Aquaculture
Research & Development

Harlina et al., J Aquac Res Development 2015, 6:10
http://dx.doi.org/10.4172/2155-9546.1000372

Research Article Open Access

Potential Study of Kopasanda (*Chromolaena odorata* L.) Leaves as Antibacterial Against *Vibrio harveyi*, Disease Causative Agent of Tiger Shrimp (*Penaeus monodon* Fabricius) Post Larvae

Harlina Harlina[1], Arief Prajitno[2], Eddy Suprayitno[2], Happy Nursyam[2] and Rosmiati[3]

[1]*Faculty of Fisheries and Marine Sciences, Muslim University of Indonesia, Makassar, Indonesia*
[2]*Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia*
[3]*Research and Development Institute for Coastal Aquaculture, 129 Makmur Daeng Sitakka Street, Maros 90512, South Sulawesi, Indonesia*

Abstract

Vibriosis caused by *Vibrio harveyi* is still being a serious bacterial disease problem in the black tiger shrimp *Penaeus monodon* aquaculture. Due to this disease, the mortality of tiger shrimp post larvae can reach 100%. Attempt has been made for this bacterial disease prevention by using mangrove associates secondary metabolites. This study aims to determine potential of kopasanda (*C. odorata L.*) leaves as natural antibacterial against *Vibrio harveyi*. Potential study was carried out by determinination of the best solvent used to extract the bioactive secondary metabolites of kopasanda leaves, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Toxicity study was also done by using soaking method to determine the save concentrations of *C. odorata* leaves on tiger shrimp post larvae. *C. odorata* leaves was extracted with methanol to extract all the active components, then, partitioned into n-hexane, ethyl acetate, methanol and aqueous extract. The highest antibacterial activity against *V. harveyi* was shown by methanol extract. The results suggest that *C. odorata* leaves have a potential to be developed as a raw material source for vibriosis prevention. It is due to its strong anti-vibrio activity showed by the low MIC and MBC of 0.625 and 1.250 mg/ml, respectively. Furthermore, the methanol extract did not show any toxicon tiger shrimp (*P. monodon*) post larvae up to 2.500 mg/ml.

Keywords: *Chromolaena odorata* L; *Vibrio harveyi*; *Penaeus monodon*; Vibriosis; Toxicity

Introduction

One of the main problems of the tiger shrimp aquaculture is the presence of mass mortality due to disease attack [1]. The disease considered as a serious problem has been caused by pathogenic bacteria infection from vibrio genus, especially *V. harveyi* [2]. This bacterium infects almost all cultivated marine organisms such as crustacean, mollusca and fish [3]. Crustacean including shrimp, crab, lobster and artemia, is very susceptible to this opportunistic pathogenic bacterium [4-10]. *V. harveyi* is also well known as a bacterial pathogen in almost all cultured marine fish species [11,12].

The prevention and control efforts of vibriosis have been carried out by maintaining adequate water quality with low bacterial biomass, sterilizing or filtering recirculated water together with routine monitoring of shrimp and ponds for early diagnosis of problems. Besides that, the most common methods carried out to overcome the problems were by using chemicals, such as formalin and malachite green as well as antibiotics, such as chloramfenicol, oxytetracyclin and prefuran [13]. Since the bactericides residue can be accumulated in water effecting shrimp quality and be harmful to consumer [14], cause resistance on bacteria and decrease the aquatic environmental quality, these treatments have become less effective. Based on the problems, some researchers have attempted other alternative methods to control the diseases.

One of method which can be applied is the use of natural bioactive compounds with a broad spectrum without dangerous side effect. Several species of sponge such as *Geodia* sp. [3], *Dendrillanigra* [15], *Gracillaria verrucosa* [16], and *Aaptos aaptos* [17] were reported to contain bioactive compounds which are able to inhibit the growth of *Vibrio harveyi*, the causative agent of vibriosis. *Halimeda opuntia* was also proved to have an antibacterial activity against *V.harveyi* [18]. Several species of mangrove have been also used for vibriosis prevention such as *Avicennia marina* and *Sonneratia caseolaris* [19,20]. Kopasanda (*Chromolaena odorata* L.) is one of mangrove associates which can be used for multiple purposes. Extract of *C. odorata* leaves is able to treat injured skin and restrain blood. Besides, its root is obtained to have antipyretic activity and function as anesthesia drug and flower is used as insecticide. The ability of *C. odorata* leaves to treat a variety of diseases is caused by its active compounds such as flavonoids, alkaloids, tannins, and phenolic [1,21,22]. Nevertheless, potential of *C. odorata* for controlling of *V. harveyi* has been not reported yet. In the present paper, we reported the antibacterial activity of *C. odorata* methanol extract against *V. harveyi* and its toxicity on tiger shrimp *P. monodon*.

Materials and Methods

V. harveyi strain and medium

V. harveyi used was collection of Research Institute of Coastal Aquaculture (RICA), Maros, South Sulawesi, Indonesia. Prior to testing, a loopful of Vibrio harveyiinoculums was zigzag streaked on TCBSA (Thiosulfat Citrate Bile Sucrose Agar) and incubated for 24 hours at 28°C. One colony of the bacterium growth was sub-cultured

***Corresponding author:** Harlina Harlina, Faculty of Fisheries and Marine Sciences, Muslim University of Indonesia, Makassar, Indonesia, Tel: +62-85255640891; E-mail: linausman1965@yahoo.com

Received May 18, 2015; **Accepted** June 11, 2015; **Published** August 15, 2015

Citation: Harlina H, Prajitno A, Suprayitno E, Nursyam H, Rosmiati (2015) Potential Study of Kopasanda (*Chromolaena odorata* L.) Leaves as Antibacterial Against *Vibrio harveyi*, Disease Causative Agent of Tiger Shrimp (*Penaeus monodon* Fabricius) Post Larvae. J Aquac Res Development 6: 372. doi:10.4172/2155-9546.1000372

in 50 ml of nutrient broth medium (Sigma, FRG) and incubated for 4 hours using a shaker at 150 rpm and 28°C to produce the density of 107 CFU/Ml [23].

Sample of *C. odorata*

The leaves of *C. odorata* were collected from around pond area of Research Institute of Coastal Aquaculture (RICA), Maranak, Maros, South Sulawesi, Indonesia. Plant identification was conducted at Centre for Plant Conservation-Bogor Botanical Garden, Bogor, Indonesia, and herbarium specimen was deposited there and RICA.

Extraction of *C. odorata*

Leaves of *C. odorata* were cleaned and dried by using herbs dryer at temperature below 40°C. Fifty hundred gram of dried leaves was ground into powder form by grinder as described by Salosso and Jusmanindar [24] and repeatedly extracted with methanol at room temperature till the residue was colourless. The methanol extracts were filtered through Whatman No. 1 filter paper fitted in a Buchner funnel using suction and collected for concentration under reduced pressure by a rotary evaporator (Buchi type) to yield a dark gummy (81 g).

Solvent partitioning of methanol extract

The methanol extract was added distilled water and solvent partitioned with n-hexane, ethyl acetate and methanol to give n-hexane (7.5 g), ethyl acetate (20.0 g), methanol (42.5 g) and aqueous extract (11.0 g). All the extracts were assayed against *Vibrio harveyi* as described below. The extract exhibiting a strong antibacterial activity was again evaluated toward *V. harveyi* to determine its minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and toxicity.

Antibacterial activity of *C. odorata* extracts against *V. harveyi*

The antibacterial activity of n-hexane, ethyl acetate, methanol, and aqueous extracts was tested by using agar well diffusion technique in Mueller-Hinton agar as described by Shekhan and Sabeeh Al-Hussaini [25]. 100 µl of bacterium inoculum were spread on Mueller Hinton agar.The filter paper discs (6 mm in diameter) were individually impregnated with 20 µl of the extracts (10 mg/ml), dried in a laminar air flow and then placed onto the agar plates previously inoculated with *V. harveyi*. The plates were incubated at 30°C for 24 h. The diameters of the inhibition zones were measured in millimetres. All the tests were performed in triplicate. Streptomycin was served as positive controls. The antibacterial activity is interpreted as follow; the diameter of inhibition zone>15.0 mm – strong; 10.0 to 14.5 mm – moderate and <10 mm – weak [26].

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the most active extract (methanol extract) against *V. harveyi* was tested by using disc diffusion method as mentioned above. Two-fold dilution of the most active extract of *C. odorata* leaves from 10 mg/ml were be used to evaluate their minimum inhibitory concentration (MIC). The final concentrations were 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 mg/ml.

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the most active extract (methanol extract) against *V. harveyi* was also tested by using disc diffusion method as described above. Concentrations used to determine minimum bactericidal concentration (MBC) were based on the minimum inhibitory concentration obtained namely; 0 × MIC, 0.5 × MIC (0.313 mg/ml), 1 × MIC (0.625 mg/ml), 2 × MIC (1.25 mg/ml), 4 × MIC (2.5 mg/ml), 8 × MIC (5 mg/ml), and 16 × MIC (10 mg/ml).

Toxicity test of methanol extract on tiger shrimp *P.monodon* post larvae

Toxicity (bioactive) of the most active extract was monitored by tiger shrimp *P.monodon* post larvae based on procedure previously described by Rosmiati [17]. The most active extract was dissolved in sterilized seawater. Prior to starting of experiment, post larvae were acclimatized in the temperature controlled wet laboratory and reared for 7 days to be 14 days of post larvae (PL-14). The post larvae that were free of white spot (based on PCR test) and that were +1 cm in length size were selected. Twenty of post larvae were exposed with *C.odorata* leaves active methanol extract at the concentrations of 0 × MIC (control treatment), 0.5 × MIC (313 ppm), 1 × MIC (625 ppm), 2 × MIC (1250 ppm), 4 × MIC (2500 ppm), 8 × MIC (5000 ppm), and 16 × MIC (10000 ppm) in triplicate in 2 L sterilized sea water (30 ppt)) using glass containers provided with gentle, continuous aeration. During the study, the post larvae were fed with commercial feed at 10% of body weight two times a day (06:00 and 18:00). Feed residue and feces were removed by siphoned out. The enumeration of mortality/survivability was performed at hours 1, 3, 6, 9, and 12 and every 24 hours until seven days of rearing.

Histophatological observation

To determine any adverse effect of the extracts after seven days, three post larvae from each experiment were harvested and fixed in a 10% buffered formalin solution for fixation and were subjected to standard histology processing and procedures as described by Zhang, [27] and Maftuch [28] with some modification.

Results and Discussions

In this study, ethyl acetate, methanol and aqueous extract of *C. odorata* leaves were found to give the antibacterial activity against *V. harveyi* (Figure 1). The highest antibacterial activity was shown by methanol extract with the inhibition zone of 19 mm, followed by ethyl acetate and aqueous extract with the inhibition zone of 9 and 7 mm, respectively. Meanwhile, its n-hexane extract did not show any antibacterial activity against *V. harveyi*. The strong antibacterial showed by methanol extract was suspected due to the presence of polar compounds such as flavonoids and alkaloids. As reported, *C. odorata* leaves contain bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic which can be used for a variety of diseases [1,21,23]. The antibacterial activity is interpreted as follow; the diameter of inhibition zone>15.0 mm – strong; 10.0 to 14.5 mm – moderate and <10 mm – weak [28-30]. The inhibition zone exhibited by *C.odorata* methanol extract against this bacterium was lower than that of streptomycine with the inhibition zone of 28 mm. Compared

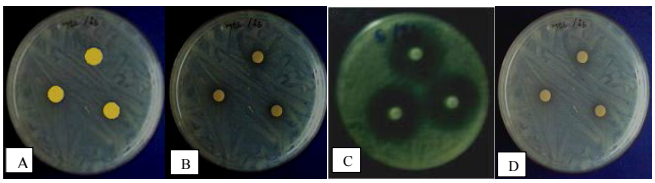

Figure 1: The antibacterial activity of n-hexane (A), ethyl acetate (B), methanol (C), and Aqueous (D) extract of *C.odorata* leaves against *V.harveyi* at the concentration of 10 mg/ml.

to the antibacterial activity shown by butanol extract of marine sponge, *Aaptos aaptos* [17,26,31], the antibacterial activity displayed by *C. odorata* methanol extract was also lower. Nevertheless, methanol extract of this plant showed the higher antibacterial activity than that of sea grass, *Halimeda opuntia* extract [18] (Figure 1).

Furthermore, the MIC value shown by the most active methanol extract of *C. odorata* leaves was 0.625 mg/ml. The low MIC value indicated that *C. odorata* methanol extract was very potent against *V. harveyi*, the causative agent of Vibriosis (luminescence disease). As reported, extracts with the MIC value<1 mg/ml are considered to be strong active extract [29]. *C. odorata* has been reported as the source of several bioactive substances including antibacterial, antiplasmodic, antiprotozoal, antifungal, anti-hypertension, and anti-inflammation [32].

The bactericidal concentration showed by *C. odorata* methanol extract using paper disc diffusion was 1.250 mg/ml (2 × MIC). It was proved by the absence of *V. harveyi* growth on TCBSA medium after 48 hours whilst, the concentration of 0.313 mg/ml (0.5 × MIC) had a stable antibacterial activity. It was different to *C. odorata* methanol extract at the concentration of 0.625 mg/ml (1 × MIC) in which the growth of *V. harveyi* was inhibited until 24 hours thereafter, the bacterium had again grown after 48 hours.

As previously reported, methanol extract of *C. odorata* leaves contains flavonoid showing antimicrobial activity [21,31]. Flavonoid is a substance group of phenol derivates which has a function to prevent body from microbes, insects and herbivores. Furthermore, reported that flavonoid shows the important biological activity used in bacteriology, pharmacology and medical due to its bactericidal activity [33]. The presence of hydroxyl (OH) functional group in flavonoid will increase the antibacterial activity [34]. The existence of carbonyl group (C=O) functional group also influences on the antibacterial activity. The antibacterial activity shown by flavonoid is through direct interaction to enzymes via hydrogen bonding [34]. As bactericidal compound, flavonoid disturbs cytoplasm membrane function. The presence of H^+ ion in phenol and its derivatives will attack polar group (phosphate group) causing phospholipid molecule of bacterium cell wall, then scattered to be glycerol, carboxylate acid and phosphate acid. In these forms, phospholipid is not able to prevent cytoplasm membrane form causing leak membrane and inhibited bacterium growth or died bacterium [35].

Mortality observation of tiger shrimp *P. monodon* pasca larvae treated with various concentrations of *C. odorata* leaves methanol extract after 168 hours of rearing period in toxicity test is shown in Figure 2.

Figure 2 shows that treatment of post larvae with various concentrations of *C. odorata* leaves methanol extract gave the different effect on mortality rate of the post larvae. The lowest mortality rate was exhibited by the post larvae treated with 0 ppm (control), followed by 313, 625 and 1250 ppm with the mortality rate of 11.67, 13.33, 13.33 and 15.00%, respectively. At the higher concentrations namely 2500, 5000 and 10000 ppm, the mortality rate of the post larva drastically increased with the mortality of 61.70, 91.67 and 100%, respectively. At the concentration of 10000 ppm all post larvae treated were obtained died. It indicated this *C. odorata* leaves methanol extract at this concentration was very toxic on post larvae with the highest mortality (68.30%) occurred on 9 hours pasca treatment.

The presence of mortality at the control (0 ppm) was suspected post larvae in stress condition and several post larvae treated could not be

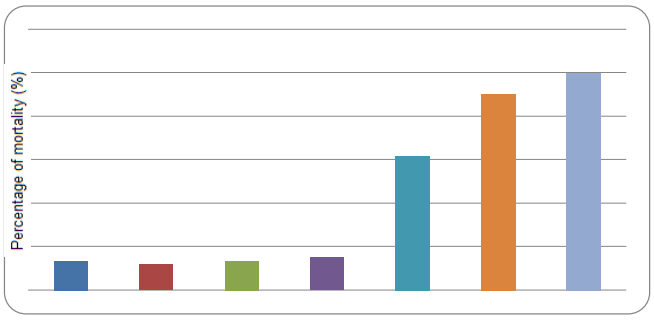

Figure 2: Mortality of tiger shrimp *Penaeousmonodon* post larvae treated with various concentrations of *C.odorata* leaves methanol extract after 168 hours rearing period time.

Concentration (ppm)	Mortality (%)
0 (kontrol)	11,67[a]
313	13,33[a]
625	13,33[a]
1250	15,00[a]
2500	61,67[b]
5000	91,33[c]
10000	100.00[c]

Note: Different word in the similar column shows the significant difference (*P<0.05*).

Table 1: Mortality statistic analysis of tiger shrimp post larvae treated with various concentrations of *C.odorata* methanol extract after 168 hours of rearing.

well adapted. Another factor causing the mortality was the existence of molting in which the molting post larva in a weak condition. As well known that tiger shrimp post larvae has a cannibalism characteristic in which the weak post larvae will be eaten by the others healthy post larvae (Table 1).

Statistical analysis (Table 1) on mortality rate of post larvae after 168 hours of rearing showed that mortality rate of post larvae treated with methanol extract at the concentrations of 2500, 5000 and 10000 ppm gave a significant difference compared to control (0 ppm) (P<0.05). Meanwhile, post larvae treated with methanol extract at the concentrations of 313, 625 and 1250 ppm showed a similar mortality effect to control (0 ppm). It indicated that these concentrations resulted in no any toxic effect on tiger shrimp post larvae. The high mortality occurred at the concentrations of 2500, 5000 and 10000 ppm was suspected to be due to the presence of an unpleasant smell and lather formation. The presence of an unpleasant smell will cause a reduced appetite. Meanwhile, the formation of lather will disturb the respiration process of post larvae.

Post larvae treated with various concentrations of methanol extract showed the different clinical sign response. Post larvae treated with the concentration of 10000 ppm on the first day displayed abnormality movement as often shown by the healthy post larvae namely the post larvae often swam to the surface thereafter, several time later (6 hours post treatment) post larvae upside-down and finally died. Mortality of 100% was occurred on 12 hours post treatment of methanol extract at the concentration of 10000 ppm. It showed that at this concentration, methanol extract was very toxic on post larvae. The similar condition was also observed for post larvae treated with methanol extract at the concentrations of 5000 and 2500 ppm. Whilst, post larvae treated with methanol extract at the concentrations of 313, 625 and 1250 ppm displayed normal behavior such as normal swimming and increasing appetitive.

Histological analysis on post larvae haepatopancreas treated with various concentrations of *C. odorata* leaves methanol extract is shown Figures 3a and 3b.

Histological analysis showed no any toxic effect or abnormal change on post larvae haepatopancreas treated with methanol extract at the concentrations of 313 ppm, 625 ppm and 1250 ppm compared to control (0 ppm) after 168 hours of rearing period time. Post larvae treated with methanol extract at the concentrations of 313, 625 and 1250 ppm displayed the lumen containing a granular material and the lumen-facing surface on the tubule was covered with a microvillus border. In addition, the tubular apex contained undifferentiated embryonic cells (E cells). R cells, B cells and F cells were also observed and F cells nuclei were larger than those of R cells, which characteristically contained numerous nuclei [36]. In turn, post larvae treated with methanol extract at the concentrations of 2500, 5000 and 10000 ppm showed abnormal change such as cell necrosis (N) and multifocal fat degeneration (D) causing the loss of B cell. Nevertheless, cell necrosis on the post larvae treated with methanol extract at the concentration of 5000 and 10000 ppm was higher than that of 2500 ppm.

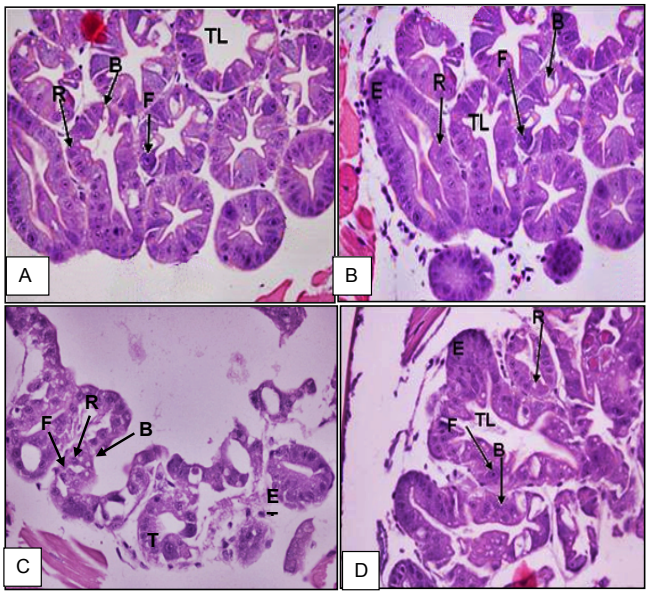

Figure 3a: Effect of *C.odorata* leaves methanol extract on haepatopancreas of post larvae treated with various concentarions: 0 ppm (A); 313 ppm (B); 625 ppm (C); 1250 ppm (D). TB: Tubule lumen; E: Embryonic cell; B: Blasenzellen cell; R: Restzellen cell; F: Fibrillazellen cell. Haematoxylin and Eosin Staining, Magnification=40X.

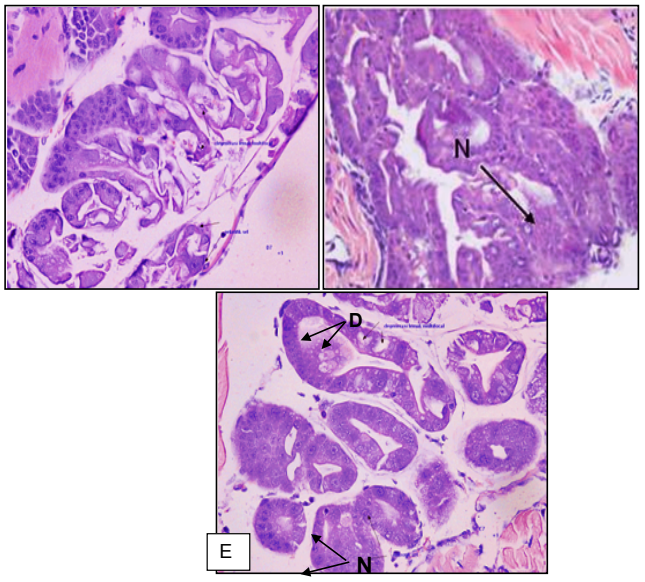

Figure 3b: Effect of *C.odorata* leaves methanol extract on haepatopancreas of post larvae treated with various concentarions: 2500 ppm (E); 5000 ppm (F); dan 10000 ppm (G).Cell Necrosis (N), multifocal fat degeneration (M). Haematoxylin and Eosin Staining, Magnification=40X continued.

The observation result of water quality during this study showed that salinity, pH, temperature, and dissolved oxygen were suitable for the growth of tiger shrimp post larvae. Salinity, temperature, pH, and dissolved oxygen obtained were 27.50-29.04 ppt, 26.5-27.5°C, 7.50-7.86, and 6.65-8.00 mg/L, respectively. It indicated that the water quality did not give any effect on the mortality of post larvae. The mortality of post larvae was clearly caused by effect toxic of *C. odorata* leaves methanol extract. As previously reported that for the optimum growth post larvae need salinity of 15-25 ppt, pH of 8.0-8.5, temperature of 25-31°C and dissolved oxygen of 4-8 mg/ml [37].

Conclusion

Kopasanda *(C. odorata)* leaves have a potential to be developed as a raw material source for vibriosis prevention. It is due to its strong anti-vibrio activity showed by the low minimum inhibitory concentration and minimum bactericidal concentration of 0.625 and 1.250 mg/ml, respectively. Furthermore, it did not give any toxic effect on tiger shrimp *(Penaeusmonodon)* post larvae.

Acknowledgement

The authors thanked Rector Muslim University of Indonesia, Makassar and Director of Research Institute for Coastal Aquaculture (RICA) Maros, South Sulawesi for his permit to do the research in Pathology and Fish Health Laboratory.

References

1. Harlina H, Prajitno A, Suprayitno E, Nursyam H (2013) The identification of chemical compound and antibacterial activity test of kopasanda (chromolaena odorata l.) leaf extract again vibriosis-causing vibrio harveyi (mr 275 rif) on tiger shrimp. Aquatic Science and Technology 1: 15-29.
2. Mariyono, A Wahyudi, Sutomo (2002) Teknik penanggulangan penyakit udang menyala melalui pengendalian populasi bakteri di laboratorium. Buletin Teknik Pertanian 7: 25-27.
3. Isnansetyo A, Trijoko, Setyowati EP, Anshory HH (2009) In vitro antibacterial activity of methanol extract of a sponge, geodia sp. against oxytetracyclin-resistent vibrio harveyi and its toxicity. J Biol Sci 9: 224-230.
4. Jiravanichpaisal P, Miyazaki T, Limsuwan C (1994) Histopathology, biochemistry and pathogenicity of vibrio harveyi infecting black tiger prawn penaeusmondon. J Aquat Ann Health 6:27-35.
5. Karunasagar I, Pai R, Malathi GR, Karunasagar I (1994) Mass mortality of penaeus monodon larvae due to antibiotic resistant vibrio harveyi infection. Aquaculture 128:203-209.
6. Liu PC, Lee KK, Yii KC, Kou GH, Chen SN (1996) Isolation of vibrio harveyi from diseased kuruma prawns penaeus japonicus. Curr Microbiol 33: 129-132.
7. Robertson PAW, Calderon J, Carrera L, Stark JR, Zherdmant M, et al. (1998) Experimental vibrio harveyi infections in penaeusvannamei larvae. Dis Aquat Organ 32: 151-155.
8. Diggles BK, Moss GA, Carson J, Anderson CD (2000) Luminous vibriosis in rock lobster jasus verreauxi (decapoda: palinuridae) phyllosoma larvae associated with infection by vibrio harveyi. Dis Aquat Organ 43: 127-137.

9. Soto-Rodriguez SA, Roque A, Lizarra ga-Partida ML, Guerra-Flores AL, Gomez-Gill B (2003) Virulence of luminous vibrios to artemia franciscana nauplii. Dis Aquat Organ 53: 231-240.

10. Bourne D, Hoj L, Webster N, Payne M, Skindersoe M, et al. (2007) Microbiological aspects of phyllosoma rearing of the ornate rock lobster panulirus ornatus. Aquaculture 268: 274-287.

11. Gauger E, Slomowitz R, Uhlinger K, Casey J, Gomez-Chiarri M (2006) Vibrio harveyi and other bacterial pathogens in cultured summer flounder,paralichthys dentatus. Aquaculture 260:10-20.

12. Oh MJ, Kim WS, Kitamura SI, Lee HK, Son BW, et al. (2006) Change of pathogenicity in olive flounder, paralichthys olivaceus by co-infection of vibrio harveyi,edwarsiella tarda and marine birnavirus. Aquaculture 257:156-160.

13. Raffi SM, Suresh TV (2011) Screening of chloramphenicol in wild and cultured shrimp penaeus monodon by competitive enzyme linked immunosorbent assay. International Conference on Chemical, Biological and Environment Sciences, Bangkok.

14. Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P (2007) Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. Trends in Biotechnology 25: 472-479.

15. Selvin J, Lipton AP (2004) Dendrilla nigra, a marine sponge, as potential source of antibacterial substances for managing shrimp diseases. Aquaculture 236: 277-283.

16. Maftuch, Toban MH, Risjani Y (2012) Administration of marine algae (gracillaria verrucosa) immunostimulant enhances some innate immune parameters in black tiger shrimp (penaeus monodon fabricus) against vibrio harveyi infection. Journal of Applied Sciences Research 8: 1052-1058.

17. Rosmiati, Habsah M, Sifzizul T, Najiah M, Aziz A, et al. (2011) In vitro antagonistic activities of indonesian marine sponge aaptosaaptos and callyspongia pseudoreticulata extracts and their toxicity against vibrio spp. Indonesian Aquaculture Journal 6: 173-182.

18. Prajitno A (2006) Prevention of vibrio harveyi.by sea weed (halimeda opuntia) on tiger shrimp (penaeus monodon fab.). Brawijaya University, Malang, Indonesia.

19. Maryani, Dana D, Sukenda (2002) Peranan ekstrak kelopak dan buah mangrove sonneratia caseolaris (l) terhadap infeksi bakteri vibrio harveyi pada udang windu (penaeus monodon fab.) Jurnal Akuakultur Indonesia1: 129-138.

20. Zulham R (2004) Potensi extract mangrove sonneratia caseolaris dan avicenia marina untuk pengendalian bakteri vibrio harveyi pada larva udang windu (penaeus monodon fabr.). Master Thesis, Institute Pertanian Bogor, Indonesia.

21. akinmoladun ac, ibokun eo, dan-ologe ia (2007) phytochemical constituents and antioxidant properties of extracts from the leaves of chromolaena odorata. Scientific Research and Essay 2: 191-194.

22. Vital PG, Rivera WL (2009) Antimicrobial activity and cytotoxicity of chromolaena odorata (l.f.) king and robinson and uncaria perrottetii (a. rich) merr. extracts. Journal of Medicinal Plants Research 3: 511-518.

23. Kadariah IAK (2012) Development of rapid detection methods of vibriosis diseases in penaeid shrimp. Aquacultur. Ph.D. Thesis. Institute Pertanian Bogor, Bogor.

24. Saloss Y, Jasmanindar Y (2014) Potential of patikan kerbau (euphorbia hirta) as antibacterial on aeromonasb hydropilla and vibrio alginolitycus in fish culture. Aquatic Science and Technology 2: 63-72.

25. Shekhan MI, Sabeeh Al- Hussaini JA (2012) Study of the inhibitory effect of the ethanolic extract of coriandrum sativum,vitis vinifera, and zingiber officinale on the growth of staphylococcus aureus isolated from milk of cows infected with clinical mastitis. Bas J Vet Res 11: 93-107.

26. Habsah M, Zalilawati Mat R, Khozirah S, Jalifah, L, Nordin HL, et al. (2009) Antibacterial and dpph free radical scavenging of methanolic extracts of aaptos sp. (marine sponges). Pertanika 32: 43-50.

27. Zhang JZ (2008) An approach to analyzing histology segmentations using shape distributions. Master Thesis, Drexel University, USA.

28. Maftuch. (2007) Exposure to vibrio alginolyticus grouper rat intestine histopathology (cromileptes altivelis) and the number and activity macrophage cells. Journal of Fisheries Research 10: 66-70.

29. Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB (2001) Composition and antimicrobial activity of the essential oils of two origanum species. Journal of Agricultural and Food Chemistry 49: 4168-4170.

30. Jyothirmayi N, Prasad SHKR (2012) Efficacy of methanol and chloroform extracts of euphorbia hirta linn in inhibiting pathogens. Journal of Chemical, Biological and Physical Sciences 2: 334-337.

31. Phan TT, Wang L, See P, Grayer RJ, Chan SY, et al. (2001) Phenolic compounds of chromolaena odorata protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull 24: 1373-1379.

32. Cody (1988) Crystal and molecular structure of flavonoid in plant flavonoid in biology and medicine ii: biochemical, cellular and medicinal properties. Alan R Liss, New York.

33. Alcaradz LE, Blanco SE, Puig ON, Tomas F, Feretti FH (2000) Antibacterial activity of flavonoids againt methicillin-resistant staphylococcus aureus strains. J theor Biology 205: 231-240.

34. Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC, et al. (1996) Antioxidant properties of hydroxylflavones. Free Radic Biol Med 20: 35-43.

35. Volk WA, Wheeler (1988) Mikrobiologi Dasar. Penerjemah Soenartomo Adisoemarto. Airlangga. Surabaya 398 hal, Indonesia.

36. Sreeram MP, Menon NR (2005) Histopathological change in the hepatopancreas of the penaeid shrimp metapenaeusdobsoni exposed to petroleum hydrocarbons. J Mar Biol Ass India 47: 160-168.

37. Jang CS, Liang CP, Wang SW (2013) Integrating the spatial variability of water quality and quantity to probabilistically assess groundwater sustainability for use in aquaculture. Stochastic Environmental Research and Risk Assessment 27: 1281-1291.

Citation: Harlina H, Prajitno A, Suprayitno E, Nursyam H, Rosmiati (2015) Potential Study of Kopasanda (*Chromolaena odorata* L.) Leaves as Antibacterial Against *Vibrio harveyi*, Disease Causative Agent of Tiger Shrimp (*Penaeus monodon* Fabricius) Post Larvae. J Aquac Res Development 6: 372. doi:10.4172/2155-9546.1000372