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The Performance of Mangrove Leaf Extract (*Sonneratiaalba*) in Combating Bacterial Associated with Ice–Ice Disease of Seaweed (*Kappaphycusalvarezii*)

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The outbreaks of ice–ice disease since 1999 at various seaweed culture in Indonesia were still very high with significant losses. Moreover, very scanty of the research on the cause and control methods has been developed. Meanwhile, Mangrove leaf extract (*Sonneratiaalba*) contained bacteriostatic that might potentially effective to combat a such disease. This research was aimed to demonstrate the performance of mangrove leaf extract to control bacteria causing ice–ice disease. Mangrove leaf (*S. alba*) was extracted by methanol. Nine bacterial collection from the ice–ice were then *in vitro*-exposed to mangrove extract at concentration of 2500, 5000 and 10,000 mg/l respectively. Research results demonstrated that mangrove leaf extract at all concentrations tested were able to prevent the growth of 9 bacterial strains associated with ice–ice disease. The diameter of clear zone was between 7.47 and 16.17 mm after 24 hours incubation. Thus, it can be concluded that alkaloid, saponin, tannin, triterpenoid/steroid, and flavonoid in the mangrove leaf at concentration as low as 2500 mg/l was able to prevent the growth of 9 bacterial associated with ice–ice disease.

Keywords: Mangrove Leaf Extract, Bacteriostatic, Ice–Ice, Seaweed.

1. INTRODUCTION

The red seaweed, *Kappaphycusalvarezii* is the most important carrageenophytes, a commercial commodity in Indonesia, the Philippines, Malaysia, and Tanzania.[1] In 2009, the world market for carrageenan has increased about 4% per year, with Indonesia producing more than 40% of the carrageenan material.[2, 3] Aquaculture is an effort to increase source of raw material, to increase food nutrition, to generate employment opportunities and income improvement for coastal communities in less developed countries, and to preserve aquatic biological resources. A number of problems are still occurring in *Kappaphycus* farming and the production is often dependent on culture condition and disease outbreak. Diseases such as ice–ice is mainly caused by un-favorable environmental condition and the involvement of some opportunistic bacterial pathogens.[4] Previously research have been reported so far, for example Largo et al.[5] found *Vibrio-Aeromonas* complex and *Cytophaga-Flavobacterium* complex to be the causative factor of early ice–ice whitening of *K. alvarezii*. Vairappan et al.[1] also described, they were 3 strain (*Alteromonas* sp., *Flavobacterium* sp. and *Vibrio* sp.) bacteria associated had been isolated from *K. alvarezii* from cultivation farms at Philippines, Indonesia, Malaysia and Zanzibar. On the other hand, there were 2 strains bacteria (*Stenotrophomonasmaltophilia* and *Vibrio alginolyticus*) to be the causative agent of white bleaching disease.[6]

Mangrove have been a source of interest for their novel natural products as an antibacterial agent. Members of the Sonneratiaceae family have been found *in vitro* to have antibacterial property as they are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids.[7, 8] *Sonneratiaalba* has been used in fish and shrimp farm as non-chemotherapeutic method such as the use of vaccine, probiotics, immune stimulants and natural therapeutics without negative effects on growth, nutrient utilization and carbohydrate and protein digestion.[9–11] However, there have been no studies so far on the role of these mangrove plant extract particularly on Sonneratiaceae family in prevention the occurrence of pathogenic bacteria causing ice–ice disease on *K. alvarezii*. The prevention

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and treatment of these infectious ice–ice diseases by applying products from mangrove plants especially from Sonneratiaceae family extract appears as a possible alternative. Thus, the study is aimed to evaluate the antibacterial activity of mangrove leaf (*S. alba*) extract against bacteria causing ice–ice disease.

2. EXPERIMENTAL DETAILS

The fresh mangrove leaves of *S. alba* were collected from Ujung Piring mangrove stand, located in Jepara, Central Java on April 2014. A four hundred grams of mangrove leaves were shade dried at room temperature and cut into small pieces, pulverized and packed separately in 3 erlenmeyer flasks and then immersed in to methanol until submerged for 24 hours. The solvent was evaporated by using a rotary evaporator to obtain concentrated extracts.[12]

Nine associated bacteria strains isolated from ice–ice thallus of *K. alvarezii* namely *Alteromonasmacleodii* (107), *Bacillus oceanisediminis* (H2), *Pseudomonas stutzeri* (ATCC 17588), *Pseudoalteromonasissachenkonii* (KMM 3549), *Bacillus hunanensis* (JSM 081003), *Bacillus megaterium* (ATCC 14581), *Alteromonas marina* (SW-47), *Aurantimonascoralicida* (WP1), and *Rhodococcusrhodochrous* (DSM 43241) were obtained from Tropical Marine Biotechnology Laboratory, Diponegoro University. All the pathogens were maintained on Zobell 2216E marine agar medium at 33 °C for 96 hours and sub cultured into Zobell 2216E marine broth for 48 hours prior to testing.

Inhibitory interaction test of extract *S. alba* against pathogenic bacteria associated with ice–ice disease was performed by using the agar disk diffusion method according to Refs. [13, 14] with slight modification. Antibacterial assay plates were prepared by pouring 20 ml of Zobell marine agar and allowed to solidify. Plates were dried and 0,1 ml culture of target microorganism in the logarithmic phase (ca. 10^8 cells ml-1) was spread on to agar medium. Blank paper disk (6 mm; Advantec, Toyo Roshi, Ltd., Japan) were impregnated with 15 μl of the extracts (concentration: 2,5 mg/ml; 5 mg/ml; 10 mg/ml). PBS was used as negative control. The plates were then incubated at 37 °C for 48 hours. Each sample was tested in triplicate and the diameter (mm) for the zone of inhibition was measured. Phytochemical test were carried out on all extracts using standard procedures for qualitative determination of phytochemical constituents.[15, 16]

3. RESULTS AND DISCUSSION

Antibacterial is a diverse group of naturally occurring or laboratory-synthesized chemicals at very low concentrations, are able to kill or inhibit the growth of microorganisms.[17] The results show that the leaf extract possessed strong antibacterial activities against almost all associated bacteria at the various concentrations (2,5 mg/ml; 5 mg/ml; 10 mg/ml) as indicated in (Table I). Among the concentration, maximum activity was observed in 10 mg/ml whereas at the concentration 2,5 mg/ml showed the least activity against all the bacteria strains. The antibacterial activity was increased with the increasing concentration. The highest activity was recorded with the methanol leaf extract of *S. alba* against *A. marina* (SW47) ($16,17 \pm 2,97$) followed by *B. hunanensis* (JSM 081003) ($12,10 \pm 3,82$) and *P. issachenkonii* (KMM 3549) ($11,90 \pm 2,29$) respectively. The negative control, PBS did not produce any zone of inhibition.

In this study revealed that the methanol extract from *S. alba* leaf was the effective antibacterial agent against various seaweed pathogens. Many review and research papers shown the information of the biological activities among the members of Sonneratiaceae family. According to Refs. [8, 18] noted that methanolic extract of *S. alba* and *S. apetala* had growth inhibition against gram positive and gram negative bacteria. It has also been tested for antibacterial activity against shrimp pathogen (*Vibrio harveyi*).[9] Mangrove species such as *S. caseolaris* were reported that the methanol extract from leaves contained the highest antibacterial activity and could even kill the MRSA, ESBL, MDR, and the PDR.[14, 19] This species was also tested for enhanced the immune resistance of shrimp by promoting both phagocytic and phenol-oxidase activities and lowering bacterial survival.[10] The highest antibacterial activities are believed to be the presence of high content of secondary metabolites, such as alkaloids, saponins, tannins, triterpenoids/steroids, flavonoids (Table II).

Plant-derived compounds that are potential for antibacterial activity according to Gyawali et al.[20] include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, terpenoids, and

Table II. Phytochemical screening of methanol extract from *S. alba* leaf.

Chemical Group	Methanol extract
Alkaloids	+
Saponins	+
Tannins	+
Triterpenoid/steroids	+
Flavonoids	+

Notes: (+): Present; (−): Absent.

Table I. Antibacterial activity of methanol *S. alba* leaf extract against bacteria cause ice–ice disease.

	Zone of inhibition (mm)			
Pathogens	Control (PBS)	2,5 mg/ml	5 mg/ml	10 mg/ml
A. macleodii (107)	0 ± 0,00	8,37 ± 1,36	8,63 ± 2,11	10,97 ± 1,88
B. oceanisediminis (H2)	0 ± 0,00	7,47 ± 0,75	9,67 ± 2,59	11,23 ± 4,47
P. stutzeri (ATCC 17588)	0 ± 0,00	8,70 ± 2,13	8,10 ± 2,05	10,80 ± 2,07
P. issachenkonii (KMM 3549)	0 ± 0,00	8,37 ± 0,55	9,90 ± 1,77	11,90 ± 2,29
B. hunanensis (JSM 081003)	0 ± 0,00	8,97 ± 1,38	11,17 ± 2,25	12,10 ± 3,82
B. megaterium (ATCC 14581)	0 ± 0,00	10,30 ± 1,90	9,40 ± 0,36	10,00 ± 3,47
A. marina (SW47)	0 ± 0,00	12,20 ± 4,04	14,40 ± 1,55	16,17 ± 2,97
A. coralicida (WP1)	0 ± 0,00	8,87 ± 0,85	8,40 ± 1,71	8,57 ± 1,07
R. rhodochrous (DSM 43241)	0 ± 0,00	8,77 ± 1,08	8,70 ± 0,36	9,30 ± 0,20

akaloids and effectiveness of antibacterial action they produce against pathogens depends on their structural configuration and variations in the chemical composition.[20, 21] Plant-derived antibacterial compounds are thought to cause damaging lipids in the plasma membrane of bacteria, damage the cytoplasmic membrane causing leakage metabolites and besides that inactivate a number of enzymes.[22] Disorder or damage the structure of the plasma membrane can inhibit or impair the ability of the plasma membrane as an osmotic barrier and disrupt a number of bio-synthetics processes required in the membrane.[22] This compound proved to be rich in the members of Sonneratiaceae family.[7]

4. CONCLUSION

The research demonstrated that mangrove leaf extract (*S. alba*) at concentration of 2.5–10 mg/mL were able to prevent the growth of 9 bacteria associated with ice–ice disease of seaweed. Furthermore, to the best of our knowledge, this is the first finding of antibacterial performance of *S. alba* extract to combat ice–ice disease *in vitro*. It is suggested that, methanol extract of *S. alba* leaves may possess promising therapeutic action in the treatment of infectious disease caused by these microorganisms associated with ice–ice disease.

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