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Antagonistic activity of leaf extracts of *Rhizophora mucronata* and *Avicennia officinalis* against *Vibrio cholerae*

V. P. Sruthi[1, 2] and K. R. Sreenath[2*]
[1]Department of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Panangad-682 506, Kerala, India.
[2]ICAR-Central Marine Fisheries Research Institute, Kochi-680 018, Kerala, India.

*Correspondence email: sreenath.ramanathan@icar.gov.in

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Original Article

Abstract

Bacterial diseases cause serious illnesses that may even lead to mortality. In the present study, the antagonistic activity of mangrove leaf extract was tested against the human pathogen. This study aims to evaluate the *in vitro* antibacterial activity of the leaf extract of *Rhizophora mucronata* and *Avicennia officinalis* against the pathogenic bacteria species, *Vibrio cholerae*. We used ethyl acetate, ethanol and aqueous extract of the leaves and the activity was evaluated with the agar well diffusion assay. The pure solvent was used as a control. The results have shown that extracts of both *A. officinalis* and *R. mucronata* in ethyl acetate and ethanol gave inhibition against test pathogens. None of the aqueous extracts showed antagonistic activity. Maximum activity was observed in the ethyl acetate extract of *A. officinalis* (15.6 ± 0.36 mm). The leaf extracts of *A. officinalis* exhibited significantly higher antibacterial activity than *R. mucronata*. Our findings show that the selected mangrove leaf extracts have potential antibacterial activity. Further research needs to be undertaken to extract and identify the potential bioactive compounds causing such biological activity.

Keywords: *Antibacterial activity, plant extracts, growth inhibition, Rhizophora, Avicennia*

Introduction

Mangroves are trees or large shrubs that grow within the intertidal zone of tropical and subtropical regions, and they have particular adaptations to survive in this specific environment (Hogarth, 1999). They provide significant ecosystem services, including carbon sequestration, sustenance livelihood, coastal protection and biodiversity (Macintosh and Ashton, 2002). Mangroves are well adapted to their extreme environmental conditions, especially high salinity, change in sea level, high temperature and anaerobic soils by specific characteristics such as pneumatophores roots, stilt roots, and salt excreting leaves. They comprise one of the most biologically complex ecosystems (Ali *et al*., 2002; Salini, 2014).

The Indian mangroves are diverse, with 125 species out of which 39 are mangroves and 86 are mangrove associates. About 56% of the world's mangrove species occur in India (Kathiresan, 2010). These mangroves are of great ecological importance, and they have socioeconomic significance for the tropical marine biotopes of the region. Kathiresan and Sandilyan (2012) report that 90% of marine organisms spend at least some part of their life in the mangrove ecosystem, and 80% of the global fish catches depend on mangroves. The Kerala coastline is blessed with vast mangrove areas and associated microflora abundantly present in the coastal wetlands. The mangrove diversity of Kerala represents 0.19% of the total mangroves of India. However, they constitute 41% of the true mangrove species in India (George *et al*., 2019). The potential of mangroves as alternative sources of bioactive compounds of medicinal value, in addition to their environmental significance and socioeconomic importance, is well documented (Harcourt, 2018).

Mangroves have popularly been used for medicinal remedies. A previous study has indicated that mangrove leaf extract of *Bruguiera cylindrica* and *Sonneratia caseolaris* showed antibacterial and antioxidant properties (Gawali and Jadhav, 2011). Lim *et al.* (2020) have reported the potential anticancer effect of endophytic fungi isolated from marine plants. *A. ilicifolius* leaf extract possesses free radical scavenging activity (Babu *et al*., 2001). The leaves of *R. mucronata* also contain several phytochemical compounds. As compared with other parts of the plant, alkaloid content is extremely high in the leaves (Nurdiani *et al*., 2012).

V. cholerae is the primary bacteria responsible for causing cholera, a severe diarrheal disease affecting millions globally. The World Health Organization estimates 1.3-4 million cholera cases and 21,000-143,000 deaths annually (Ali *et al*., 2015). *V. cholerae* is an important pathogenic bacterium that induces vibriosis in cultured fish species also (Devi *et al*., 2022). Therefore, continued research on novel antibacterial strategies, like exploring natural plant extracts, remains crucial for effectively combating cholera and its causative agent, *V. cholerae*. The antagonistic activity of *R. mucronata* and *A. officinalis* has not been tested against *V. cholera* to date. Hence, this work aims to investigate these mangrove leaf extract's antagonistic potentials against *V. cholerae*.

Material and methods

Samples were collected from the mangroves near Kumbalam, Kochi, India (9° 54′ 41.96″ N, 76° 18′ 32.36″ E). These mangroves were washed under tap water and subsequently with sterile distilled water. The species were then identified using the Manual on Mangrove Ecosystems (Jayasurya *et al*., 2005) as *R. mucronata* and *A. officinalis* (Fig. 1). These were then dried in shade for 15 days and powdered using a mixer grinder. Later, leaf extracts were prepared by dissolving 50 mg of powdered mangroves in 100 ml of the organic solvent having different polarities such as ethyl acetate, ethanol, and aqueous solvents. The containers were sealed and stored for five days. The crude extract mixtures were filtered using Whatman No.1 filter paper, and the filtrate was obtained using a rotary evaporator. This extract was transferred into air-tight bottles and stored at 4 °C till further use. Soxhlet extraction was performed to obtain the crude extract and the resulting solvent extract was filtered and concentrated in a vacuum concentrator (Kumar *et al*., 2011).

Fig 1. Mangrove leaf samples (a) *A. officinalis* (b) *R. mucronata*

Screening for antimicrobial activity

Agar well diffusion assay (Babu, 2014) was used for the antagonistic activity of crude mangrove extracts. Required wells of about 6.0 mm diameter were made on plates using a well cutter, and 100 μl of the crude extract of different mangrove extracts was transferred into each well. Pure solvent was used as a control for each bacterial strain. The plates were incubated for 24 hrs at 30 °C and the clear inhibition zone around the well was examined.

Statistical analysis

The Analysis of Variance test was performed to find the significant difference between the mangroves and the solvents using R 4.1.1 (R Core Team, 2021).

Results

The agar well diffusion assay (Babu, 2014) revealed varying degrees of in vitro anti-*Vibrio cholerae* activity amongst the extracts. Clear inhibition zones surrounding the inoculated wells containing mangrove extracts were observed, indicating antagonistic properties (Fig. 2). Pure solvent controls showed no inhibitory effect. This analysis aimed to identify any statistically significant differences in the inhibitory effect of *R. mucronata* and *A. officinalis* extracts against *V. cholerae*. The leaf extracts of *A. officinalis* in ethyl acetate exhibited more promising antibacterial activity than *R. mucronata* against *V. cholerae* (15.46 mm) (Fig. 3). None of the other aqueous extracts showed antagonistic activity.

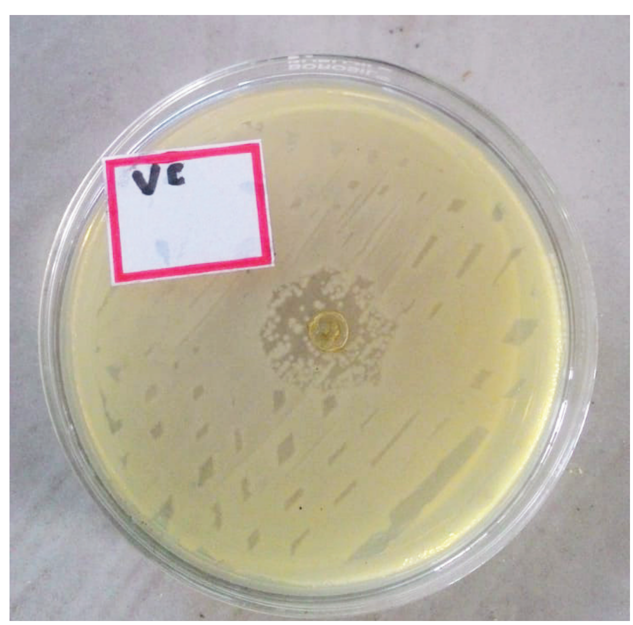

Fig. 2. Antibacterial activity of *A. officinalis* against the tested pathogen

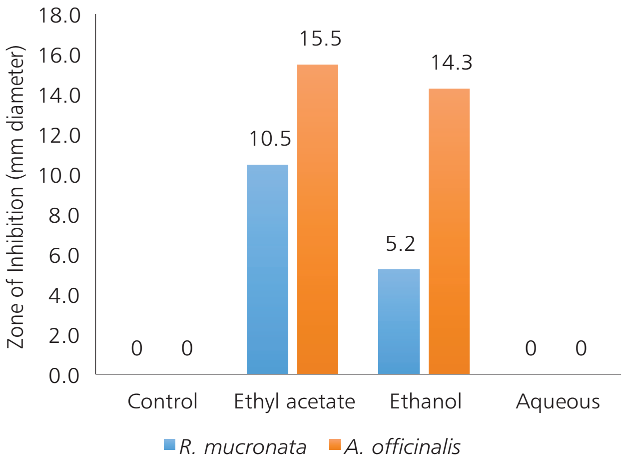

Fig. 3. Extent of inhibition of mangrove extracts against the tested pathogen

The analysis assessed whether the choice of solvent used for extraction significantly impacted the observed antimicrobial activity. The present investigation revealed that mangrove ethyl acetate extract showed more potent activity against pathogenic bacterial strains. Statistical analysis revealed a significant difference ($P<0.05$) between the means of antagonistic activity of mangroves and between the ethyl acetate and ethanol as solvents.

Discussion

Marine organisms, particularly marine flora, hold significant promise in offering potent, cost-effective, and safer alternatives for anticancer drugs, a prospect that is being rigorously explored. Despite their potential as a source of anticancer compounds, marine flora remain underexplored. Mangroves, for instance, have contributed to 28.12% of the anticancer compounds extracted thus far (Boopathy and Kathiresan, 2010). The inaccessibility of mangrove habitats has limited the exploration of all mangrove species for their bioactive potential. Our study aimed to investigate the antagonistic activity of leaf extracts from two mangrove species found along the southwest coast of India against the human pathogen *Vibrio cholerae*. The findings reveal that the leaf extract of *A. officinalis* demonstrated higher biological activity against the tested pathogenic bacteria, suggesting the presence of potential antibacterial compounds effective against *V. cholerae*. Additionally, ethyl acetate emerged as a more effective solvent than ethanol and water for extracting these compounds.

Previous studies identified phytochemical compounds such as triterpenoids, betulinic acid, lupeol, and betulinaldehyde in the ethyl acetate extract of the stem bark of *A. officinalis* (Haque, 2006). Notably, Rhizophrine, an alkaloid found in the leaves of *R. mucronata* and *R. stylosa*, demonstrated positive efficacy. Acanthicifolin, identified in *A. ilicifolius*, along with its extensive use in treating various ailments and its rich composition of bioactive compounds, underscores the medicinal value of mangrove species (Boopathy and Kathiresan, 2010). Moreover, the discovery of 2-Benzoxazoline and a new triterpenoidal saponin from *A. ilicifolius* further highlights the diverse bioactive potential of these plants (Jongsuvat, 1981; Minocha and Tiwari, 1981). Comparative analysis with previous studies on the antibacterial activity of *R. mucronata* and *A. officinalis* leaf extracts revealed significant efficacy against a range of human pathogenic bacteria, including *E. coli* and *S. aureus* (Bhimba *et al*., 2010; Joel and Bhimba, 2010). The identification of 1,4-dihydroanthraquinone, or Quinizarin, in the methanolic extract of *R. mucronata*, known for its antimicrobial, antioxidant, and cytotoxic activities, showcases the potential of these extracts in developing therapeutic drugs against bacterial infections and cancer (Sachithanandam *et al*., 2021).

Conclusion

The comprehensive antibacterial nature as identified in this study necessitates further biochemical analysis to pinpoint the secondary metabolites responsible for these effects. This research sets a foundation for future studies focused on the isolation and characterization of compounds with activity against pathogens. In light of climate change and anthropogenic pressures, conserving mangrove ecosystems becomes increasingly crucial. Recognizing their potential impact on human health emphasizes the need to protect these valuable plants, highlighting their significant economic value.

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