD8xP/BiQ0) has been reported *Avicennia marina* leaf extracts, rich in phenolics, saponins, and terpenoids, exhibited strong inhibition against *Pseudomonas aeruginosa* but weaker effects against *Aeromonas hydrophila* and *Vibrio alginolyticus*. Similarly, *Acanthus ilicifolius* methanol extracts demonstrated significant antibacterial activity against *A. hydrophila* and other pathogens, along with notable antioxidant properties. Furthermore, treatment with *A. ilicifolius* extracts enhanced the survival rates of *A. hydrophila*-infected *Labeo rohita* fingerlings, suggesting its potential as a natural therapeutic agent in aquaculture [(Sravya et al., 2023)](https://paperpile.com/c/PTD8xP/lAsf). [(Achenbach et al., 2022)](https://paperpile.com/c/PTD8xP/rdfk) highlight the toxicokinetic profiling in zebrafish larvae assays, and also author integrating toxicokinetic assessments will improve the accuracy of zebrafish-based toxicity evaluations for regulatory and environmental studies. Followed by [(Andre C. Grisola & G. Fuentes, 2017)](https://paperpile.com/c/PTD8xP/xfvI) highlighted the potential of mangrove extracts in influencing melanogenesis, with *S. alba* bark extract showing the most significant effects. *S. alba* bark extract showed a dose-dependent effect on embryo pigmentation, with higher concentrations altering melanogenesis.

# Conclusion

From this study, it can be inferred that *R. apiculata* ethanolic leaf extract can be used as an immunostimulant in aquaculture. Additionally, it has the potential to provide resistance against the bacterial pathogen *V. alginolyticus* & *V. vulnificus*. The enhancement in the immune responses was correlated with the improved survivability of *D. rerio* larvae. Further research on elucidating the molecular mechanisms are needed to explore the impact on *R. apiculata* crude extract on the physiological status of other ornamental fishes.

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