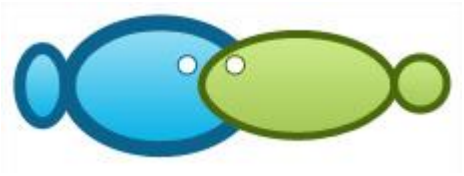

The application of *Sonneratia alba* leaves extracts at different concentrations to enhance the survival and immune reaction of tiger shrimp, *Penaeus monodon*, postlarvae

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Abstract. In recent years, research on the potential and utilization of mangrove plants as alternative materials for disease prevention and immunostimulants in the field of aquaculture has been widely conducted. *Sonneratia alba* is a type of mangrove that is widely studied because it has antibacterial properties and has been used to treat infections in fish and shrimp. Based on this, the current study aimed to determine the potential immunostimulant properties of *S. alba* using extracts in tiger shrimp feed. This study evaluates the use of methanol and diethyl ether extracts of *S. alba* leaves at different concentrations in tiger shrimp, *Penaeus monodon*, survival and immune response. An aquarium, with a volume of 20 L, was filled with 15 L of 28 ppt seawater and stocked with 30 PL45 tiger shrimp as an animal test. The treatments tested were: A) methanol extract 0.1% kg^{-1} feed; B) methanol extracts 1% kg^{-1} feed; C) methanol extracts 10% kg^{-1} feed; D) diethyl ether extracts 0.1% kg^{-1} feed; E) diethyl ether extracts 1% kg^{-1} feed; F) diethyl ether extracts 10% kg^{-1} feed; G) control, without any mangrove extract in their feed. Each treatment was applied with three replications. The parameters monitored were: total hemocyte count (THC), propanol oxidase (ProPO), differential hemocyte count (DHC), total organic matter (TOM), ammonium (NH_4-N), and survival rate of tiger shrimp. The highest survival rate of tiger shrimp was observed in treatment F and the lowest in treatment G, and they were significantly different ($P<0.05$). The use of both methanol extract and diethyl ether extract in feed has a positive influence on increasing tiger shrimp's survival. At the end of the study, total hemocytes and ProPO activity of post-larvae were highest in treatment F and lowest in C, and they were significantly different ($P<0.05$). This study shows that adding *S. alba* diethyl ether extract to a 10% kg^{-1} feed boosts the immune system and makes tiger prawns 48% more likely to survive than the control group.
Key Words: mangrove plant, extraction solvent, alternative feed, immunostimulant, disease prevention.

Introduction. Mangrove plants are native vegetation of coastal ecosystems whose existence needs to be protected because they have an ecological function, namely protecting beaches from abrasion, accelerating sedimentation, controlling seawater intrusion, protecting the area behind mangroves from high waves and strong winds, and providing a place for fish and another biota to live, lay eggs, and look for food (Akram et al 2023; Batubara et al 2024; Su et al 2021; Wang & Gu 2021; Zhang et al 2022; Zhuang et al 2020). Apart from these ecological functions, in recent years mangroves have begun to be developed as ingredients for food, drinks, medicines, and natural dyes which have quite high economic value (Budiyanto et al 2022; Duryat et al 2023). In several areas, both within the country and in other countries, mangrove ecosystems have been used as ecotourism destinations that can contribute to regional income (Spalding & Parrett 2019; Veettil et al 2023).

Mangrove plants have long been explored and studied because of their ecological importance, their use in various medical industries, as previously explained, and their use for ecotourism. In recent years, studies regarding the potential and use of mangrove

plants as alternative disease prevention and immunostimulant materials in the field of aquaculture have also been carried out by several researchers (Alam et al 2022; Azis et al 2022; Kannappan et al 2018; Kumar et al 2023; Manik et al 2020; Muliani et al 2020, Muliani et al 2022; Nurhidayah et al 2020; Sravya et al 2023; Susianingsih et al 2022; Syawal et al 2020; Yoswaty et al 2021).

Numerous investigations have been conducted to ascertain the antimicrobial characteristics of diverse mangrove species (Acharya et al 2020; Baskaran & Mohan 2012; Ramesh et al 2014; Ravikumar et al 2011). According to their research, mangroves contain organic molecules with antibacterial capabilities. This revelation sparked more interest and prompted more research. It has been reported that *Salicornia brachiata* and *Rhizophora mucronata* were found to be very efficient against various *Vibrio* species, including *V. harveyi*, *V. vulnificus*, *V. alginolyticus*, *V. anguillarum,* and *V. loci*. It was further reported that these mangrove species demonstrated their ability to fight bacterial infections, both on a laboratory and field scale. In addition, these mangrove plants not only demonstrated effectiveness against *Vibrio* species but also demonstrated their usefulness in reducing fish diseases caused by other bacterial infections by *Serratia* sp., *Aeromonas hydrophila*, *Bacillus subtilis*, and *V. harveyi*. This is important for aquaculture because bacterial infections can cause large financial losses (Baskaran & Mohan 2012).

Another mangrove species, *Excoecaria agallocha*, has been studied for its capacity to ward off *Flavobacterium* spp., the organisms that cause bacterial infections in fish. *Excoecaria agallocha* is beneficial in avoiding these infections, underscoring the adaptability of mangrove plants in the fight against aquatic bacterial diseases (Laith & Najiah 2014; Sabu et al 2022). Meanwhile, *Avicenia marina* has been studied for its use in preventing ice-ice disease in seaweed, *Kappaphycus alvarezii* (Rahman et al 2023). Another type of mangrove that has high economic value is the *R. apiculata species.* This type of mangrove has been reported to contain compounds 1, 2-diacetate, cyclododecane, 2-chloropropionic acid, and squalene. These substances are in charge of the mangroves' extract antagonism against *V. harveyi*. It further explained that crude extract of *R. apiculata* can be applied as a substitute for antibiotics to prevent shrimp disease (Kannappan et al 2018). The method of extraction also has a significant impact in determining the effectiveness of mangrove extracts against particular bacterial strains, in addition to the choice of mangrove species. It is clear that *Acanthus ilicifolius*, with a resistance diameter of 16.4 mm, displays more potential as an anti-*V. harveyi* VSH5 agent, compared to leaf extracts from *A. marina*, *A. officinalis*, and *R. mucronate* (Ramesh et al 2014). Furthermore, when the chloroform extraction method from the leaves of *E. agallocha* was used, dangerous bacteria such as *A. hydrophila*, *V. parahaemolyticus*, and *V. harveyi* were unable to multiply and grow in fish (Ravikumar et al 2011). This demonstrates how crucial the extraction method is to improving the antibacterial effects of mangroves.

A kind of mangrove known as *Sonneratia* spp. has drawn attention for its antibacterial characteristics, and have been used to treat infections in fish and shrimp, as well as in the context of human health (Limbago et al 2021; Manilal et al 2015; Pratiwi 2021; Thuoc et al 2018). One form of mangrove that has been studied and is being developed as a replacement for chemical treatments to prevent shrimp infections is *S. alba.* This type of mangrove is found growing around river flows and up to river mouths. This plant has brownish-white stems that resemble trees. The bisexual blooms on the wrinkly bark measure 5–12.5x39 cm and bloom alone or in clusters of three or more. The petals consist of 6-8 with a bell shape with a length of 2-2.5 cm. It has many stamens which are white at the tips and yellow at the base. Often referred to as "Mangrove Apple" because it has round fruit like an apple (Avenido & Serrano 2012). It has been established that some *Sonneratia* species, notably *S. alba*, *S. caseolaris*, and *S. lanceolata*, are efficient antibacterial agents (Nguyen et al 2024; Pratiwi 2021). *S. alba* has been reported to exhibit inhibitory effects against a range of bacteria, including Gram-positive species such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, and *Sarcina lutera*, as well as gram-negative bacteria commonly associated with human diseases, including *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*,

Salmonella typhi, *V. parahaemolyticus*, and *V. mimicus* (Avenido & Serrano 2012; Cahyadi et al 2020; Saad et al 2012). Nevertheless, *S. caseolaris* has been demonstrated to be antibacterial against gram-positive bacteria, specifically *Bacillus subtilis* and *Bacillus coagulans*, as well as gram-negative bacteria like *E. coli* and *Proteus vulgaris*, as well as the fungus *Saccharomyces cerevisiae* (Simlai et al 2014). The methanol extract of *S. caseolaris* was reported to have minimum inhibitory concentrations (MIC) of 3.90 mg mL^{-1} for *B. subtilis* and 7.81 mg mL^{-1} for *B. coagulans*, respectively. Notably, the moderate MIC value for *P. vulgaris* was 62.5 mg mL^{-1}, indicating that *S. caseolaris* has a greater efficacy against gram-positive bacteria than gram-negative ones. Previous research results show that the MIC values of *S. alba* methanol extract against *V. harveyi* and *V. parahaemolyticus* were 1 mg L^{-1} and 0.1 mg L^{-1}, respectively. This shows that the antibacterial activity of mangrove extracts of *S. alba* and *B. gymnorrhiza* is higher against *V. parahaemolyticus* than *V. harveyi*. Additionally, it is claimed that the butanol fraction of *B. gymnorrhiza* and the diethyl ether fraction and methanol extract of *S. alba* exhibit comparable antibacterial properties. Apart from being antibacterial, *S. alba* has also been identified as potential as an anti-WSSV. Diethyl ether extract and methanol extract from *S. alba* and methanol extract from *B. gymnorrhiza* are reported to be effective in inactivating WSSV, thereby increasing shrimp survival (Effendi et al 2023; Muliani et al 2017; Muliani & Susianingsih 2018).

Considering the possible applications of *S. alba* extract as an antimicrobial, antifungal, and antioxidant both in terms of human health and in aquaculture, as previously reported (Saptiani et al 2020), researchers are interested in conducting studies related to the use of *S. alba* extract in shrimp feed, which is expected to improve the immune system and survival. This study's objective was to assess the efficacy of adding *S. alba* mangrove methanol and diethylene extract to tiger prawn (*Penaeus monodon*) feed at different concentrations. The scientists predict that the *S. alba* extract in shrimp feed will have a positive effect on the immune response, therefore increasing the survival rate of postlarvae rearing in the nursery or growing out in a brackish water pond.

Material and Method

Sample preparation. *S. alba* leaves were collected from the Bone district, South Sulawesi. S*. alba* leaves were further cleaned and dried for approximately 2 weeks. Dried leaves of *S. alba* were mashed using a blender and sifted using a sieve with holes about 1 mm in size to obtain a flour-shaped sample.

Preparation of methanol and diethyl ether extracts. Maceration was the extraction method used. The dried *S. alba* powder (1,000 g) was soaked in 1,000 mL of 80% methanol for 1x24 hours while occasionally shaking and then filtered with a sieve cloth. With Whatman's No. 1 filter paper (GE Healthcare, Buckinghamshire, UK), the filtrate was re-filtered and a Buchner funnel. The residue was re-soaked twice or until a less solvent-colored filtrate was obtained and the same treatment was obtained. A dark-colored concentrated methanol extract was prepared by combining and condensing the three filtrates using a rotary evaporator (EYELA N-N, Tokyo, Japan) at a temperature below 40°C. Using a split mouthpiece, the 10 g dark methanol extract was diluted in 50 mL of distilled water and then progressively extracted with equal quantities of diethyl ether to get diethyl ether. To prepare them for use as samples, all fractions were freeze-dried, powdered, and evaporated at low temperatures and low pressure.

Experimental diet preparation. The methanol and diethyl ether extracts from *S. alba* leaves were chosen for this investigation to assess their potential as immunostimulants and to increase the shrimp's survival rate. As a reference diet, commercial shrimp feed containing 30–40% protein was utilized. To prepare the experimental feed, approximately 500 g of the commercial pellets, both with and without the extracts, were ground using a grinder. These ground pellets were then combined with a predetermined

quantity of mangrove extract and further bound together using a binder called Progol (at a concentration of 10%). The feed that has been re-peletted is dried for further use.

Feeding experiment. A set of 21 aquarium units (30x45x30 cm^3) was used as an experimental container for each shrimp and was filled with 15 L of seawater (30 ppt). Aeration was provided in each aquarium for oxygenation. Before use, the aquarium and seawater were disinfected with 150 ppm chlorine solution for 24 hours and neutralized with 75 ppm sodium thiosulfate. *P. monodon* post-larvae (PL_{45}) previously acclimatized for seven days were used as test animals under laboratory conditions. Post-larvae were randomly distributed to each aquarium at a density of 30 ind. per aquarium. Post-larvae were fed on an experimental diet three times a day at doses of 3.5% of body weight at 8:00 a.m., 12:00 p.m., and 4:00 p.m. A fully randomized design was employed in the experiment, with the treatments being: (A) methanol extract (0.1% kg^{-1} feed); (B) methanol extracts (1% kg^{-1} feed); (C) methanol extracts (10% kg^{-1} of feed); (D) diethyl ether extracts (0.1% kg^{-1} of feed); (E) diethyl ether extracts (1% kg^{-1} of feed); (F) diethyl ether extracts (10% kg^{-1} of feed); and (G) control. Each treatment is repeated three times, with a duration of maintenance of one month.

Variables and measurement method. Hemolymph was collected on the first, 15, and 13 days following the challenge to ascertain the impact of the test on the immune response. Prophenoloxidase (ProPO) activity and total hemocyte count (THC) are examples of immunological responses that have been seen. To test THC, 0.1 mL of hemolymph was extracted from the second abdominal segment using a 26-gauge needle and a 1 mL capacity syringe that contained 0.3 mL of an anticoagulant and 3.8% Na-citrate. Observation of total hemocyte cells was carried out by flowing hemolymph fluid into the hemacytometer and then observed under a microscope with 100x magnification. Hemocyte cell differentiation was determined using 400x magnification and was distinguished into 3 cell forms, namely hyaline, semi-granular and granular. Using a spectrophotometer, proPO activity was assessed concerning dopachrome formations brought about by L-dihydroxyphenylalanine (L-DOPA). The spectrophotometric method was utilized to evaluate the activity of phenoloxidase by producing dopachrome from L-dihydroxyphenylalanine (L-DOPA) through a procedure that had been previously described by multiple researchers (Hernández-López et al 1996; Liu & Chen 2004). As supporting data, observations of water quality parameters are also carried out, which include total organic matter (TOM) by the titrimetric method and ammonium (NH4-N) by the spectrophotometer method (Clesceri et al 1998). The immune response of tiger shrimp was carried out on days 15 and 30. Meanwhile, water quality parameters are carried out at the start and end of the research. After the study, the tiger shrimp's survival rate following the challenge test was noted.

Data analysis. The immunological response and survival rate of *P. monodon* post-larvae were examined using an analysis of variance (ANOVA) at a confidence level of 0.95 to ascertain the impact of *S. alba* leaf extract administration on the feed. LSD was then used for additional analysis.

Results

P. monodon immune response after 15 days maintenance. Table 1 presents the results of the immune system analysis on the use of methanol and diethyl ether extract of *S. alba* at different concentrations in the feed after 15 days of maintenance. The total hemocyte count (THC) for treatment G (control) was the highest, whereas treatment C (*V. harveyi*-infected shrimp were treated with a 10% kg^{-1} feed methanol extract of *S. alba*) had the lowest THC. However, the results of the statistical analysis show that the *P. monodon*'s THC value in the treatment is not significantly different from other treatments. It was the control that had the highest average THC value, but the logarithmic and statistical analysis used to normalize the data showed that results of the 10% kg^{-1} methanol extract dose and of the 1% kg^{-1} diethyl ether extract dose differed

considerably (P<0.05). The number of hyaline cells was lowest in the treatment with methanol *S. alba* extract at 10% kg^{-1} of feed. It was also lowest in the treatment with diethyl extract at 0.1% kg^{-1}, and then it was lowest in the treatment with methanol extract at 1% kg^{-1} of feed (Table 1). The statistical analysis results revealed that using a 10% kg^{-1} feed of *S. alba* methanol extract showed a noticeable difference (P<0.05) from the control.

Table 1

Immune response of *Penaeus monodon* to the use of methanol and diethyl ether extract of *Sonneratia alba* at different concentrations in feed after 15 days of maintenance

Treatments	*THC (x 10^4 cells mL^{-1})*	*Granular cell (%)*	*Semi-granular cell (%)*	*Hyalin (%)*
A. Methanol extract 0,1% kg^{-1} feed	1,088.67^{ab}	67.81^{ab}	5.85^{ab}	26.35^{ab}
B. Methanol extract 1% kg^{-1} feed	622.33^{ab}	76.94^{a}	8.70^{a}	16.03^{b}
C. Methanol extract 10% kg^{-1} feed	255.33^{b}	92.67^{a}	0^{b}	7.33^{b}
D. Diethyl ether extract 0,1% kg^{-1} feed	522.33^{ab}	72.34^{a}	2.73^{ab}	18.13^{b}
E. Diethyl ether extract 1% kg^{-1} feed	1,433.33^{a}	74.39^{a}	2.31^{ab}	37.34^{ab}
F. Diethyl ether extract 10% kg^{-1} feed	1,589.00^{a}	64.47^{ab}	0.39^{b}	35.14^{ab}
G. Control	1,722.33^{a}	39.02^{b}	6.087^{ab}	54.70^{a}

The same-column numbers with distinct superscripts indicated a significant difference (P<0.05).

The value of ProPO in tiger shrimp after 15 days of maintenance by *S. alba* extract through the feed is presented in Figure 1. The treatments that used *S. alba* extract methanol at a rate of 1% kg^{-1} feed had the highest ProPO values, whereas the control treatment had the lowest. Nevertheless, no significant difference (P>0.05) was found in the statistical analysis between the treatments.

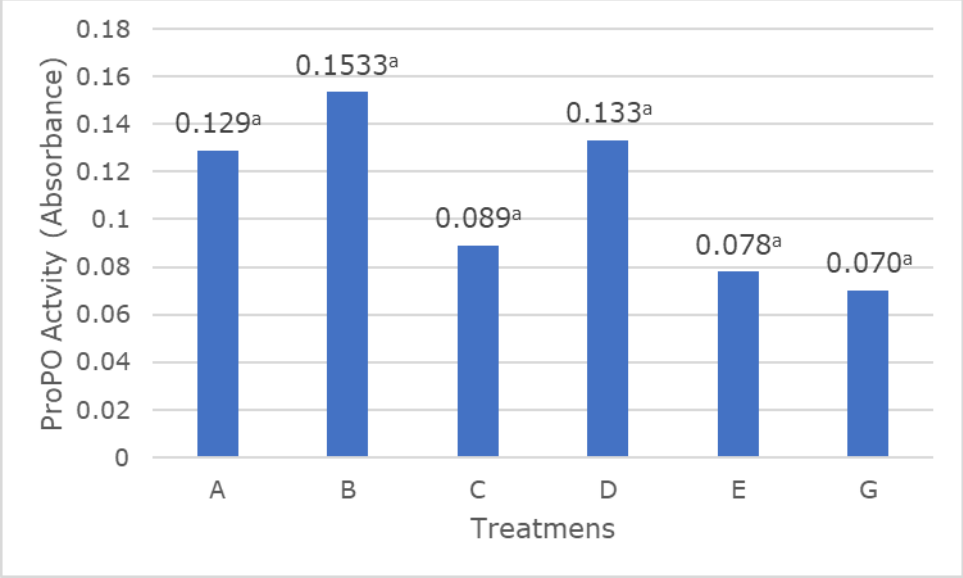

Figure 1. ProPO activity of tiger shrimp on the use of methanol extract and diethyl-ether mangroves *Sonneratia alba* at different concentrations in feed after 15 days maintenance. (A. Methanol extract 10%.kg feed, B. Methanol extract 1% kg^{-1} feed, C. Methanol extract 10%/kg feed, D. Diethyl ether extract 0,1%/kg feed, E. Diethyl ether extract 1%.kg feed, F. Diethyl ether extract 10%/kg feed, G. Control).

Tiger shrimp immune response after 30 days of maintenance. Table 2 displays the tiger shrimp's immunological response following 30 days of maintenance. The table indicates that *P. monodon* treatments with diethyl ether extract (10% kg^{-1} of feed) had the highest THC value, whereas the treatments with methanol extract (10% kg^{-1} of feed) had the lowest THC value. The THC value in the 10% kg^{-1} feed treatment using diethyl ether extract was substantially different ($P<0.05$) from the THC value in the treatment using 10% kg^{-1} feed methanol extract, but not statistically different ($P>0.05$) from other treatments, according to the statistical analysis results.

Table 2

Immune response of *Penaeus monodon* to the use of methanol and diethyl ether extracts of *Sonneratia alba* at different concentrations in feed after 30 days of maintenance

Treatments	*THC (x 10^4 cells mL^{-1})*	*Granular cell (%)*	*Semi-granular cell (%)*	*Hyaline cell (%)*
A. Methanol extract 0,1% kg^{-1} feed	$1,377.67^{ab}$	34.69^{a}	5.28^{ab}	79.83^{a}
B. Methanol extract 1% kg^{-1} feed	$1,289.00^{a}$	38.94^{a}	14.65^{ab}	46.33^{a}
C. Methanol extract 10% kg^{-1} feed	77.67^{b}	33.33^{a}	0.00^{b}	66.67^{a}
D. Diethyl ether extract 0,1% kg^{-1} feed	$1,000.00^{a}$	50.75^{a}	23.32^{a}	20.80^{a}
E. Diethyl ether extract 1% kg^{-1} feed	900.00^{ab}	38.15^{a}	23.49^{a}	38.36^{a}
F. Diethyl ether extract 10% kg^{-1} feed	$1,466.67^{a}$	94.41^{a}	14.95^{ab}	53.46^{a}
G. Control	333.33^{ab}	33.72^{a}	6.67^{ab}	59.60^{a}

The same-column numbers with distinct superscripts indicated a significant difference ($P<0.05$).

The value of ProPO in tiger shrimp after 30 days of administration of *S. alba* extract through the feed is presented in Figure 2. The highest ProPO value was in treatment B (using extra methanol *S. alba* 1% kg^{-1} feed), followed by treatment F (10% kg^{-1} feed of diethyl ether extract), and the lowest was in treatment C (10% kg^{-1} feed of methanol extract). The statistical analysis showed that there were significant differences ($P>0.05$) in the ProPO values of treatments B and F compared to treatment C.

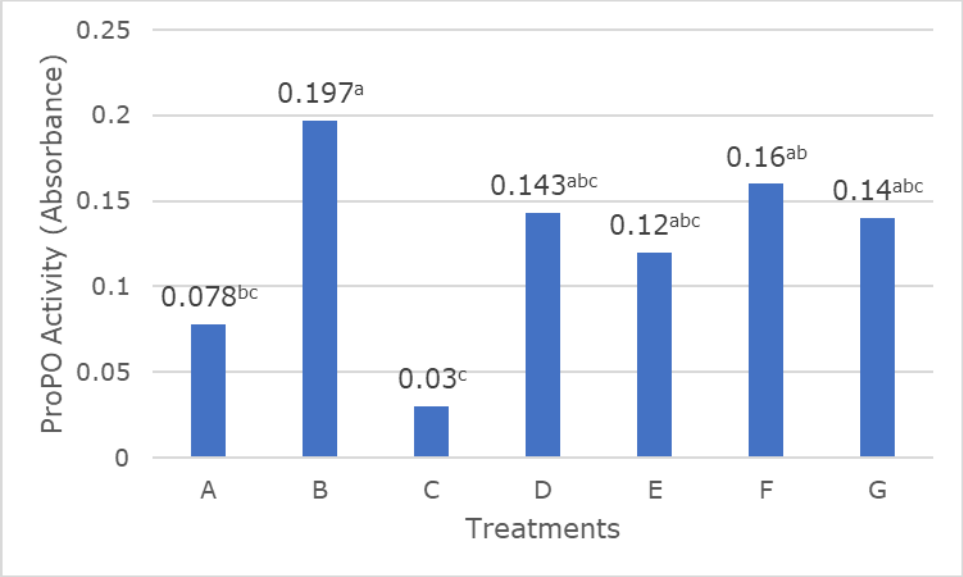

Figure 2. ProPO activity of tiger shrimp on the use of methanol and diethyl ether extracts of *Sonneratia alba* at different concentrations in feed after 30 days of maintenance (A. Methanol extract 10% kg^{-1} feed, B. Methanol extract 1% kg^{-1} feed, C. Methanol extract 10% kg feed, D. Diethyl ether extract 0,1% kg^{-1} feed, E. Diethyl ether extract 1% kg^{-1} feed, F. Diethyl ether extract 10% kg^{-1} feed, G. Control).

Survival of P. monodon. Table 3 shows the survival rate of *P. monodon* fed feed containing *S. alba* mangrove extract. The table shows that the treatments with the

highest *P. monodon* survival rates used diethyl ether of *S. alba* extract at 10% kg^{-1} of feed, followed by treatments with the lowest tiger shrimp survival rates using 1% kg^{-1} of diethyl ether of *S. alba* extract. According to the statistical analysis, the survival rate of shrimp in treatments containing 10% kg^{-1} and 1% kg^{-1} diethyl ether extract of feed differed considerably ($P<0.05$) from the control treatment, but not significantly ($P>0.05$) from the other treatments. This indicates that tiger shrimp survival is positively impacted by the use of mangrove extracts in feed, specifically methanol and diethyl ether extracts.

Table 3

The survival rate of *Penaeus monodon* on the use of methanol and diethyl ether extracts of *Sonneratia alba* at different concentrations in feed after 30 days of maintenance

Treatment	*Survival rate (%)*
A. Methanol extract 0,1% kg^{-1} feed	63.33^{ab}
B. Methanol extract 1% kg^{-1} feed	53.33^{ab}
C. Methanol extract 10% kg^{-1} feed	53.33^{ab}
D. Diethyl ether extract 0,1% kg^{-1} feed	56.67^{ab}
E. Diethyl ether extract 1% kg^{-1} feed	73.33^{a}
F. Diethyl ether extract 10% kg^{-1} feed	76.67^{a}
G. Control	40.00^{b}

The same-column numbers with distinct superscripts indicated a significant difference ($P<0.05$).

Water quality parameters. As supporting data, an analysis of the content of TOM and NH_4-N in maintenance water was carried out when the research was finished. The treatment utilizing 0.1% kg^{-1} of diethyl ether extract had the highest quantity of NH_4-N, while the control had the lowest (Figure 3).

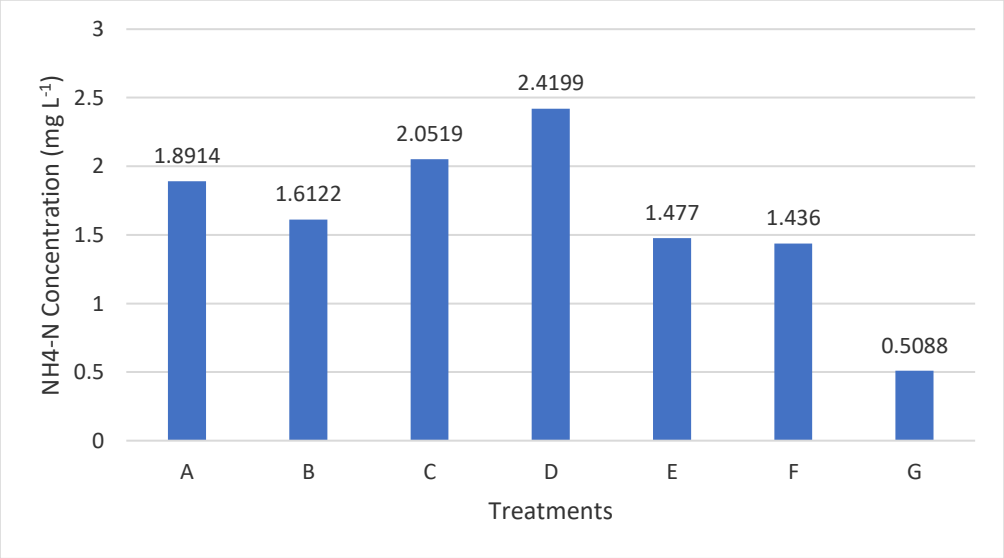

Figure 3. NH_4-N concentration (mg L^{-1}) on the use of methanol and diethyl ether extract of *Sonneratia alba* at different concentrations in feed after 30 days maintenance (A. Methanol extract 10% kg^{-1} feed, B. Methanol extract 1% kg^{-1} feed, C. Methanol extract 10% kg^{-1} feed, D. Diethyl ether extract 0,1% kg^{-1} feed, E. Diethyl ether extract 1% kg^{-1} feed, F. Diethyl ether extract 10% kg^{-1} feed, G. control)

The treatment utilizing a 1% kg^{-1} feed methanol extract had the highest TOM concentration, while the control treatment had the lowest TOM concentration (Figure 4). The use of mangrove extracts, both methanol extract and diethyl ether extract, contributes to an increase in NH_4-N and TOM content in tiger shrimp maintenance water. It can be seen that the concentration of these two parameters in the control (without

mangrove extract in feed) is lower than in treatments using mangrove extracts, both methanol and diethyl ether extracts.

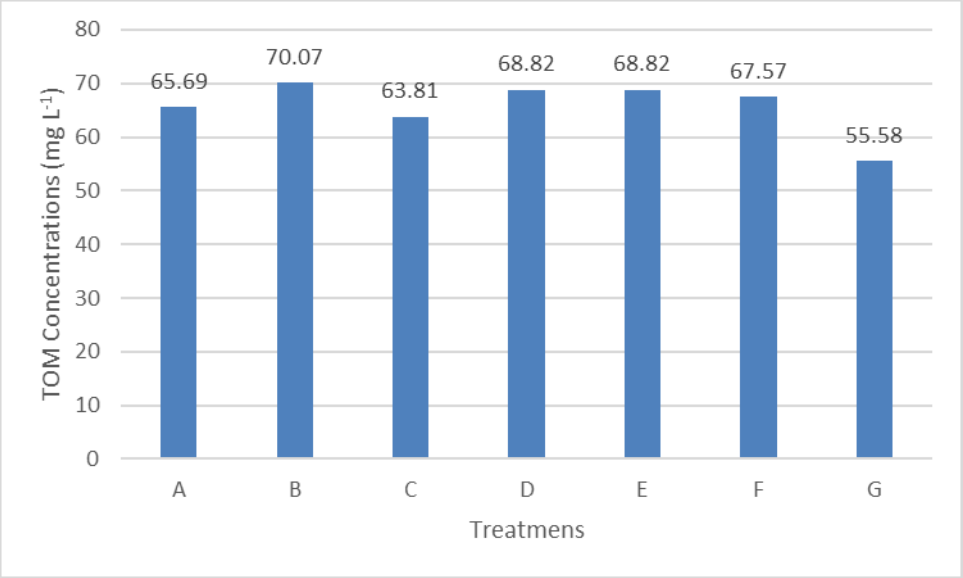

Figure 4. TOM concentration (mg L^{-1}) on the use of methanol and diethyl ether extracts of *Sonneratia alba* at different concentrations in feed after 30 days of maintenance (A. Methanol extract 10% kg^{-1} feed, B. Methanol extract 1% kg^{-1} feed, C. Methanol extract 10% kg^{-1} feed, D. Diethyl ether extract 0,1% kg^{-1} feed, E. Diethyl ether extract 1% kg^{-1} feed, F. Diethyl ether extract 10% kg^{-1} feed, G. control)

Discussion. Hemocyte differentiation, or DHC, is the comparison of hyaline, granular, and semi-granular cells respectively. The three different hemocyte cell types play crucial roles in the immune system of shrimp. Granular and semi-granular cells work together to produce and release the prophenoloxidase system, in addition to performing cytotoxic actions. Hyaline cells provide immunity through phagocytosis. Phagocytic activity is attributed to hyaline cells, whereas granular and semi-granular cells are involved in the production of antimicrobial compounds, enzyme proteases, and reactive oxygen species like hydrogen peroxide and superoxide anions (Johansson et al 2000). In crustaceans, the total hemocyte counts are composed of 4–20% granular cells, 9–30% semi-granular cells, and 50–80% hyaline cells. However, the distribution of these three cell types depends a lot on the species, stage of molting, and physiological state of the organism (Johansson et al 2000; Sung et al 1999).

P. monodon was fed mangrove extract through feed, and there were more granular cells than hyaline cells or semi-granular cells (Table 1). Granular cells in tiger shrimp fed with *S. alba* leaf extract at different doses were highest in the treatment fed with a methanol extract of 10% kg^{-1} of feed, and the lowest was in the control treatment. The results of statistical analysis of tiger shrimp granular cells fed with *S. alba* extract at different doses showed that all treatments using mangrove extract did not show a noticeable difference. However, when compared to the control (without mangrove extract in shrimp feed), the treatments using 1 and 10% methanol extract and 0.1% and 1% diethyl ether extract showed significant differences ($P<0.05$). As with granular cells, semi-granular cells in vibriosis disease prevention studies using *S. alba* mangrove extract vary according to treatment. Semi-granular cell presentation was highest in treatments using *S. alba* methanol extract of 1% kg^{-1} of feed and lowest in the use of methanol extract of 10% kg^{-1} of feed. The results of the statistical analysis showed that the percentage of semi-granular cells in the two treatments was significantly different ($P<0.05$). In addition, the percentage of semi-granular cells in treatments using 1% kg^{-1} methanol extract was significantly different ($P<0.05$) from the treatment with 10% kg^{-1} feed of methanol extract and 10% kg^{-1} feed of diethyl ether.

A comparison of granular, semi-granular, and hyaline cells in tiger shrimp after the 30th day of rearing is presented in Table 3. In the table it can be seen that the comparison of the three cell types varies according to the treatment. According to Sung et al (1999), the total hemocytes in crustaceans consist of 50 to 80% hyaline cells, 9 to 30% semi-granular cells, and 4 to 20% granular cells. The amount of granular, semi-granular, and hyaline cells in crustaceans changes a lot depending on the species, the molting phase, and the body's health. Each species exhibits a different distribution and function of hemocytes, which are also influenced by the molting phase and physiological conditions such as infection (Alvarez & Chung 2015; Johansson et al 2000; Sung et al 1999; Wu et al 2019).

Additional research is required to understand the mechanisms underlying this variation and its implications for crustacean health and immune function. The results of the statistical analysis of the percentage of granular cells and hyaline cells in tiger shrimp did not show a noticeable difference ($P>0.05$) between treatments. The percentage of semi-granular cells in tiger shrimp was highest in treatments using diethyl ether extract of 1% kg^{-1} of feed and was excluded by treatments using diethyl ether extract of 0.1% kg^{-1}, and lowest in treatments using methanol extract of 10% kg^{-1} of feed. The analysis's findings demonstrated a substantial difference between the semi-granular cell treatments with 10% kg^{-1} of methanol extract and with 0.1% kg^{-1} and 1% kg^{-1} diethyl ether extract. As with other shrimp immunity parameters, the ProPo value of tiger shrimp also differs according to the treatment. ProPO values were lowest in treatments using *S. alba* methanol extract of 10% kg^{-1} of feed and highest in treatments using 1% kg^{-1} of *S. alba* methanol extract. According to statistics, there was a significant difference ($P<0.05$) between the ProPo values in the two treatments, and the ProPO values in the two treatments did not differ significantly ($P>0.05$) from the other treatments.

The results of statistical analysis showed that both diethylene extract and methanol extract of *S. alba* were capable of raising the THC value of tiger prawns at tolerable concentrations. According to Muliani et al (2015), the maximum average hemocyte cell value in the diethyl ether extract of *S. alba* treatment was 14.80x107 cells mL^{-1}. Additional research revealed that the THC value of shrimp was highest when treated with diethyl ether extract from *S. alba* and was significantly different when treated with *B. gymnorrhiza* methanol extract and control (Kannappan et al 2018). The THC value is a sign of the state of health of shrimp. A method to increase the THC value of shrimp is by providing immunostimulants.

The use of *S. alba* leaf methanol and diethyl ether extracts had a positive influence on increasing tiger shrimp survival rates. *P. monodon* with the greatest survival rate was found in treatment F, which included 10% kg^{-1} feed of diethyl ether extract, followed by treatment E (diethyl ether extract, 1% kg^{-1} feed), and the lowest survival rate was observed in treatment G (control). The study's findings demonstrated that the use of diethyl ether extract in feed could increase tiger shrimp survival at the end of the research by 46-48% compared to the control. *P. monodon* mortality was significantly higher in treatments without *S. alba* mangrove extract than in treatments with mangrove extract, according to earlier research findings. This shows that the use of *S. alba* mangrove leaf extract can increase tiger shrimp survival (Muliani et al 2021; Nurhidayah et al 2020). The effectiveness of using mangrove extracts to improve shrimp survival has also been reported by previous researchers with different types of mangroves. It has been reported that butanol extract from the leaves of *A. ilicifolius* at a concentration of 300 mg L^{-1} increases the resistance to disease and prevents shrimp mortality (Kannappan et al 2018; Kannappan et al 2021), while *Avicennia alba* extracts at a concentration of 300 mg L^{-1} effectively acts as an immunostimulant to increase shrimp survival (Mahenda et al 2023).

The study's findings provide new information that focuses on testing the impact of methanol and diethyl ether extracts derived from *S. alba* leaves on the immunological response and survival rate of tiger shrimp. The results of this study indicate the potential use of this extract as an immunostimulant for tiger prawns, improving their immune system and consequently increasing their chances of survival. Furthermore, this study seeks to provide valuable insight into the extract concentration and type of extract that

produces the most significant immune response and increases survival rates in tiger prawns. By identifying the optimal concentration and type of extract, this research not only contributes to our understanding of the potential application of natural ingredients in aquaculture but also provides practical guidance for shrimp farmers who wish to improve the health and survival of their livestock.

Conclusions. *P. monodon* survival was highest in the treatment using 10% *S. alba* diethyl ether extract per kg feed, followed by the treatment using 1% *S. alba* diethyl ether extract per kg feed, and lowest in the control. The diethyl ether extract of *S. alba* mangrove is more likely to boost the immune system of *P. monodon* than the methanol extract. It also increases the survival rate of *P. monodon* by up to 30% more at the same concentration and by 48% compared to the control group (which did not use mangrove extract).

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