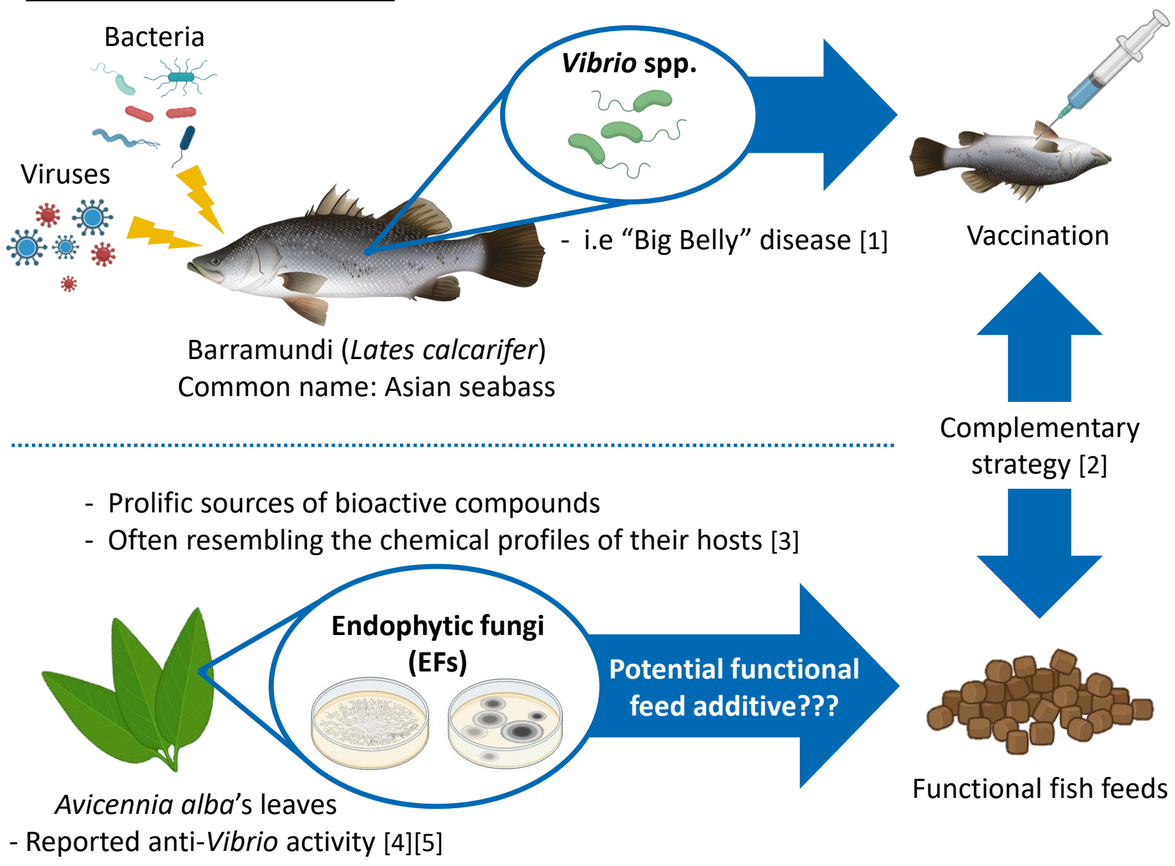
Potential of Mangrove-Derived *Phyllosticta* sp. as a Functional Feed Additive in Barramundi (*Lates calcarifer*) Aquaculture for Managing *Vibrio* spp. Infection

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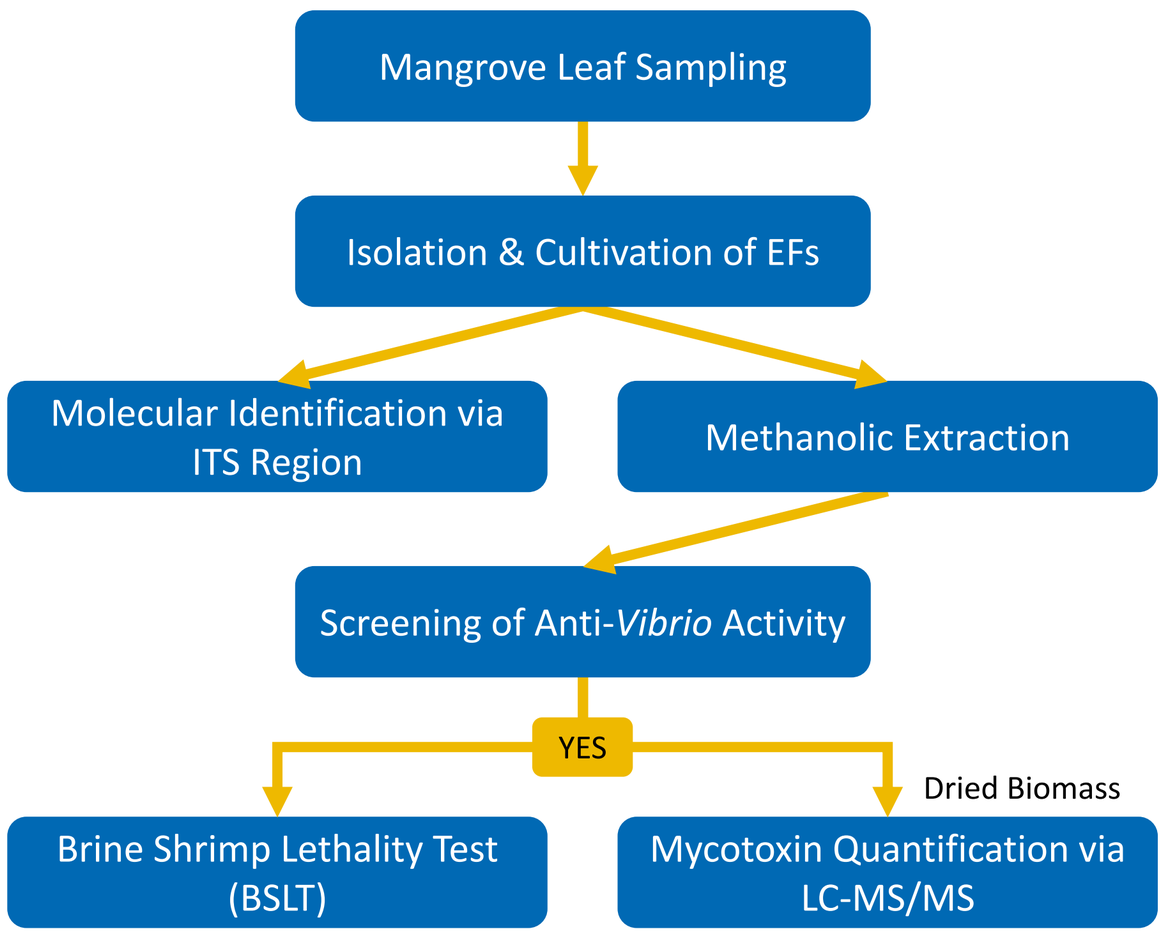
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Introduction



This study aimed to evaluate the anti-*Vibrio* effects and any potential toxicity of the methanolic extracts of EFs isolated from the *A. alba*.

Methodology



Results & Discussion

In this study, two EFs were successfully isolated from the *A. alba* leaves: *Pyrrhoderma* sp. strain MH022 and *Phyllosticta* sp. strain MH007. Photographs of plate cultures captured on day 7 are shown in **Fig. 1**.

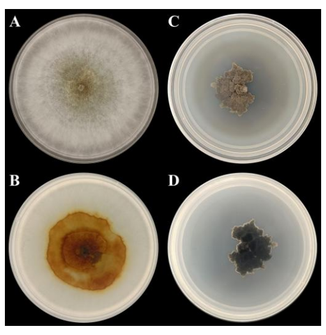


Fig. 1 Photographs of plate cultures for the EFs isolated in the study captured on day 7: (A) front and (B) reverse of *Pyrrhoderma* sp. strain MH022; (C) front and (D) reverse of *Phyllosticta* sp. strain MH007.

Fig. 2 shows that *Phyllosticta* sp. strain MH007 extract was found to exhibit broad-spectrum inhibition against all seven *Vibrio* spp., with inhibition zone diameters ranging from 25 ± 3 mm to 34 ± 1 mm, whereas *Pyrrhoderma* sp. strain MH022 showed no detectable activity. Relatively smaller inhibition zones compared with the positive control, chloramphenicol may due to the presence of a complex mixture of bioactive compounds in the extract, resulting in synergistic, additive, or antagonistic activity interactions among the compounds [6].

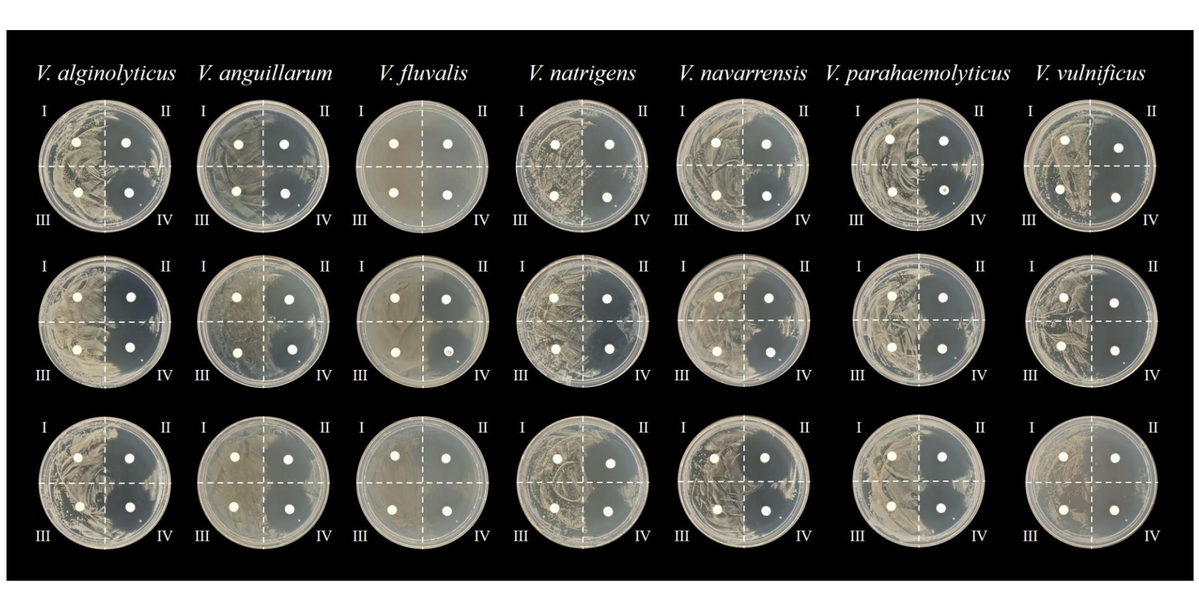


Fig. 2 Photographic results of anti-*Vibrio* screening. Antibiotic Assay (AA) discs in Quadrant "I" were loaded with ethanol (negative control); AA discs in Quadrant "II" were loaded with chloramphenicol (positive control); AA discs in Quadrant "III" were loaded with the extract of *Pyrrhoderma* sp. strain MH022; and AA discs in Quadrant "IV" were loaded with the extract of *Phyllosticta* sp. strain MH007.

Brine shrimp lethality test (BLST) (**Table 1**) indicated that the *Phyllosticta* sp. strain MH007 extract exhibited low to negligible toxicity. The median lethal concentration (LC_{50}) value could not be determined, but a previous study reported an LC_{50} value of more than 1000 µg/mL for the methanolic extract of *Phyllosticta* sp. using the BSLT [7].

Table 1: Results of brine shrimp lethality test (BSLT) experiment.

No.	Final Conc. of Extract (µg/mL)	Average Number of Surviving *Artemia* nauplii	Average Corrected Mortality Rate (%)
1.	20	16 ± 1	11.3 ± 5.6
2.	10	18 ± 2	5.6 ± 9.6
3.	5	17 ± 1	5.6 ± 5.6
4.	2.5	17 ± 1	5.6 ± 5.6
5.	1.25	17 ± 1	5.7 ± 5.6
6.	Negative control	18 ± 1	0.0 ± 0.0

Note. Negative control indicates zero concentration of *Phyllosticta* sp. strain MH007 extract in the sterile artificial seawater.

Mycotoxin quantification via LC-MS/MS (**Table 2**) confirmed that all 15 targeted common mycotoxins were below the detection limits. Currently, limited information is available on the toxicity of *Phyllosticta* spp. compared to major mycotoxin producers such as *Aspergillus* or *Fusarium* [8].

Table 2: Mycotoxin quantification of dried *Phyllosticta* sp. strain MH007.

No.	Analysis	Limit of Detection (ppb)	Concentration (ppb)
1.	Aflatoxin B1	1	<1
2.	Aflatoxin B2	1	<1
3.	Aflatoxin G1	1	<1
4.	Aflatoxin G2	1	<1
5.	Deoxynivalenol	20	<20
6.	3-acetyldeoxynivalenol	20	<20
7.	15-acetyldeoxynivalenol	20	<20
8.	Fumonisin B1	20	<20
9.	Fumonisin B2	20	<20
10.	T-2	10	<10
11.	HT-2	10	<10
12.	Nivalenol	50	<50
13.	Ochratoxin A	1	<1
14.	Zearalenone	15	<15
15.	Mycophenolic acid	2	<2

Phyllosticta sp. strain MH007 may be a promising functional feed additive, but future studies are required to evaluate its beneficial effects _in vivo_ in barramundi under disease challenge conditions.

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Acknowledgements

This study was supported by the Xiamen University Malaysia Research Fund (Grant No. XMUMRF/2019-C3/ICAM/0004). The authors would also like to thank Jing Sien Chang and Erik Yew Woh Hui for their support throughout the study.