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In Vitro Phytochemical and Inhibitory Potential Tests of Buton Forest Onion Extract (*Eleutherine palmifolia*) on *Vibrio harveyi*

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The objectives of this study were to analyze phytochemical content of buton forest onion extract and to test the inhibitory potential of buton onion extract on the growth of *Vibrio harveyi* bacteria at different doses. Test parameter included: (1) Phytochemical test through the method of color visualization, (2) Inhibitory potential test using two methods namely agar diffusion and co-culture. Treatment of dose consisted of positive control/K+ (Chloramphenicol 30 mg mL^{-1}), negative control/K- (Sterile Aquadest) and treatment of extract included A (20 mg mL^{-1}), B (40 mg mL^{-1}), C (60 mg mL^{-1}), D (80 mg mL^{-1}). Qualitatively, result of phytochemical test showed that buton forest onion extract contained flavonoid, tannin, saponin, quinone, steroid and triterpenoid compounds. Result of inhibitory potential test indicated that treatment D exhibited the highest inhibitory potential, while the minimum inhibitory potential was found in treatment A. The best co-culture test result was also found in treatment D. The extract of buton forest onion in this study the showed its capability in hibiting the growth of *V. harveyi*

Key words: *Eleutherine palmifolia,* inhibitory potential, phytochemical, *Vibrio harveyi*

Tujuan penelitian ini adalah menganalisa kandungan fitokimia dari ekstrak bawang hutan buton dan menguji daya hambat ekstrak bawang hutan buton terhadap pertumbuhan bakteri *Vibrio harveyi* dengan dosis berbeda. Parameter uji meliputi: (1) Uji fitokimia dengan metode visualisasi warna, (2) Uji daya hambat menggunakan dua metode yaitu metode *diffusi agar* dan metode kultur bersama. Perlakuan dosis terdiri dari kontrol positif/K+ (Chloramphenicol 30 mg mL^{-1}), kontrol negatif/K- (Aquades steril) dan perlakuan ekstrak meliputi A (20 mg mL^{-1}), B (40 mg mL^{-1}), C (60 mg mL^{-1}), D (80 mg mL^{-1}). Hasil uji fitokimia secara kualitatif menunjukkan bahwa ekstrak bawang hutan buton mengandung senyawa flavonoid, tanin, saponin, quinon, steroid dan triterpenoid. Uji daya hambat terbesar terdapat pada perlakuan D, sedangkan daya hambat minimum terdapat pada perlakuan A. Perlakuan terbaik dari uji kultur bersama juga ditemukan pada perlakuan D. Ekstrak bawang hutan buton pada penelitian ini dapat menghambat pertumbuhan *V. harveyi.*

Kata kunci: *Eleutherine palmifolia*, daya hambat, fitokimia, *Vibrio harveyi*

Vibrio harveyi is a bacterium that causes vibriosis disease or luminescent disease in aquatic organism (Phuoc *et al*. 2009). *V. harveyi* is a saprophytic and opportunistic bacterium, living in fish culture environment in normal condition and becomes pathogen in worsening environmental and host condition (Garrity *et al*. 2004). Vibriosis disease occurs in shrimp including in larvae stadia up to adult shrimp (Soto-Rodriguez *et al*. 2012, Munaeni *et al*. 2014), is acute and infectious. Shrimp attacked with vibriosis shows several signs such as brownish and skin damage, reddish tail and swimming legs, necrosis, black lymphoid organ, brown gills, brownish muscle, empty intestine, and weak movement (Cano-Gomez *et al*. 2009).

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Diseases caused by microorganism in aquaculture may lead to serious loss, while the use of antibiotic for disease treatment causes undesired side effect (Li *et al*. 2006) such as bacterial resistance on antibiotic. The use of natural compound originated from harmless plants has potential in fish culture as an alternative of antibiotic use (Van Hai *et al*. 2015). Medicinal plant is considered to be able to show potential effect on the growth and survival and also has antimicrobial properties on aquatic organism (Immanuel *et al*. 2004; Citarasu 2010). Phytochemical compound in medicinal plants can be used as chemotherapy in aquaculture (Chang 2000; Sivaram *et al*. 2004). Phytochemical compound from several medicinal plants contained phenolic, polyphenol, alkaloid, quinone, terpenoid, lectin, and polypeptide compounds (Harikrishnan *et al*. 2011). In addition, medicinal plant

is rich in various nutrients (Chang 2000).

The use of medicinal plants has advantages namely cheap, easy to prepare, effective with less side effect during treatment (Jian and Wu 2003; 2004), not dangerous and does not create environmental problems (Citarasu 2010). The use of alcohol solvent resulted higher efficiency in extracting secondary bioactive metabolites compared with water extraction method (Bulfon *et al*. 2015).

The aim of this study were to analyze phytochemical content of buton forest onion extract and to test the inhibitory potential of buton onion extract on the growth of *Vibrio harveyi* bacteria at different doses.

MATERIALS AND METHODS

Time and Place. This study was conducted during March-May 2017 in Testing Laboratory of Fisheries and Marine Science Faculty, Universitas Halu Oleo and Laboratory of Fish Health, Aquaculture Department, Fisheries and Marine Science Faculty and Laboratory of Biopharmaca, Institut Pertanian Bogor.

Preparation of Powder and Ethanol Extraction. The bulbs of buton forest onion that had flowered or already reached 3-4 months old were cleaned and further thinly sliced and dried in the oven for 48 h at temperature of 60 °C. Later, the slices of dry onion were grinded using blender and separated using sieve until flour/powder was produced.

Buton forest onion powder was further extracted using ethanol solvent of 96% at ratio of 1:4 (w/v). Later, maceration process was performed at room temperature for 24 h using magnetic stirrer. The product of first maceration was filtered and further re-macerated for two times. The products of maceration were collected together and filtered using Whatman filter paper no 41, then treated to generate more concentrated product using evaporator vacuum at temperature of 40 °C. The yield of dry buton forest onion extract obtained was calculated using the equation below :

$$\%\,\text{Yield} = \frac{\text{Gram of buton forest onion extract}}{\text{Gram of buton forest onion powder}} \times 100\%$$

Observational Parameter. Phytochemical test was done using color visualization method and inhibitory potential test was conducted using two methods, namely agar diffusion and co-culture.

Phytochemical Test. Phytochemical test was performed through color visualization method by the standard method of Department of Health of Republic of Indonesia (1995), which included tests of flavonoid, alkaloid, tannin, saponin, quinone, steroid, and triterpenoid.

Inhibitory Potential Test through Agar Diffusion Method. Inhibitory potential test through agar diffusion method was performed to observe the zone of inhibition around the paper disk. Buton forest onion extract were made into various concentrations, those were 20 mg mL^{-1}, 40 mg mL^{-1}, 60 mg mL^{-1}, 80 mg mL^{-1} and negative control (K-) using sterile aquadest, and positive control (K+) using 30 mg mL^{-1} antibiotic Chloramphenicol (Bernofarm).

Pure culture of *V. harveyi* amounted to one innoculation loop was taken and cultured in 25 mL liquid SWC (Sea Water Complete: 5 g bactopeptone, 1 g yeast extract, 3 mL glycerol, 750 mL sea water, 250 mL aquadest) media and further incubated in waterbath shaker at temperature of 37 °C for 24 h. Suspension of bacteria was moved into eppendorf of 2 mL and serial dilution was performed until bacterial density of 10^8 CFU mL^{-1} was obtained. Approximately 50 µL of bacterial suspension was spread on TCBS (Thiosulphate Citrate Bile-Salt Sucrose) agar media. Sterile paper disk was dipped into the stock of buton forest onion extract and further put on TCBS media in which the bacteria has been spread. Each treatment of dose was given mark with replication of four times. Furthermore, treatments were incubated for 24 h at temperature of 37 °C. After being incubated, measurement of the diameter of the zone of inhibition was done.

Inhibitory Potential Test through Co-culture Method. *In vitro* antibacterial test of buton forest onion extract on *V. harveyi* was also performed through co-culture method. Buton forest onion extract at different doses were put into a test tube of 90 µL, while for control, PBS (Phosphate Buffer Saline: 8 g NaCl, 0.2 g KH2PO4, 1.5 g Na2HPO4, 0.2 g KCl, 1000 mL *aquadest*) was filled into the sterile tube. Each tube was filled with suspension of *V. harveyi* of 10 µL. Further, it was homogenized and incubated for 24 h at temperature of 37 °C. Later, it was serially diluted and spread on TCBS media and incubated for 24 h at temperature of 37 °C. Moreover, the number of colony grew in each treatment was calculated.

Data Analysis. Research data were analyzed descriptively and statistical test was done using Microsoft Excel 2010, ANOVA (Analysis of Variance) test, if the result was significantly different, post hoc test of Duncan was performed using the program of SPSS (Statistical Program Software System) version 16.

RESULTS

Fresh buton forest onion bulbs was processed into the powder or flour of buton forest onion (Fig 1a) and further was extracted using 96% ethanol thus resulted in buton forest onion extract (Fig 1b) with yield value of 10.11% (v v^{-1}). Result of phytochemical test (Table 1) showed that the extract of buton forest onion bulbs positively contained flavonoid, tannin, saponin, quinone, steroid and triterpenoid compound, yet it was negative or did not contain alkaloid compound using the reagent of Dragendorff, Meyer's and Wagner.

Result of inhibitory potential test through agar diffusion method using TCBS media (Fig 2a) and SWC media (Fig 2b) indicated that higher dose of treatment led to larger clear zone. It can be found in the result of statistical test (Fig 3) that the treatment of buton forest onion extract generated the largest zone of inhibition in treatment D which was not significantly different (P>0.05) from treatment K+ (positive control using Chloramphenicol of 30 mg mL^{-1}) and significantly different (P<0.05) from other treatments.Treatment A was not significantly different (P>0.05) from treatment K-, yet it was significantly different (P<0.05) from other treatments. Treatment B was significantly different (P<0.05) from all treatments, so was treatment C.

Result of inhibitory potential test through co-culture method (Fig 4) also indicated the same finding as agar diffusion result that higher dose will result in decreasing density of bacteria. Treatment D (dose of 80 mg mL^{-1}) was significantly different (P<0.05) from other treatments. In this treatment, *V. harveyi* colonies were not found (0 log 10 cfu mL^{-1}) after incubation for 24 h.

DISCUSSION

The yield of buton forest onion extract produced in this study amounted to 10.11 % (v v^{-1}). This yield value was higher than the result of study conducted by Febrinda (2014) by macerating fresh bulb using water and ethanol solvent which resulted in yield of 8.11% and 6.47%. The result of phytochemical test was also different from the research finding of Febrinda (2014) where buton forest onion extract obtained from

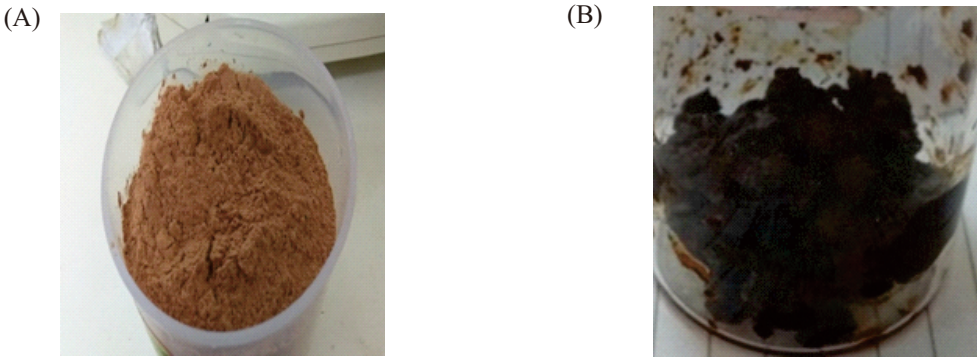

Fig 1 Powder (A) and extract of buton forest onion with yield of 10.11 % (v v^{-1}) (B).

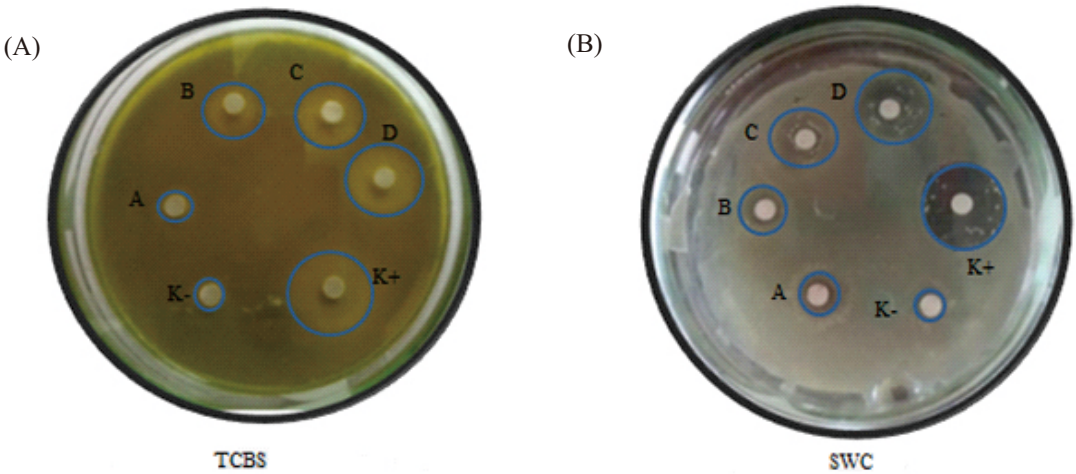

Fig 2 Inhibitory potential test of *V. harveyi* through agar diffusion method using media of TCBS (A) and SWC (B) with treatments: K+ (Positive control using Chloramphenicol of 30 mg mL^{-1}), K- (Negative control using Aquadest), A (20 mg mL^{-1}), B (40 mg mL^{-1}), C (60 mg mL^{-1}), D (80 mg mL^{-1}).

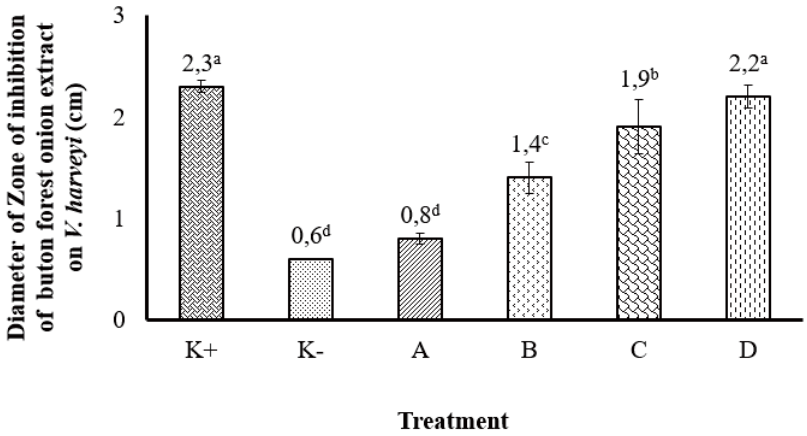

Fig 3 Diameter of zone of inhibition in *V. harveyi* through agar diffusion method. Different letters over each treatment bar (mean ± SD) indicated significant difference (Duncan; $P < 0.05$). K+ (Positive control using Chloramphenicol of 30 mg mL^{-1}), K- (Negative control using Aquadest), A (mg mL^{-1}), B (40 mg mL^{-1}), C (60 mg mL^{-1}), D (80 mg mL^{-1}).

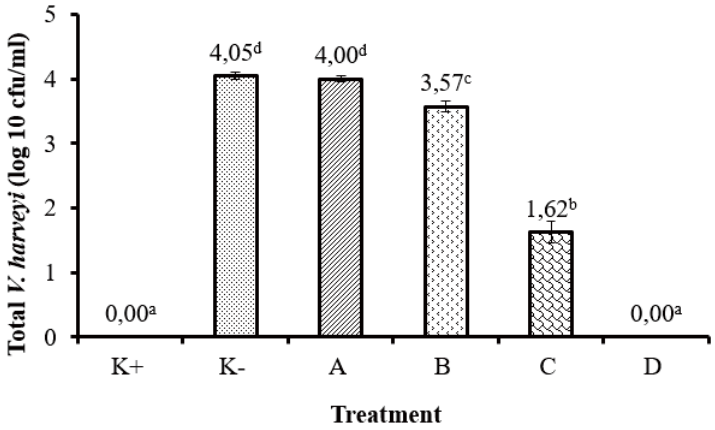

Fig 4 Total of *V. harveyi* resulted from inhibitory potential test through co-culture method. Different letters over each treatment bar (mean ± SD) indicated significant difference (Duncan; $P < 0.05$). K+ (Positive control using Chloramphenicol of 30 mg mL^{-1}), K- (Negative control using Aquadest), A (20 mg mL^{-1}), B (40 mg mL^{-1}), C (60 mg mL^{-1}), D (80 mg mL^{-1}).

Table 1 Result of phytochemical test of buton forest onion extract

Parameter	Reagent	Result
Flavonoid	-	Positive
Alkaloid	Wagner	Negative
	Dragendorff	Negative
	Meyer's	Negative
Tannin	-	Positive
Saponin	-	Positive
Quinone	-	Positive
Steroid	-	Positive
Triterpenoid	-	Positive

Kalimantan, positively contained alkaloid compound, both extracts were produced using water and ethanol solvent. The difference of this phytochemical content was expected due to the difference of the type of soil where the bulbs of buton forest onion extract used in this study grew, that was Buton forest of South East Sulawesi. Furthermore, the use of solvent also affected the compound composition of extraction product. It was explained by Stanojević *et al*. (2009) that the total phenol and flavonoid content in *Hieracium pilosella*

extract was higher by using ethanol solvent compared with water and methanol maceration. According to Febrinda (2014), buton forest onion extract produced using ethanol solvent contained higher phytochemical compound, total phenol, and total flavonoid than that of water maceration.

This study showed that higher dose of buton forest onion extract resulted in larger clear zone obtained from agar diffusion method or less bacterial density through co-culture method. Result of this research was in line with the research finding of Pandhi and Panda (2015) that the use of *E. bulbosa* extract with solvent of Ethyl acetate, Chloroform, Butanol, Ethanol, and Aqueous at dose of 30 mg mL^{-1} (w v^{-1}) was able in inhibiting Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens, Shigella boydii, Shigella dysentriae, Shigella flexneri, Salmonella typhimurium, Shigella sonnei, Vibrio alginolyticus, Vibrio cholerae*) and Gram positive bacteria (*Bacillus brevis, Bacillus subtilis, Bacillus licheniformis, Staphylococcus aureus, Staphylococcus epidermidis*). The use of extract from medicinal plants was able to penetrate the outer membrane of bacteria, disrupt cellular and metabolism function, and led to the loss of cell content that eventually resulted in death (Kang *et al*. 2011). These naturally occuring phenols and phenolic compounds of medicinal plants may accumulate in bacterial membrane which led to energy depletion (Conner 1993), inhibited bacterial cell wall synthesis (Marcucci *et al*. 2001).

extract to inhibit *V. harveyi* was due to the existence of flavonoid, tannin, saponin, quinone, steroid, and triterpenoid compounds which were expected to be able to inhibit metabolism and caused *V. harveyi.* cell bacteria to lyse since polyphenolic, flavonoid, terpenoid, and otThe ability of buton forest onionher volatile compounds have strong antimicrobial properties (Gulluce *et al*. 2007; Tassou *et al*. 2000).

Lin and Tang (2007) mentioned that phenolic and flavonoid content in fruits and vegetables functioned as immunomodulator and was able to kill microorganism. Extract of medicinal plants rich of phenolic compound contained high level of antimicrobial activity with the ability to inhibit the growth of various Gram-negative and Gram positive bacteria (Silici *et al*. 2010; Negi 2012). Phenolic compound may cause bacterial cell wall to lyse, disrupt cytoplasmic membrane, and lead to leakage in cellular structure of bacteria which later resulted in cell death (Negi, 2012). According to Kim *et al.* (1995), medicinal plant compound was able to hinder the permeability of cell membrane and caused changes in cell structure and function, cell damage until cell death.

In conclusion, the use of buton forest onion extract resulted in the death of *V. harveyi* cell. It was shown by the result of inhibitory potential test through co-culture method in this study, in which the colony of *V. harveyi* was 0 cfu mL^{-1} at dose treatment of D (80 mg mL^{-1}). Whereas, in dose treatment of B and C (60 mg mL^{-1} and 40 mg mL^{-1}), there was still *V. harveyi* some colonies, yet it surpressed the *V. harveyi* growth significantly higher compared with control treatment.

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