

Antibacterial Efficacy of Mangrove *Rhizophora mucronata* Extracts on Aquatic Bacterial Pathogens

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Introduction

Medicinal plants contain components of therapeutic value and are used as remedies for human/animal diseases. They are environmentally friendly due to less toxicity and have minimal health hazards (Opara and Wokocha, 2008). Mangrove plant extracts and its major chemical classes displayed various levels of antibacterial, antifungal, antifouling, antiviral and free radical scavenging effects due to their bioactive compounds (Bandaranayake, 2002). Bacterial infections cause severe economic losses to the aquaculture industry which increased the use of antimicrobial agents significantly as a treatment (Alderman and Michel, 1992). Improper usage and presence of drug residues lead to the development of resistant strains of pathogenic bacteria. Therefore, novel antibacterial compounds of natural products which degrade easily need to be synthesized to overcome above consequences. Aim of this study was to find out the antibacterial efficacy of Mangrove *Rhizophora mucronata* extracts on aquatic bacterial pathogens, to be used as a remedy to treat fish diseases.

Methodology

Fresh matured leaves of six mangrove plant species (*Avicennia marina*, *Lumnitzera racemosa*, *Bruguiera sexangula*, *Acanthus ilicifolius*, *Excoecaria agallocha* and *Rhizophora mucronata*) were collected from mangrove reserve at Kadolkele, Negombo and were brought to the laboratory for making extractions. Initially, preliminary trials were conducted for identification of most suitable species with antibacterial properties against four aquatic pathogens (*Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Listeria monocytogenes*) *in vitro* by well diffusion method. Upon identification of *Rhizophora mucronata* having the highest inhibitory zone for tested bacteria, further analysis were performed using various parts such as roots, barks, flowers and fruits of *R. mucronata*. The solvent was extracted by mixing 8 g of each part of mangrove in powder form, with 125 ml of 95% methanol and evaporating methanol using rotary flash evaporator (model 519-00000-00-3). Antibacterial efficacy was assessed against all four species of fish pathogens, using antibiotic sensitivity testing (Mbata, *et al.*, 2008). Tetracycline 30 µgml⁻¹ was used as the positive control against *Rhizophora mucronata* extractions of 350 mgml⁻¹. After 24 hour incubation at 25 °C, the diameters of the inhibitory zones were obtained and statistically analyzed by using One way-ANOVA incorporated in Minitab version 16.

Results and Discussion

All six mangrove species showed antibacterial activity against *Listeria monocytogenes*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa* significantly ($p < 0.05$) but insignificant for *Shigella flexneri* ($p > 0.05$). Highest inhibition zones were observed in *Rhizophora mucronata* leaf extract against all bacteria examined for the study (Table 1). *Rhizophora mucronata* extracts (root, bark, fruit and flower) also exhibited positive inhibition zones for all the tested bacterial species cultured *in vitro*.

Root and bark extracts possessed higher antibacterial potential when compared to the fruit and flower extracts. *Rhizophora* leaf extracts showed highest inhibition for *Listeria monocytogenes* and *Pseudomonas fluorescens* while root and bark extracts were effective against *Shigella flexneri* and *Pseudomonas aeruginosa* respectively (Figure 1). The overall mean comparative evaluation on antibacterial efficacy of *Rhizophora mucronata* extracts displayed the highest in leaves followed by the root, bark and fruit extracts. The minimum antibacterial activity was observed in the flower extracts.

Table 1. Antibacterial activity of six mangrove leaf extracts against fish pathogenic bacteria (Inhibition zone in mm).

Pathogens	<i>Avicennia</i>	<i>Bruguiera</i>	<i>Acanthus</i>	<i>Excoecaria</i>	<i>Rhizophora</i>	<i>Lumnitzera</i>	Tetracycline 30 µg/ml
<i>Pseudomonas fluorescens</i>	14.9 ± 1.12	13.5 ± 1.13	12.7 ± 1.27	16.1 ± 3.35	17.9 ± 0.41	14.3 ± 0.3	22
<i>Pseudomonas aeruginosa</i>	11.4 ± 0.87	13.3 ± 0.92	13.4 ± 0.86	12.5 ± 0.5	15.1 ± 0.23	15.1 ± 1.01	22
<i>Shigella flexneri</i>	10.2 ± 0.2	7.4 ± 1.10	10.2 ± 1.7	10.4 ± 0.6	12.3 ± 1.91	10.1 ± 1.85	0
<i>Listeria monocytogenes</i>	16.3 ± 0.3	14.5 ± 0.76	13.4 ± 1.25	17.4 ± 1.12	18.8 ± 1.04	14.5 ± 0.5	22

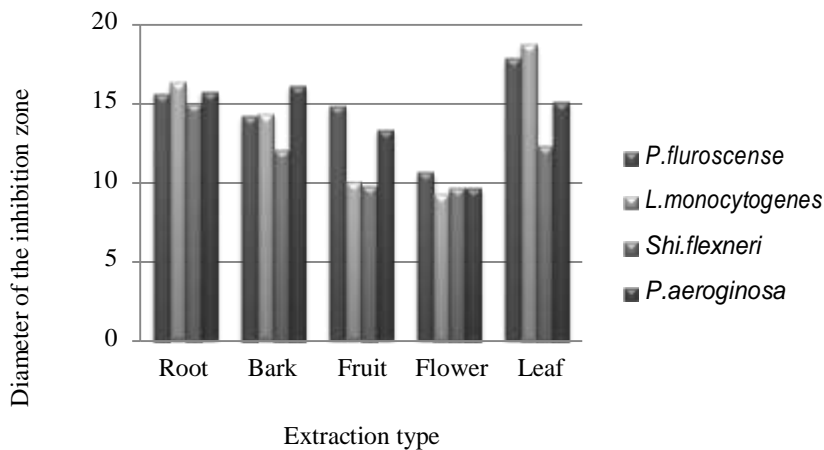


Figure 1. Bar chart for the antibacterial susceptibility test results of *Rhizophora* parts extract.

Rhizophora mucronata leaf extract possesses significantly higher antibacterial activity against all the tested fish pathogens where as other parts were also positive against pathogenic bacteria. The antibacterial activity is due to the presence of high content of polyphenols which include tannins.

However, some mangrove extracts did not exhibit higher inhibition zones as leaf extract which could be due to the resistant mechanisms such as enzymatic inactivation and target site modification (Schwarz and Noble, 1999). *Shigella flexneri* was resistant to Tetracycline, although it was moderately susceptible for almost all the mangrove leaves extractions. It implies

that mangrove leaves extracts have the capability of inhibit the both Gram positive and Gram negative bacteria which include antibiotic resistant strains.

The natural antibiotic compounds like polyphenols, tannins are rapidly degraded in natural environment while synthesized antibiotic like Quinolones and Tetracyclines were persistent, with half lives approaching 24 hour. Fate of this persistent, leads to plant uptake and bioaccumulation, ultimately it will affect food safety and human health and accumulation of residues will pose challenges to environment matrix (Joanne et al., 2008). Therefore, further attention should be paid to develop the novel drugs from such natural products. Tannin is responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility in organisms which ultimately affect for the growth inhibition of fungi, viruses and bacteria (Chung, et al., 1998). Moreover, antibacterial property of tannins which obtained from the extractions can be used in food processing industries to increase the shelf-life of certain foods, such as fish fillets. Since tannins are edible and water soluble polyphenols, toxicity compounds do not exist in the food products. However, more detailed investigation is essential to find out the active fraction of the mangrove plants.

Conclusion

Mangrove extractions of *Rhizophora mucronata* have potent antibacterial activity, particularly in the leaf extracts, which can be used to treat aquatic bacterial pathogens as an environmental friendly natural compound.

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