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The effectiveness of methanol extract and fractionations from mangrove leaves *Sonneratia alba* and *Bruguiera gymnorrhiza* to prevent white spot syndrome virus (WSSV) infection in black tiger shrimp *Penaeus monodon*

Efektivitas ekstrak metanol dan hasil fraksinasi dari daun *mangrove Sonneratia alba* dan *Bruguiera gymnorrhiza* untuk pencegahan infeksi *white spot syndrome* virus (WSSV) pada udang windu *Penaeus monodon*

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ABSTRACT

The study aimed to determine the effectiveness of mangrove leaves (*Sonneratia alba* and *Bruguiera gymnorrhiza*) to prevent WSSV infection in black tiger shrimp, *Penaeus monodon*. The mangrove leaves were taken from Maros and Pangkep Regency. Mangrove leaves dried for two weeks, made into flour, extracted and then evaporated. The yield partitioned used two solvents, butanol and diethyl ether. Anti WSSV activity test was done by injection method with the ratio of mangrove extract and WSSV was 2:1 as much as 100 µL/ind. The treatments were; A) Water fraction of *S. alba* + WSSV suspention; B) Butanol fraction of *S. alba* + WSSV suspention; C) Diethyl ether fraction of *S. alba*+ WSSV suspention; D) Methanol extract of *S. alba* + WSSV suspention; E) Water fraction of *B. gymnorrhiza* + WSSV suspention; F) Butanol fraction of *B. gymnorrhiza* + WSSV suspention; G) Diethyl ether fraction of *B. gymnorrhiza* + WSSV suspention; H) Methanol extract of *B. gymnorrhiza* + WSSV suspention; I) Positive control (WSSV suspention without mangrove extract). Each treatment was repeated three times with 10 days of rearing period. The results showed that the highest activity of anti-WSSV from *S. alba* was in diethyl ether fraction and the methanol extract, while the highest activity of anti-WSSV from *B. gymnorrhiza* was in butanol fraction. The diethyl ether fraction and the methanol extract of *S. alba* and the butanol fraction of *B. gymnorrhiza* were capable to stimulate immune response of shrimp, deactivating WSSV, and it increased the black tiger shrimp survival rate.

Keywords: antiviral, white spot syndrome virus, mangrove, *S. alba, B. gymnorrhiza, Penaeus monodon*

ABSTRAK

Penelitian ini bertujuan menguji efektivitas daun bakau *Sonneratia alba* dan *Bruguiera gymnorrhiza* untuk mencegah infeksi WSSV pada udang windu *Penaeus monodon*. Daun *mangrove S. alba* dan *B. gymnorrhiza* diambil dari Kabupaten Maros dan Pangkep. Daun *mangrove* dikeringanginkan selama dua minggu, dibuat tepung, diekstraksi dengan metanol 80%, dan dievaporasi. Rendemen dipartisi menggunakan dua jenis pelarut yaitu butanol dan dietileter. Uji aktivitas anti-WSSV dilakukan metode penyuntikan dengan perbandingan ekstrak *mangrove* dengan WSSV 2:1 sebanyak 100 µL/ekor. Perlakuan yang diuji adalah; A) Fraksi air *S. alba* + suspensi WSSV; B) Fraksi butanol *S. alba* + suspensi WSSV; C) Fraksi dietileter *S. alba* + suspensi WSSV; D) Ekstrak metanol *S. alba*+ suspensi WSSV; E) Fraksi air *B. gymnorrhiza* + suspensi WSSV; F) Fraksi butanol *B. gymnorrhiza* + suspensi WSSV; G) Fraksi dietileter *B. gymnorrhiza* + suspensi WSSV; H) Ekstrak metanol *B. gymnorrhiza* + suspensi WSSV; I) Kontrol positif (suspensi WSSV tanpa ekstrak *mangrove*). Tiap perlakuan diulang tiga kali dan lama pemeliharaan 10 hari. Hasil penelitian menunjukkan bahwa aktivitas anti-WSSV *S. alba* tertinggi pada fraksi dietileter dan ekstrak metanol, sedangkan aktivitas anti-WSSV *B. gymnorrhiza* tertinggi pada fraksi butanol. Fraksi dietileter dan ekstrak metanol *S. alba* serta fraksi butanol *B. gymnorrhiza* mampu menstimulasi respons imun udang, efektif dalam menonaktifkan WSSV, dan meningkatkan sintasan udang windu.

Kata kunci: antiviral, *white spot syndrome virus*, *mangrove, S. alba, B. gymnorrhiza*, udang windu

INTRODUCTION

The case of shrimp mortality in brackish-water pond nowadays is still occurring continously and is one of the causing-agent of white spot syndrome virus (WSSV) infection (Martorelli *et al*., 2010; Salehi, 2010; Sanchez-Paz, 2010; Tendencia *et al*., 2010; Cavilla *et al*., 2011; Hoa *et al*., 2011a; Iqbal *et al*., 2011; Stentiford & Lightner, 2011; Ashokkumar *et al*., 2012; Selvam *et al*., 2012; Arafani *et al*., 2016). Rahma *et al*. (2014) reported that by then of WSSV, the productivity of shrimp decreases up to 30.5% during the past five years, from 180,000 tons in 1995 to 125,000 tons in 2000.

White spot virus (WSV) is one of the DNA virus, rods shaped, the genus is Whispovirus, the family is Nimaviridae. WSSV has a double strand DNA sizing from 292.9 to 307.2 kb (Sanchez-Martinez *et al*., 2007). Beside of infecting shrimp WSSV in brackish-water pond, it has been reported found infecting the broodstock shrimp (De Mello *et al*., 2011; Sethi *et al*., 2011), juvenile shrimp, and even wild organisms lived in brackish-water pond, such as greasy back shrimp *Metapenaeus* sp., jawla paste shrimp *Acetes* sp., Mozambique tilapia *Tilapia mosambica*, crab *Scylla* sp., and some of molluscs as a carrier (Cavalli *et al*., 2013; Macías-Rodríguez *et al*., 2014; King *et al*., 2015), microalgae, and zooplankton (Esparza-Leal *et al*., 2009), plankton, larvae, and insects (Corsin *et al*., 2005).

White spot syndrome viruses doesn't only attack shrimp that reared in ponds (Hoa *et al*., 2011b & 2014; Bosma *et al*., 2014; Sivasankar *et al*., 2015), but also has been detected infected tiger shrimp *P. monodon* broodstock from its nature or that have been spawned. In addition, it has also been reported that WSSV infects crayfish, freshwater shrimp *Cherax* sp., juvenile shrimp, tiger shrimp *P. monodon*, and even wild organisms that live in the ponds (Soowannayan & Phanthura, 2011).

Tackling white spot disease attack can be with by using probiotics (Lakshami *et al*., 2013; Pham *et al*., 2016; Sivasankar *et al*., 2017), vaccines (Nguyen *et al*., 2010; Amar *et al*., 2011; Syed & Kwang, 2011; Valdez *et al*., 2014; Chen *et al*., 2016), immunostimulants (Chen *et al*., 2010; Ermantianingrum *et al*., 2013; Velmurugan *et al*., 2013), and the management quality of the environment (Bosma *et al*., 2014), but until now, the mortality of shrimp in pond and hatchery caused by the disease attack is still occurring continously. The use of natural materials including mangrove and others associated with mangroves that used to tackle the disease in fish have started though it was still in laboratory scale as an antibacterial and antivirus.

The potency of the herbal ingredients extract for bacterial disease prevention and white spot disease tackling at shrimp rearing has been reported by some researchers (Ahilan *et al*., 2010; Haq *et al*., 2011; Banerjee *et al*., 2012; Immanuel *et al*., 2012; Sahu *et al*., 2012; Ghosh & Chakraborty, 2013; Chakraborty & Ghosh, 2014; Chakraborty *et al*., 2014; Declarador *et al*., 2014; Maikaeo *et al*., 2015; Muliani *et al*., 2015; 2016; & Jha *et al*., 2016).The water fraction of the mangrove *Ceriopstagal* was effective as anti-WSSV in tiger shrimp (Sudheer *et al*., 2012.). Further, it mentioned that the survival rate of the tiger prawn fed 1% feeding rate per day with the addition of mangrove *C.tagal* water fraction as much as 500 mg/body kg/day reached 100% (Sudheer *et al*., 2011). In addition, some researchers have reported the potential of mangrove as an immunostimulant on shrimp and fish (Avenido, 2012; Rajeswari *et al*., 2012; Govind *et al*., 2012).

S. alba is a kind of mangrove grew excessively around entire Indonesia. This plant-shaped tree, green colored, grows scattered, sometimes the height reached 15 m with dark-brown bark colored. It has wires shaped root and appear on the ground surface as a blunt cone-shaped chicken-claw root reaches 25 cm. The leaves of *S. alba* has spherical upside eggs shaped with a rounded edge (Noor *et al*., 2012).

This type of another mangrove that very potential as an anti-WSSV is *B.gymnorrhiza*. This mangrove lived excessively all around Indonesia. This plant-shaped tree, green colored, grows scattered, and sometimes the height reached 30 m. The root is wide-board like to its side and has some of theknee-roots. The leaves have layer, dark-green colored on its top layer and yellowish green on its bottom with black splotches and have an ellipse-shaped (Noor *et al*., 2012).

B. gymnorrhiza and *S. alba* have already studied as antibacterial and anti-WSSV (Milon *et al*., 2012). Besides of these two species of mangrove, *Avicenia* sp. has been studied for the prevention of WSSV infection. The level of highly pathogenic WSSV was relatively decreased after soaking in some concentration of *Avicennia* sp. and *Sonneratia* sp. mangrove trees extract (CEPM) (Wahjuningrum *et al*., 2006). Furthermore, it was said that at doses of 250 mg/L, the survival rate of shrimp that challenged with white spot syndrome virus (WSSV) was 98.4%.

According to that, the study aimed to examine the effectiveness of *S. alba* and *B. gymnorrhiza* mangrove extracts to prevent WSSV infection in tiger shrimp *P. monodon*.

MATERIALS AND METHOD

The methanol extract preparation of *S. alba* and *B. gymnorrhiza*

The available flour of each *S. alba* and *B. gymnorrhiza* weighed as much as 500 g, then it put in 2000 mL of beacker glass. After that, it added with 80% methanol and it stirred until the flour soaked. Soaking was done three times over the past 24 hours and it filtered every 24 hours depending on its level of turbidity, if it already looked clear then the soaking stopped. It was intended to maximize the active ingredients of the plant extract by methanol. Next, it held in a bottle sample and it heated by using a rotatory evaporator. The methanol extracts that were obtained is for fractionation and as challenge test for anti-WSSV.

The water fraction, butanol, and diethyl ether preparation of *S. alba* and *B. gymnorrhiza*

The methanol extract of *S. alba* and *B. gymnnorrhiza* weighed to determine the amount of solvent to be used, then it put into a cup glass and it gave diethyl ether, it homogenized and put in a separator funnel. It left until settles and shaped in two layers. The lower layer was removed through the bottom of the separator funnel and the clear layer was removed through the top of the separator funnel. A similar thing was done three times until the diethyl ether layer was already clear. Furthermore, the rest of the sediment is dissolved again with butanol. The process was done as the same as the diethyl ether separation. After the butanol layer was clear, the separation of butanol was stopped and the insoluble sediment was collected as a water fraction and was heated by using dryer, while the diethyl ether and butanol fraction using a rotatory evaporator.

The preparation of rearing container and experimental shrimp

As many as 27 of 40 L aquariums were used as an experimental rearing container, filled with the sea water with salinity of 28 g/L and as many as 30 L/container that has been previously disinfected with 150 mg/L of calcium hypochlorite and neutralized with 75 mg/L of sodium thiosufat. Tiger shrimp WSSV free as experimental shrimp was taken from Instalasi Perbenihan Udang Windu (IPUW), Balai Riset Perikanan Budiaya Air Payau dan Penyuluhan Perikanan (BRPBAP3). Every container had density of 10 shrimps with average weight of 6 ± 1 g/shrimp.

Anti-WSSV test and shrimp rearing

Methanol extracts solutions (rough extracts) with a concentration of 0.1 g/0.01 L NTE buffer (0.2 M NaCl, 0.02 M Tris-HCL, and 0.02 M EDTA, pH 7.4) and water fraction, butanol fraction, and diethylether fraction of *S. alba* and *B. gymnorrhiza* with a concentration of 0.05g/0.01 L NTE buffer (Either & Isnansetyo, 2013) mixed with WSSV suspension derived from the haemolymph of shrimp infected by WSSV in ratio 2:1 (10 µL of extract solutions + 5 µL WSSV/shrimp suspension).The mixture incubated at a temperature of 29°C for three hours (Velmurugan *et al*., 2012; Chakraborty *et al*., 2014). After that the mixture is injected in a healthy tiger shrimp through intramuscular injection with injecting dose of 100 µL/shrimp.

The experimental design that used for this study was a randomized complete design (RAL) with treatment: A)The water fraction of *S. alba* + WSSV; B)Butanol fraction of *S. alba* + WSSV; C)The diethyl ether fraction of S. alba + WSSV; D)The methanol extract of *S. alba*+ WSSV; E)The water fraction of *B.gymnorrhiza*+ WSSV; F)Butanol fraction of *B. gymnorrhiza*+ WSSV; G)The diethyl ether fraction of *B. gymnorrhiza*+ WSSV; H)The methanol extract of *B.gymnorrhiza* + WSSV; I)Positive control (shrimp was injected with the mixture of 10 µL NTE buffer + 5 µL WSSV suspension without mangrove extract). Each treatment was repeated three times. Then, the shrimp reared with stocking density of 7 shrimps/aquarium for 10 days and fed with a commercial feed (contained 36–38% of protein) twice a day in the morning and evening.

Parameters of observation

The observations towards shrimp mortality and the clinical symptoms from the morphology of tiger shrimp infected by WSSV (its movement and white patches in its body) was done every day (Velmurugan *et al*., 2012), the counting of total hemocyte count (THC) following methods developed by Braak (2002), and differential hemocyte count (DHC) calculated from the shrimp hemolymph at the beginning and end of the study with the Martine and Graves method (1985).

Data analysis

Mortality and immune parameters data were analyzed the variance and continued with least significant different test (LSD) on a 95% of confidence interval while the WSSV infection data were analyzed descriptively.

RESULTS AND DISCUSSIONS

The mortality of tiger shrimp

The mortality of tiger shrimp that injected with WSSV and mangrove *S. alba* and *B.gymnorrhiza* extract is presented in Figure 1. It was observed that from day 1 to day 10, the mortality of shrimp in the treatment of water fraction of *S. alba* and *B. gymnorrhiza* occured gradually, and at the end of the study (day 10), in the treatment of the water fraction of *S. alba,* the mortality of shrimp reached 40%, whereas in the treatment of water fraction of *B. gymnorrhiza,* the mortality of shrimp reached 60%. Butanol and diethyl ether fraction from both species of mangrove is effective on WSSV inactivation, it has seen until the end of the study that showed the mortality of shrimp on butanol fraction of *S. alba* and diethyl ether fraction of *B.gymnorrhiza* amounted to 6.67%. In the control treatment (WSSV injection without mangrove extract) the total mortality of shrimp occurred on the day 3 and there was white patched on the head carapace. The mortality of shrimp occurred for three days since the presence of infection and followed by 100% of mortality was the main characteristic of the WSSV attacks. Munn (2004) reported that WSSV can lead to mortality of 80% for two to three days in juvenile of shrimp and seven to ten days in the broodstock shrimp, while Peinado-Guevara and Lopez-Meyer (2006) reported that WSSV can cause mortality in shrimp to 100% within three to ten days. The other researchers reported that WSSV can cause 90–100% of mortality among three to ten days after the clinical signs showed (Haq *et al*., 2015; Hossain *et al*., 2015).

The PCR results of WSSV at the beginning of

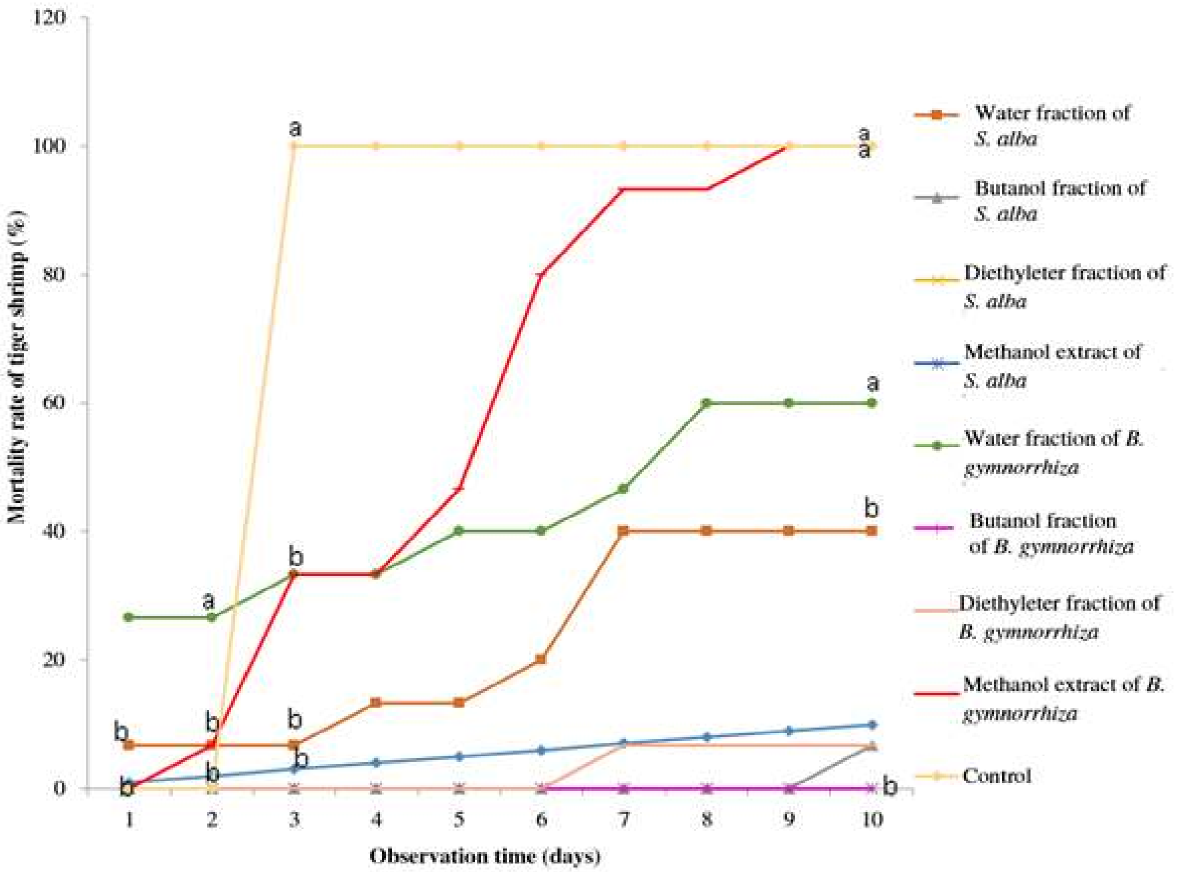

Figure 1. The mortality of tiger shrimp *Penaeus monodon* in challenge test of WSSV used mangrove extract of *S. alba* and *B.gymnorrhiza*. The figures on the same vertical line followed the same superscript showed that the results did not differ significantly (P>0.05).

the study before the injection showed a negative results (Table 1). After injection of WSSV and mangroves extract showed that fraction of methanol and diethyl ether extract of *S. alba*, and butanol fraction of *B. gymnorrhiza* were not detected the WSSV as on the other treatments in case the survival rate of shrimp increased to 100%. The potential of mangrove as an anti-WSSV has previously been reported by Chakraborty and Ghosh (2013a), Chakraborty *et al*. (2014) reported that MP07X (a mixture of water extract and ethanol extracts) from *Rhizophoira mucronata* has a potential as to be a producer of anti-WSSV. Further, it can be said that with 1000 mg/kg of body weight/day of MP07X through oral increased the survival rate of vannamei shrimp up to of 85%. The previous report stated that MP07X was not toxic to shrimp against WSSV at an effective doses (Chakraborty & Ghosh, 2013b).

The results of statistical tests showed that the highest mortality of tiger shrimp at day 1 and day 2 was occur at water fraction treatment of *B. gymnorrhiza* (Figure 1) and it was significantly different (P<0.05) with other treatments. In the control treatment, shrimp was injected with WSSV without mangrove extract, then the mortality of tiger shrimp occurred at the third day reached 100% and it was significantly different (P<0.05) with other treatments. The mortality of shrimp at the sixth day of *B. gymnorrhiza* extract injection treatment was continuously increase up to 80% and reached 100% after the day 10, whereas the water fraction injection treatments of injected was 40% and increased to 60% at day 10. The statistic result analysis indicated that mortality shrimp on both treatments at the end of the study (day 10) was significantly different (P<0.05) with other treatments except with the control (Figure 1). The results of this study showed that methanol extract and water fraction extract from *B. gymnorrhiza* were ineffective as anti-WSSV, obtained by the results of WSSV infection analysis that showed a positive results.

The immunity response and WSSV infection of tiger shrimp

The total hemocyte count and differential hemocyte count at the beginning of the study (before WSSV injection) were $15.75 \times 10^7 \pm 3.78 \times 10^7$ and 52.5 ± 10.32 of granule cells, 17.62 ± 8.13 of semi granule cells (%), and 29.88 ± 12.19 of hyaline cells (%). After 10 days of rearing, the total hemocyte count and differential hemocyte count of shrimp showed at Table 1. THC of shrimp before WSSV injection and mangrove extracts was lower than after injection, this showed that shrimp have the ability to increase or multiply its hemocyte against WSSV in its body. Otherwise, on the treatment that used methanol extract, the mortality of shrimp occurred since the second

Table 1. Immunity parameter and WSSV infection of tiger shrimp *Penaeus monodon* before and after challenge test

Treatments	THC ($\times 10^7$cells/mL)	Differential hemocyte count (%)			WSSV infection
		Granula cells	Semigranula cells	Hyalin cells	
A. Water fraction of *S. alba*+WSSV	8.10 ± 2.52^{ab}	55.95 ± 27.38^{a}b	8.35 ± 8.33^{a}	35.7 ± 35.71^{a}	Heavy positive
B. Butanol extract *S. alba* + WSSV	7.60 ± 1.59^{ab}	73.65 ± 13.98^{a}	19.02 ± 21.07^{a}	7.33 ± 7.15^{ab}	Heavy positive
C. Diethyl ether extract of *S. alba*+WSSV	14.80 ± 4.39^{a}	85.04 ± 15.67^{a}	10.80 ± 9.69^{a}	4.16 ± 7.22^{ab}	Negative
D. Methanol extract of *S. alba*+WSSV	8.80 ± 5.25^{ab}	69.77 ± 25.94^{a}	10.23 ± 9.30^{a}	20 ± 34.64^{ab}	Negative
E. Water fraction of *B. gymnorrhiza*+WSSV	7.20 ± 4.70^{ab}	79.05 ± 21.44^{a}	16.19 ± 14.66^{a}	4.76 ± 8.25^{ab}	Medium positive
F. Butanol extract of *B. gymnorrhiza*+WSSV	10.07 ± 3.97^{a}	86.54 ± 12.61^{a}	9.29 ± 8.18^{a}	$4\ 17 \pm 7.22^{ab}$	Negative
G. Diethyl ether extract of *B. gymnorrhiza*+WSSV	12.27 ± 5.35^{a}	65.98 ± 13.39^{a}	14.02 ± 13.39^{a}	0^{b}	Light positive
H. Methanol extract of *B. gymnorrhiza*+WSSV	0^{b}	0^{b}	0^{b}	0^{b}	Heavy positive
I.WSSV (positive control)	0^{b}	0^{b}	0^{b}	0^{b}	Heavy positive

The numbers in the same column followed by the same superscript showed the results did not significantly different (P>0.05) day and the 10th day after the mortality reached 100%.

The highest average value of THC indicated by treatment using *S. alba* diethyl ether extract was 14.80 × 10^7 cells/mL and statistically it was significant different ($P<0.05$) with THC value on a treatment using *B. gymnorrhiza* methanol extract and positive controls, then it followed by the treatments using the diethyl ether fraction of *B. gymnorrhiza* (12.27 × 10^7 cells/mL) and butanol fraction (10.07 × 10^7 cells/mL). Total haemocyte count (THC) is an indicator of shrimp health status and one of the ways to increase the THC value of shrimp by giving immunostimulant (Tampangallo, 2012).The diethyl ether fraction and methanol extract from *S. alba*; and butanol and diethyl ether fraction from *B.gymnorrhiza* assumed to kill WSSV and can be an immunostimulant for tiger shrimp, this can be seen at the treatment that used a of diethyl ether fraction and methanol extracts of *S. alba* has THC value respectively of 14.80 × 10^7 cells/mL and 8.80 × 10^7 cells/mL and WSSV infection test results showed negative results and gave a positive impact against the survival of tiger shrimp reached 100% at the end of the study. Similarly to treatment that used diethyl ether fraction and butanol fraction of *B.gymnorrhiza,* the THC value respectively was 12.27 × 10^7 cells/mL and 10.07 × 10^7 cells/mL with the survival rate of tiger shrimp was 93.33% and 100% at the end of the study.

The results of this study showed that both species of mangroves was tend to be examined further as an anti-WSSV and immunostimulant for shrimp to enhance the body's defenses against pathogens such as bacteria and WSSV. Beside of producing anti-WSSV, *S. alba* also has been reported as a powerful anti-bacterial causing agent of diseases on shrimp with MIC values of each was 1 mg/L for *V. harveyi* and 0.1 mg/L for *V. parahaemolyticus* (Muliani *et al*., 2015). Some species of mangrove have been reported in inactivated WSSV, such as *Rhizophora mucronata*, *Sonneratia* sp., and *Ceriops tagal* (Sudheer *et al*., 2011). The water fraction from the mangrove can inactivate WSSV after it incubated at room temperature with ratio of 1:1 for three hours. While the potential of mangrove as immunostimulant extracts has been reported by Avenido *et al*. (2012) stated that the methanol extract of *S. caseolaris* can enhance the immunity of shrimp by increasing the immune response, phagocytic, and phenoloxidase activity therefore this type of mangrove can be a natural ingredient of immunostimulant for shrimp.

A comparison between granular cells, hyaline cells, and semigranular cells known as haemocyte or DHC (differential haemocyte count). All this haemocyte cell types played a role in the immunity of shrimp. Hyaline cells responsible for immune system as phagocytosis, whereas semigranular and granular cell collectively responsible for cytotoxic activities and the production and release of prophenoloxidase system (Kakoolaki *et al*., 2010; Chen *et al*., 2015). According to Johansson *et al*. (2000) hyaline cells responsible for phagocytic activity, whereas granular and semi-granular cells is for protease enzymes activities, antibacterial substances formation, and reactive oxygen such as superoxide anion and hydrogen peroxide.

The DHC value of tiger shrimp before injection with mangrove extract was lower than those after injection (Table 1). In general, the granular cells and semigranular cells on shrimp was higher than with hyaline cells. In this study, granular cells and semigranular cells on the tiger shrimp that injected with diethyl ether fraction of *S. alba* and butanol fraction of *B. gymnorrhiza* was higher than another. As has already been explained previously that this two cell types is responsible for the release process and vault for prophenoloxsidase which is one of the body's humoral defense form on crustacea, including tiger shrimp.

Sung *et al*. (1999), reported that 50% to 80% of total haemocyte on crustacea was hyaline cells, 9% to 30% is semigranular cells, and 4% to 20% is granular cells. However the proportion between this three types of cells depends on the species, the molting phase, and physiological condition of the organism (Winotaphan *et al*., 2005). The ability of the injected shrimp with the diethyl ether fraction of *S. alba* and butanol fraction of *B.gymnorrhiza* to form humoral defense through the vault of prophenoloxidase is one of the causes of the high survival rate of tiger shrimp on that two treatment. The results of this study indicated that the diethyl ether solvent and butanol were more effective to attract the existing active ingredients in mangrove plants, further besides of being able to inactivate the WSSV, it has also had an impact on enhancing the immune system of shrimp to increase the survival rate of shrimp.

CONCLUSION

The diethyl ether fraction and methanol extract of *S. alba* and the butanol fraction of *B. gymnorrhiza* with ratio of 2:1 between the mangrove extract and WSSV suspension with injecting dose of 100 µL/individual can inactivate the WSSV therefore it can increase the survival rate of shrimp up to 100%. Leaves extract of *S. alba* and *B. gymnorrhiza* was effective to prevent WSSV infection in tiger shrimp.

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