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Potential Antimicrobial Activity of Extracts of the Mangrove Leaves (*Avicennia marina* and *Rhizophora mucronata*) Against the Isolated Microbes

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Abstract: Mangrove leaves have antimicrobial compounds that help to resist pathogenic microorganisms, benefiting aquaculture. The present study aimed to identify fungal strains isolated from dead gourami fish (*Osphrone midae*) and experimental feed stored for six months. It also investigated the antimicrobial potential of aqueous extracts from *Avicennia marina* and *Rhizophora mucronata* against the three isolated fungal strains, *Aspergillus niger*, *Phoma* sp. and *Fusarium solani* and five bacterial strains, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The fungal strains *Aspergillus niger* and *Phoma* sp. were identified morphologically, while *Fusarium solani* was confirmed through DNA sequencing, a blast search of ITS sequences in NCBI with 100% query coverage of MN729431.1. Aqueous extracts (fresh and dried powder, 1mg/mL) from leaves were tested for antifungal and antibacterial activities. Antifungal and antibacterial activities were determined using the agar plate assay and agar well diffusion methods. The results showed that *R. mucronata* exhibited higher antifungal activity than *A. marina*, particularly against *Phoma* sp (53.9%). In contrast, *A. niger* demonstrated the lowest sensitivity to the dried powder extract of *A. marina*. Both species showed notable antibacterial effects, with fresh extracts generally outperforming dried ones. Specifically, the fresh extract of *A. marina* was most effective against *S. aureus* (14.08 ± 1.01%), while the dried extract of *A. marina* showed reduced activity against *B. subtilis*. These results imply that fresh leaf extracts may be more effective in controlling microbial strains and could provide potential solutions for infection management in the ornamental fish industry, although further research is necessary to confirm their efficacy and safety for practical use.

Keywords
- Aquaculture
- Ornamental fish
- Pathogenic microorganisms

1. INTRODUCTION

In recent years, there has been a growing interest in exploring natural sources of potential antimicrobial agents to combat the rise of drug-resistant microbes (Subramani et al., 2017). Mangrove forests, recognized as one of the most productive tropical ecosystems on our planet,are known to possess a rich diversity of bioactive compounds that exhibit antimicrobial properties (Bandaranayake, 2002). Mangroves are highly resistant to tidal fluctuations and salinity (Bakshi and Chaudhuri, 2014) and possess a unique morphology and physiognomy compared to other higher terrestrial plants (Bandaranayake, 2002). They have been widely used in coastal folk medicine for many centuries due to their healing abilities against various diseases (Rastegar and Gozari, 2017). Many scientific studies have found that mangrove plants carry great potential against numerous human, animal, and plant pathogens (Arivuselvan et al., 2011).

Ornamental fish can be affected by various

diseases caused by fungi and bacteria (Cardoso et al., 2019). Pathogenic infections in ornamental fish can result in economic losses for fish keepers. To prevent the spread of diseases and minimize economic losses, fish keepers must maintain good water quality, practice proper hygiene, and quarantine new fish. Antimicrobial approaches are routinely employed to address pathogenic challenges within the ornamental fish industry (Preena et al., 2020). It is essential to employ effective treatments to ensure the health and well-being of the fish and to maintain a safe and sustainable environment.

Several studies have shown the antimicrobial activity of the mangrove leaf extracts against edible fish and aquatic pathogens. These studies include research on *R. mucronata* and *A. officinalis* against *Vibrio cholera* (Sruthi and Sreenath, 2024), *R. mucronata* and *R. apiculate* against *S. agalactiae*, *A. hydrophila*, *V. harveyi*, and *V. parahemolyticus* (Vittaya et al., 2022), ethanolic extract of *R. mucronata* and *A. marina* against *Salmonella arizonae* isolated from *Carassius auratus*; *R. mucronata* against *V. harveyi* (Saptiani et al., 2019; Mulyani et al., 2020), and *Saprolegnia sp.* (Saptiani et al., 2019). It is worth noting that there are limited records available on the antimicrobial effects of these extracts against ornamental fish pathogens, *A. marina* against *V. alginolyticus* in clownfish, *Amphiprion sebae* (Dhayanithi et al., 2012). Ethanolic leaf extracts of *R. mucronata* and *A. marina* species were tested against *Salmonella arizonae*, which was isolated from *Carassius auratus* (Limbago et al., 2021). Raju and Sreeramulu (2017) tested ethanolic extracts of *A. marina* and *R. mucronata* against fish pathogens: *E. coli*, *Citrobacter freundii*, *V. harveyi*, *S. aureus*, and *Shigella flexneri* isolated from *Mugil cephalus*. Abeysinghe (2012) studied the antibacterial activity of aqueous and ethanol extracts of *A. marina* and *R. mucronata* against *Shigella sp*., *Pseudomonas sp*., and two antibiotic-resistant pathogens; *S. aureus* and *Proteus sp*.

Research on the antimicrobial efficiency of *A. marina* and *R. mucronata* against the tested fungal and bacterial species was scarce, and literary works are lacking in Sri Lanka. Given this knowledge gap, the present study investigates the antimicrobial activities of aqueous leaf extracts of *A. marina* and *R. mucronata* against selected fungal species isolated from dead ornamental fish and formulated fish feed, along with reference bacterial species.

2. MATERIALS and METHODS

2.1. Plant material and Extracts preparation

Fresh, healthy, mature and tender leaves of *A. marina* and *R. mucronata* were collected, with a sample size of 100 leaves from *A. marina* and 50 leaves from *R. mucronata*, sourced from the Mandaitivu area (09˚38'N-79˚59'E), Jaffna, Sri Lanka. The experiments were conducted in triplicate. The leaves were individually placed in sterile polythene bags and transported to the laboratory for further studies. The taxonomic identities of the plants were confirmed.

The fresh extracts were prepared following the method described by Saravanan and Radhakrishnan (2016). The collected leaves were washed twice with running tap water and once with sterile distilled water (SDW) to remove debris. The washed leaves (200 mg) were then air-dried and crushed using a sterilized motor and pistol and stored for future use. A one-hour sterilisation was done under UV-A and UV-B light before plant extract preparation to prevent the contamination of fungal species on mangrove leaves (Ebrahimi et al., 2024; Tian et al., 2024). The crushed leaf powder (200 mg) was mixed with 200 mL of SDW (1 mg/mL) and filtered through a muslin cloth to remove fine debris.

For dried powder aqueous extract, leaves were powdered and stored separately in an oven at 60 °C for 24 - 48 hours (Sajeevan and Krishnapillai, 2018). Before extraction, the powder was sterilized under UV light for one hour. The sterilized leaf powder (100 mg) was then soaked with 100 mL of SDW (1 mg/mL) and stirred well. The same procedure was carried out for both extracts, and the mixtures were allowed to settle for 24 hours at room temperature (RT) after covering them with aluminium foil. Finally, the mixtures were filtered using muslin cloths (Alo et al., 2012).

2.2. Bacterial and fungal test organisms

2.2.1. Fungal strains

Fungal species were isolated using dead gourami fish (*Osphronemus goramy*) and dry formulated fish feed. The main ingredients of the formulated fish feed were fish meal, corn flour, rice bran, Palmyra pulp, and soybeans, which were mixed with water and stored for more than six months. The feed was ground into fine

powder, and 1 mg was weighed and mixed with 9 mL of sterilized peptone water (SPW). The mixture was then homogenized in a vortex mixer for 1 minute. To collect a sample from a dead gourami fish, the 1 cm2 surface area of the fish skin was wiped with a cotton swab. The swab was then dipped into a test tube containing 10 mL of SPW and stirred well. Next, the sample was homogenized for 1 minute (Kavitha et al., 2017).

A 0.1 mL sample was transferred onto the chloramphenicol-added Potato Dextrose Agar (PDA) plates, and the spread plate method was carried out. Each isolation procedure was carried out in triplicate to ensure reproducibility. Then all the plates were incubated for seven days in a shaded area at room temperature (32 ± 5 °C).

Subsequently, the subculture technique was employed to obtain pure fungi cultures (Ambeng et al., 2019). Stock cultures were maintained at 10 °C in PDA slants for further studies.

Fungal identification was conducted by observing macroscopic morphological characteristics: growth rate, topography, texture, opacity, colony color, sulcation, and exudates. The agar slide culture technique described by Patrick et al. (2010) was utilized to study microscopic features, including hyphae type, hyphae colour, spore type, spore shape, and spore colour. Furthermore, the morphologically identified fungal species were confirmed at the molecular level by comparing their DNA sequences with those available at NCBI (National Center for Biotechnology Information).

2.2.2. Bacteria species

The bacterial species *B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and *S. aureus*, obtained from the Department of Botany, University of Jaffna, Sri Lanka, were used for antimicrobial testing. New bacterial cultures of *B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and *S. aureus* were made by transferring subcultured bacterial specimens onto freshly prepared Nutrient Agar (NA) plates, which were then incubated overnight at 37 °C. Following incubation, bacterial suspensions were prepared by transferring a loopful of inoculum into 10 ml of SDW. A small amount of chloramphenicol was added gradually to 10 mL of SDW for the positive control treatment.

2.3. Antimicrobial Assay

2.3.1 Antifungal activity by Agar plate assay

The PDA plates were prepared by adding 1 mL of leaf extract. For the negative control, 1 mL of SDW was used instead of plant extracts. A 0.5 cm diameter mycelial disc, punched with a cork borer from an actively growing (5-7 days old) fungal culture, was aseptically placed at the center of a freshly prepared PDA plate. These old fungal cultures were initially isolated from dry fish feed and the dead gourami fish. Triplicates were prepared, and the same procedure was repeated for all extracts. Then, the plates were incubated at room temperature for seven days (Al-Manhel and Kareem Niamah, 2012), and the growths of mycelia were measured and recorded. Inhibition was determined according to Krishnapillai (2003).

$$Inhibition\ (\%) = \left[\frac{Growth\ area\ in\ reference - \ Growth\ area\ in\ sample}{Growth\ area\ in\ reference}\right] x\ 100$$

2.3.2. Antibacterial activity by well diffusion method

A 0.1 mL bacterial suspension was pipetted at the center of the chloramphenicol-added NA plate and spread over the surface. Then wells (0.5 cm in diameter) were aseptically punched into the agar surface. 200 µL of the plant extract was introduced into each well, while chloramphenicol (200 µL) served as the positive control. The procedure was repeated for all the mangrove extracts and triplicates were prepared for each bacterial suspension. The NA plates were incubated at 37 °C for 24-48 hours. Then, the inhibitory zones formed around each well were measured and recorded using a ruler (Nagendran and Krishnapillai, 2018).

2.4. Preparation of plant extracts for phytochemical screening

2.4.1. Determination of total phenols

The total phenolic content (TPC) of each plant extract was determined using the Folin–Ciocalteu colorimetric method with slight modifications. A volume of 20 µL of each plant extract was pipetted into individual boiling tubes, followed by the addition of 100 µL of Folin–Ciocalteu reagent and 1.58 mL of distilled water. The mixture was vortexed thoroughly and allowed to stand for 8 minutes at room temperature. Then, 300 µL of 7.5% sodium carbonate (Na_2CO_3) solution was added to each tube, and the mixtures were mixed well. All tubes were placed in a water bath at 40 °C for 30 minutes, followed by

cooling to room temperature. After cooling, the solutions were vortexed again, and absorbance was measured at 765 nm using a UV–Vis spectrophotometer. A calibration curve was prepared using gallic acid as a standard at concentrations ranging from 10 to 100 µg/mL. The total phenolic content of each extract was calculated from the standard curve and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW). All measurements were performed in triplicate. Reagent blanks were prepared using ethanol, methanol, or distilled water in place of plant extracts (Nikolaeva et al., 2022).

2.4.2. Determination of total alkaloids

The alkaloid content was estimated using the method proposed by Harborne (1973). A total of 5.0 g of dried, powdered plant material was placed in a 250 mL beaker containing 200 mL of 10% acetic acid in ethanol. The mixture was covered and left to stand at room temperature for 4 hours. The resulting solution was filtered, and the filtrate was concentrated to one-quarter of its original volume using a water bath. Concentrated ammonium hydroxide was added dropwise until complete precipitation was achieved. The mixture was allowed to stand to facilitate settling, and the precipitate was collected by filtration, washed with dilute ammonium hydroxide, dried, and weighed. The residue was regarded as the total alkaloid content and expressed in mg/g dry weight (Prajapati et al., 2024).

2.5. Statistical analysis

The mean % of inhibition data and mean diameter of the inhibition zone for each tested fungus and bacterium, respectively, were statistically analyzed using Minitab 19. The normality of data was confirmed using the Anderson–Darling test prior to applying ANOVA. The General Linear Model—Univariate Test (ANOVA) was carried out at the 0.05 significance level. The significance of each level was analyzed using the mean separation technique (Tukey test).

3. RESULTS

3.1. Isolation and Identification of Fungi

Three fungal species were isolated from the dry fish feed (Strains 2 and 3) and dead fish (Strain 1). Each isolation procedure was performed in triplicate to ensure reproducibility. The subcultures of isolated fungal species were maintained to ensure perfect isolation and for further studies. Initially, using the colony morphological and microscopic (Table 1) characters, fungal strains 1, 2, and 3 were identified as *Fusarium sp*., *Aspergillus niger*, and *Phoma sp*., respectively.

Table 1. Colony morphology and microscopic features of fungal isolates grown on Potato Dextrose Agar in this study.

Characteristic	Strain 1	Strain 2	Strain 3
Yeast/Mold	Mold	Mold	Mold
Rate of growth	Rapid	Rapid	Slow
Colony Form	Filamentous	Filamentous	Circular
Elevation	Umbonate	Umbonate	Flat
Margin	Filiform	Entire	Lobate
Texture	Cottony	Cottony	Velvety/Suede-like, often powdery
Opacity	Opaque	Translucent	Translucent
Colony Colour (Front)	White	Black	White → pinkish white
Colony Colour (Back)	Red	Pale Yellow	Red
Sulcation (Front)	Absent	Absent	Present
Sulcation (Back)	Absent	Present	Present
Exudates	Absent	Absent	Present
Hyphae Type	Aseptate	Septate	Septate
Hyaline/dematiaceous	Hyaline	Hyaline	Hyaline
Hyphae Colour	Colourless	Colourless	Colourless
Spore Type	Conidia	Conidia	Conidia
Cells per Spore	Multi-celled	One-celled	One-celled
Spore Shape	Falcate/Oval	Spherical	Oval
Spore Colour	Colourless	Dark-brown	Colourless

Fusarium sp. showed rapid growth on the PDA plate, producing a full-growth colony (6.8 mm in diameter) after six days of incubation. The well-grown colony looked like cotton wool in the PDA medium. The front view of the colony was white (Figure 2a), and the reverse side was reddish (Figure 2b). The macroconidia are typically falcate and oval. The strain identified as *Fusarium* sp. by morphological examination was confirmed as *Fusarium solani* (MN729431.1) by DNA sequences available at NCBI (Figure 1).

5’GCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTT
TCTTCTGAGTAAA
ACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAG
AACGCAGCGAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCT3’

Figure 1. The DNA sequence of Fungal strain 1 (*Fusarium solani*)

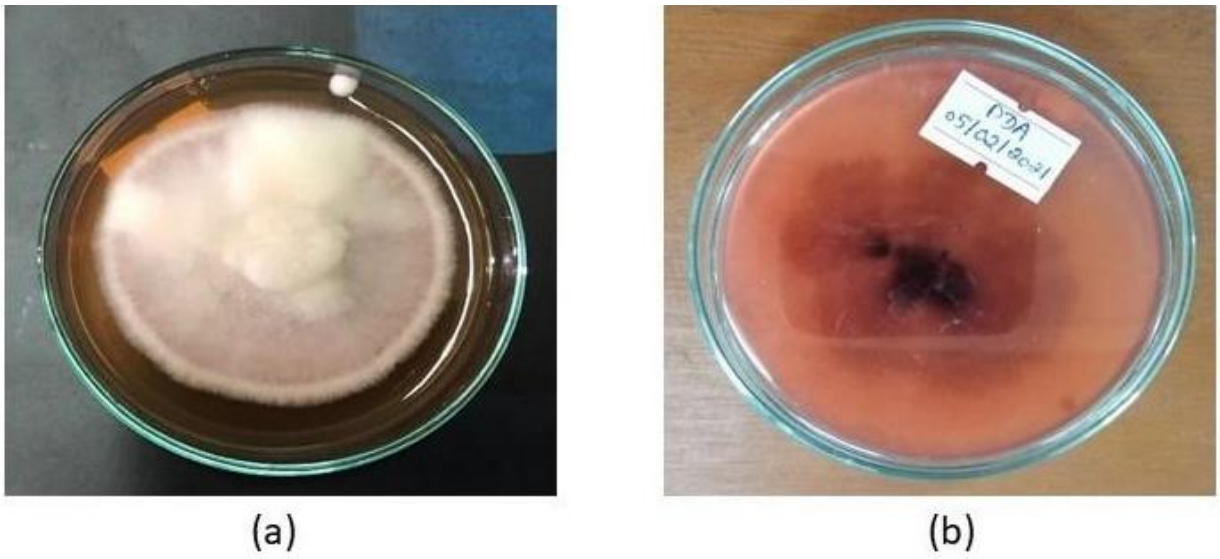

Figure 2. Front (a) and reverse views (b) of *Fusarium solani* colony on Potato Dextrose Agar-after six days of incubation.

A. niger proliferated on the PDA plate, and it showed a full-growth colony (6.8 mm in diameter) only after four days of incubation. The colonies of the *A. niger* on the PDA medium appeared in a cottony form. Initially, the colony was white and followed the black colour (Figure 3a). The reverse side was pale yellow with radial fissures (Figure 3b). The observed T-shaped foot cells of conidiophores were used as the vital identification factor for *A. niger*.

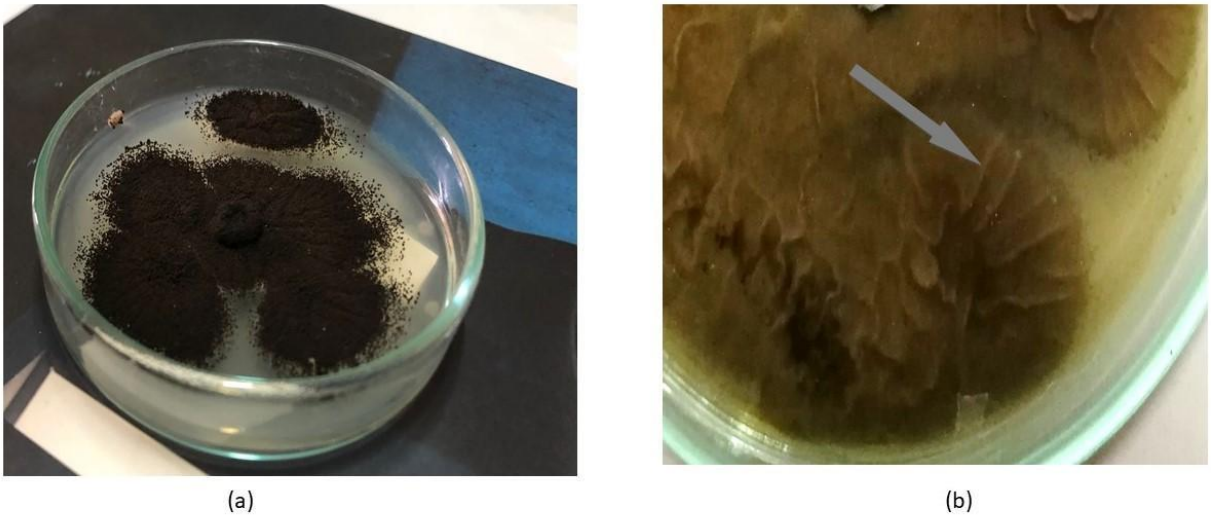

Figure 3. Front (a) and reverse views (b) of *Aspergillus niger* colony on Potato Dextrose Agar after four days of incubation.

On the PDA plate, *Phoma* sp. showed slow growth, and after six days of incubation, it obtained only a 4.7 mm diameter colony. The front view of the colony was white (Figure 4a) after 72 hours of the incubation period, followed by pinkish white (Figure 4b). The reverse side was red (Figure 4c). The texture of the colony was velvety/suede, which often became powdery. Parallel grooves (sulcation) were seen on both sides of the colony. Exudates (Figure 4b) can be seen on the front view of the plate. The macroconidia are colourless and globose in shape.

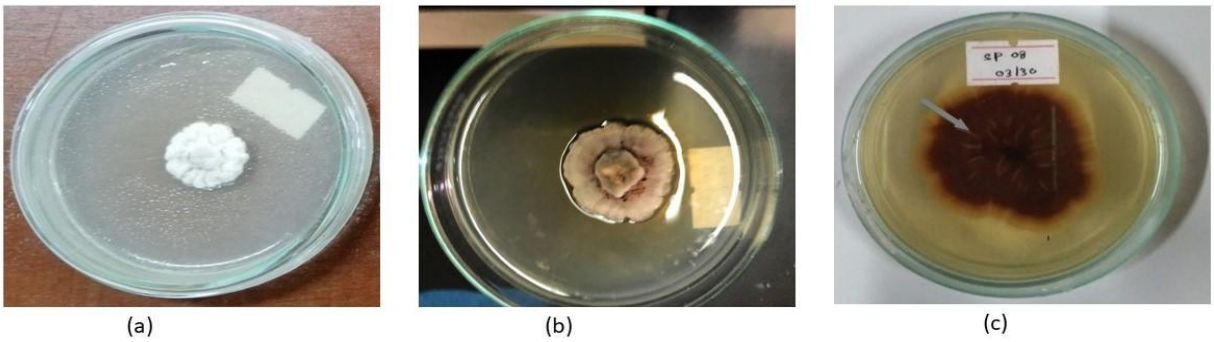

Figure 4. After 3 days' incubation, front view (a), after 5 days' incubation, front view (b), reverse view and exudates (c) of *Phoma* sp. colony on Potato Dextrose Agar

3.2. Antifungal activity of extracts

Four different aqueous extracts (*A. marina* dried powder, *A. marina* fresh, *R. mucronata* dried powder, and *R. mucronata* fresh) were used to test the antifungal activities, and all extracts exhibited different degrees of growth inhibition against the tested fungal strain. The results obtained from the agar plate assay showed that there had been a significant effect of both plant extract type and fungal strain on growth inhibition (p=0.0001). All fungal strains showed more inhibition against the fresh extract of *R. mucronata* than the fresh extract of *A. marina*. Among the tested fungi species, *Phoma* sp. showed the highest inhibition rate (53.95 %) with the fresh extract of *R. mucronata*, while the lowest inhibition (2.92 %) against *A. niger* was demonstrated by *A. marina* dried powder extract.

The dried powder extract of *R. mucronata* showed more promising growth inhibition than the *A. marina* dried powder extract against the tested species, except for *F. solani* (16.77 %). According to Figure 5, fresh aqueous extracts of both plants possessed the highest antifungal activity compared to their dried powder extracts against all tested fungal strains.

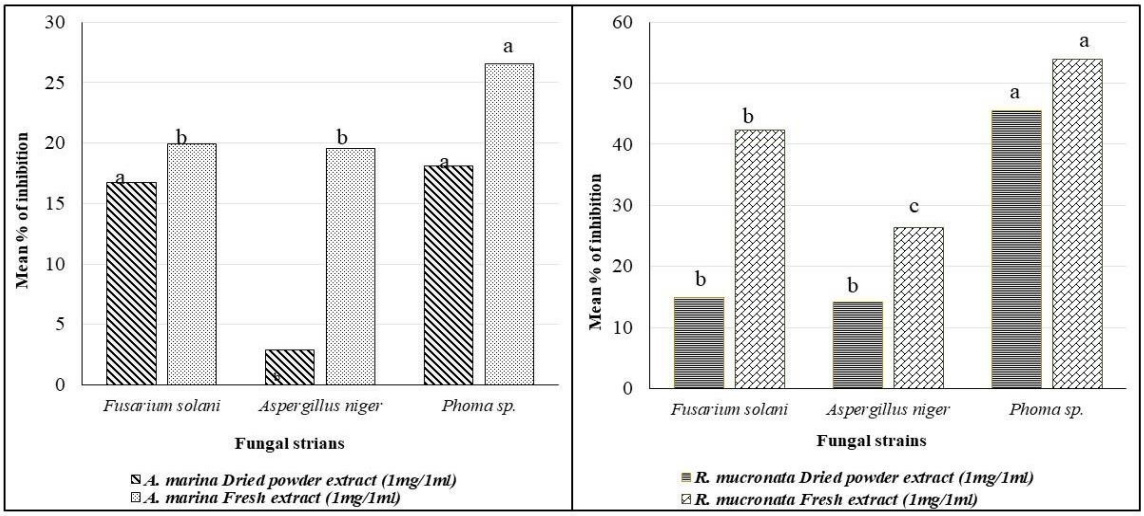

Figure 5. Comparison of the antifungal activity between fresh and dried extracts of *A. marina* and *R. mucronata* Different letters for each treatment indicate significant differences at $P < 0.05$ (Turkey Test) against three fungal species (*Fusarium solani*, *Aspergillus niger* and *Phoma* sp.) isolated from dead fish and formulated feed.

3.3. Antibacterial activity of extracts

A significant difference in sensitivity can be seen between the four prepared plant extracts (p=0.0001). According to the results obtained from the agar well diffusion method, the fresh extract of *A. marina* showed the highest inhibition against *S. aureus* (14.08 ± 1.01 %), while the dried powder extract of *A. marina* showed the lowest inhibition (3.42 %) against *B. subtilis* . When comparing the fresh extracts, *A.*

marina showed a higher inhibition rate against *E. coli* (9.9 ± 0.00 %), *P. aeruginosa* (12.06 ± 0.05%), and *S. aureus* (14.08 ± 1.01%), whereas *B. subtilis* (7.13 ± 0.05%) and *E. faecalis* (7.63 ± 0.06%) were more sensitive to *R. mucronata*. As shown in Figure 6, the fresh aqueous extracts demonstrated stronger antibacterial activity than the dried powder extracts against all tested bacterial species.

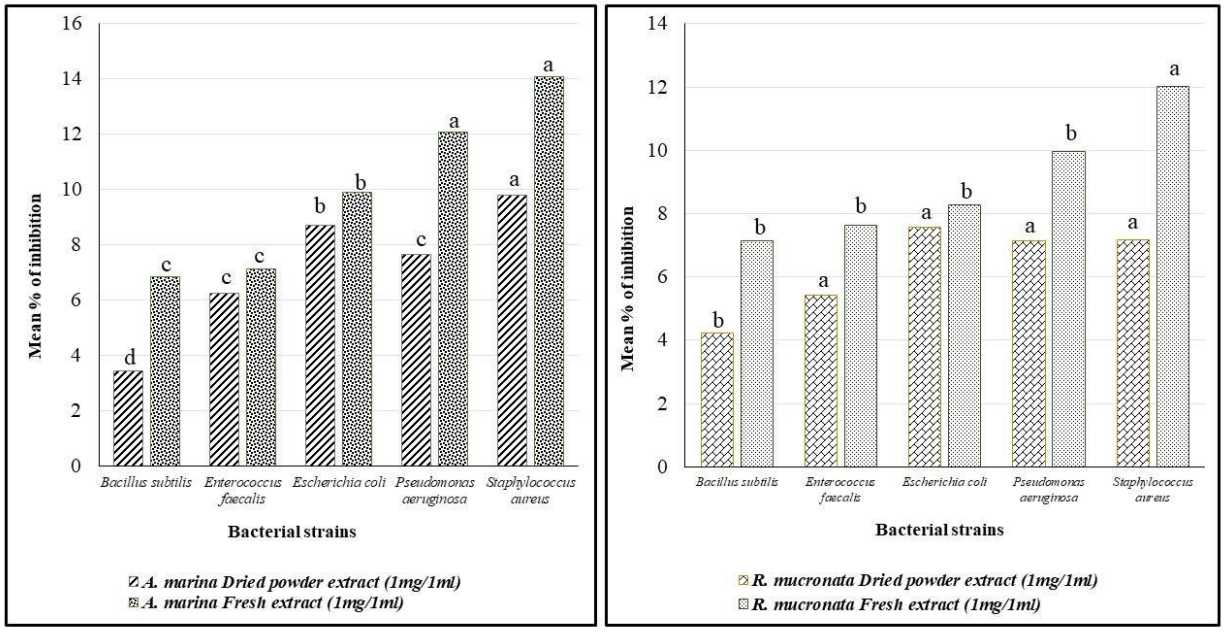

Figure 6. Comparison of the antibacterial activity between fresh and dried aqueous extracts of *A. marina* and *R. mucronata.* Different letters for each treatment indicate significant differences at $P < 0.05$ (Turkey Test) against five bacterial species (*Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*).

3.4. Phytochemical screening of plant extracts

3.4.1. Total phenolic content

The total phenolic content (TPC) of the dried leaf extracts from the two mangrove species studied exhibited notable differences. Specifically, *Avicennia marina* recorded a TPC of 24.919 ± 0.001 mg GAE/g DW, whereas *Rhizophora mucronata* demonstrated a significantly higher concentration at 58.963 ± 0.006 mg GAE/g DW. These findings clearly indicate that both dried leaves contain total phenolic content (TPC); however, *R. mucronata* possesses a significantly greater amount of phenolic compounds compared to *A. marina*.

3.4.2. Total alkaloid content

Both dried leaves contain total alkaloid content, which was notably lower compared to the total phenolic content (TPC). Specifically, *Avicennia marina* exhibited a TPC of 1.45 ± 0.009 %, while *Rhizophora mucronata* demonstrated a significantly higher concentration of 2.09 ± 0.001 %.

4. DISCUSSION

The present study has focused on the antimicrobial potential of mangrove leaf extracts, specifically from *A. marina* and *R. mucronata* against bacterial and fungal strains isolated from ornamental fish and formulated fish feed. These extracts have shown promising activity against a variety of isolated microbes.

The present study's analysis of the phytochemical content in the extracts of *A. marina* and *R. mucronata* reveals significant antimicrobial properties, such as alkaloid and phenolic compounds, that align with findings from previous research (Mouafi et al., 2014; Roy et al., 2023). Studies have consistently highlighted the presence of bioactive compounds in these mangrove species, which contribute to their antibacterial and antifungal activities. The

total phenolic content (TPC) measured for *Avicennia marina* is in close alignment with the value previously reported for *A. marina* leaves from West Sumatra, which was 24.51 ± 0.056 mg GAE/g (Yanti et al., 2021). In contrast, the TPC of *Rhizophora mucronata* was significantly higher, corroborating previous studies that indicated elevated phenolic levels ranging from 100.00 ± 0.54 to 187.53 ± 8.75 mg GAE/g under conditions of salinity stress (Kodikara et al., 2020). The results of the present study are in accordance with earlier findings of Chandrasekaran, et al. (2006), who reported that a 0.25 mg/ml of methanol extract from *R. mucronate*.

Several studies have indicated that the leaves of mangrove species contain bioactive compounds with antimicrobial properties, which could be utilized in developing new antimicrobial agents. In the studies by Ibrahim et al. (2022) and Sohaib et al. (2022), the phytochemical extracts of *A. marina* leaf exhibited significant antimicrobial activity against a range of pathogenic bacteria and fungi. The results demonstrated the potential of these mangrove species as a source of natural antimicrobial agents.

The findings on the antifungal activity of extracts from mangrove leaves, specifically those of *A. marina* and *R. mucronata*, are significant given the increasing issue of fungal infections in fish populations. In our study, the leaf extracts exhibited notable antifungal properties against common fish pathogenic fungi. This aligns with previous research indicating that mangrove-derived compounds can inhibit the growth of fungal pathogens. For instance, Kathiresan and Ramanathan (1997) reported that mangrove extracts, including Avicennia and Rhizophora species, exhibited antifungal activity against fish and shrimp pathogenic fungi. More recently, Rastegar and Gozari (2016) demonstrated that ethanolic extracts of *A. marina* and *R. mucronata* exhibited antifungal activity against various fungal species, including *A. niger* and Penicillium species.

The mechanisms underlying this antifungal activity may be attributed to the presence of secondary metabolites such as flavonoids, tannins, and phenolic compounds, which have been documented for their antifungal properties (Aguilar et al., 2023). Our results support the hypothesis that the utilized mangrove extracts contain these bioactive substances that effectively inhibit the growth of fungal pathogens, thereby offering a natural alternative for managing fungal infections in aquaculture settings.

The antibacterial activity of these mangrove leaf extracts against fish pathogenic bacteria deserves particular attention. The extracts demonstrated varying levels of antibacterial efficacy, with *R. mucronata* showing stronger activity than *A. marina*. This finding corroborates findings by Mangrio et al. (2016), who highlighted that extracts from Rhizophora and Avicennia species tend to possess a broader antibacterial spectrum due to the higher concentration of active phytochemicals.

The effectiveness of the extracts may be attributed to significant components such as alkaloids and phenolic compounds, which have been shown to disrupt bacterial cell membranes and inhibit protein synthesis (Prajapati et al., 2024; Aguilar et al., 2023). Moreover, the observed inhibitory effects on specific pathogens, such as *Staphylococcus aureus* and *Salmonella typhi* imply a potential role for these extracts as preventive measures against bacterial infections in aquaculture.

Both the antifungal and antibacterial activities of *A. marina* and *R. mucronata* highlight the promising potential of mangrove leaf extracts in aquaculture. These results advocate for further investigation into the isolation and characterization of specific bioactive compounds, which could pave the way for developing natural antimicrobial agents for fish health management. The exploration of such natural remedies is essential for sustainable aquaculture practices, particularly in the face of rising antibiotic resistance in fish pathogens.

In the current study, two fungal species, *A. niger* and *Phoma sp*. were identified in the stored formulated fish feed. These findings are consistent with previous studies by Barbosa et al. (2013) and Marijani et al. (2017), which also identified a prominent fungal species, *A. niger*, in fish feed formulated from corn flour. One of the ingredients used in the formulated fish feed in our study is corn flour, which can influence the fungal growth of the feed. While *Phoma sp*. infrequently contaminates fish feed, it was identified in the present study. It may cause granulomatous disease, particularly in the kidney. The morbidity rate in salmonid outbreaks rarely exceeds 5%, and the disease usually affects juveniles (Roberts, 2012). In the case of the dead gourami fish, *F. solani* was isolated, and the

species was confirmed with the DNA sequences. A similar fungus was identified by Andreas et al. (2021) in gouramy (*Osphronemus gouramy*) in modern market Surabaya. This infection may be due in large part to the stressful conditions of fish farming and some other environmental factors (water quality problems, changes in salinity or temperature, trauma) or pathogens (bacterial disease or parasites) (Cutuli et al., 2015).

The results obtained in the present study on the antimicrobial activities show that the plant extracts from fresh leaves of *A. marina* and *R. mucronata* were more effective in inhibiting the growth of all tested fungal and bacterial strains compared to extracts from dried plant materials. This is consistent with a similar case reported by Abeysinghe (2010), and it was revealed that the aqueous extract of fresh *A. marina* exhibited the highest antibacterial activity against *S. aureus* and the reason is because the fresh plant parts typically exhibit higher concentrations of antimicrobial compounds compared to their dried counterparts. It is suggested that fresh plant parts contain higher levels of antimicrobial compounds compared to dried parts, likely due to higher concentrations of labile bioactive compounds such as phenols, flavonoids, and alkaloids, which may degrade during drying or prolonged storage. Additionally, fresh plant material may facilitate more efficient release of water-soluble antimicrobial compounds during extraction., Among the tested microbes, *S. aureus* and *Phoma sp*. showed the highest inhibition against the fresh aqueous extract of *A. marina* and *R. mucronata*, respectively. Past studies have also reported similar activity (Abeysinghe, 2012; Saravanan and Radhakrishnan, 2016). Several limitations should be noted. The study involved a limited number of microbial isolates, which may affect the generalizability of the results. Variability in extract preparation, including leaf maturity, handling, and extraction time, may influence antimicrobial efficacy. Environmental factors, such as seasonal changes and salinity stress, that affect mangrove plants can also alter the concentrations of bioactive compounds. Future studies with larger sample sizes, standardized extraction protocols, and seasonal monitoring are recommended to confirm these findings and explore the mechanistic basis of antimicrobial activity in mangrove extracts.

5. CONCLUSION

The study revealed that the aqueous extracts exhibited significant antimicrobial activity against the tested bacterial and fungal species. This suggests that these extracts could potentially be used as natural alternatives to synthetic antimicrobial agents. It is recommended to use fresh leaf extracts to maximize antimicrobial activities, particularly against *S. aureus* and *Phoma* sp., highlighting the potential of these mangrove species as natural sources of antimicrobial agents. Fresh leaf extracts were consistently more effective than dried extracts, likely due to higher concentrations of labile bioactive compounds. The isolation of *Aspergillus niger*, *Phoma* sp. and *Fusarium solani* underscores the microbial risks in fish feed and cultured ornamental fish, emphasizing the need for effective management strategies. Understanding how these extracts exert their antibacterial activity could provide valuable insights for the development of new antibacterial agents. Additionally, further research could focus on identifying the specific compounds within the aqueous extracts that are responsible for their antimicrobial properties. The potential applications of these findings should also be discussed, and larger sample sizes and standardized extraction methods are recommended to validate their efficacy.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Fiction: AMPR; Literature: AMPR, NK; Methodology: AMPR, NK, SS; Performing the experiment: AMPR; Data analysis: AMPR, NK; Manuscript writing: AMPR, SS, Supervision: NK, SS. All authors approved the final draft.

ETHICAL STATEMENTS

The research ethical committee of the Faculty of Science, University of Jaffna, Sri Lanka (No:

AERC/202/101) reviewed and approved all procedures involving the use of microbiological analysis to prevent the spreading of tested bacteria and fungi from the in vitro conditions.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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