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Short Communication: Antibacterial activity of *Boesenbergia pandurata, Zingiber zerumbet* and *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp.

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Abstract. *Hardi EH, Kusuma IW, Suwinarti W, Agustina, Nugroho RA. 2016. Antibacterial activity of* Boesenbergia pandurata, Zingiber zerumbet *and* Solanum ferox *extracts against* Aeromonas hydrophila *and* Pseudomonas sp. *Nusantara Bioscience 8: 18-21*. This study evaluated the potential antibacterial activity of *Boesenbergia pandurata, Zingiber zerumbet* and *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp. This paper aims to review the best concentration of the extract *B. pandurata, Z. zerumbet* and *S. ferox* to inhibit the growth of bacteria *A. hydrophila* and *Pseudomonas* sp. on tilapia in vitro test. The concentrations used range from 100-6000 ppm for *B. pandurata* and *S. ferox*, menwhile for *Z. zerumbet* extracts ranged from 25-1000 ppm. The best concentration was injected to tilapia by intraperitoneal (0.1 mL/fish) to know in vivo inhibition of extract in fish. The results showed that *B. pandurata* 600 and 900 ppm , and *Z. zerumbet* 200 and 2000 ppm revealed potent antibacterial activity against *A. hydrophila*; while the concentration of *S. ferox* at 400 and 900 ppm inhibit *Pseudomonas* sp. growth, whereas concentration of 600, 200, and 900 ppm reduced the bacteria pathogen in fish body.

Key words : *Aeromonas hydrophila*, antibacterial, *Boesenbergia pandurata, Pseudomonas, Solanum ferox, Zingiber zerumbet*

INTRODUCTION

Aeromonas hydrophila and *Pseudomonas* sp. bacteria are opportunistic Gram negative pathogens naturally occurring in aquatic environment, it cause outbreak when the normal environmental conditions changed (Austin and Austin 2007). Symptoms of infection in fish from the two bacteria include exophthalmia on fish eyes, broken fins, ulcer in infection organs and gall bladder rupture and usually causes the internal organs of fish looks pale (Hardi and Pebrianto 2012).

Prevention and treatment of fish bacterial disease using natural materials are recommended because it is safe and has no impact on fish resistant. Previous study showed that the extract of *Boesenbergia pandurata* Roxb. and *Zingiber zerumbet* (L.) Roscoe ex Sm. were able to suppress the growth of *A. hydrophilla*, while *Solanum ferox* L. suppressed the growth of *Pseudomonas* sp. (Hardi et al. 2015). Finger root (*B. pandurata)*, zerumbet ginger (*Z. zerumbet)* and mawaeng *(S. ferox)* are herbal plants that are always found at traditional markets in Samarinda, East Kalimantan. These plants are easy to growth and have been using them to support East Kalimantan communities lives. *Boesenbergia pandurata* and *Z. zerumbet* are used to make traditional medicines while *S. ferox* is, used in food traditionally by the dayak and banjar tribes.

Application of these plants in the field of fisheries has not been done, but application in human medicines has been reported such as antifungal, antibacterial, anti-inflammatory, and anti-cancer (Chahyadi et al. 2014). These plants are widely used to treat a number of ailments such as gastrointestinal (Farnsworth and Bunyapraphatsara 1992), oral, and respiratory (Hirschhorn 1983; Saralamp et al. 1996). This study was aimed to evaluate the antibacterial activity of *B. pandurata, Z. zerumbet* and *S. ferox* extract against *A. hydrophila* and *Pseudomonas* sp. pathogens in fresh water aquaculture and examine their mechanisms of action.

MATERIALS AND METHODS

Plant material

Boesenbergia pandurata, Z. zerumbet and *S. ferox* herbal materials were collected from traditional market in Samarinda. The ethanol extract of rhizome of plants were prepared according to previous study by Limsuwan and Voravuthikunchai (2008).

Bacterial isolate and culture condition

Aeromonas hydrophila (EA-01) and *Pseudomonas* sp. (EP-01) were isolated from Nile tilapia (*Oreocrhomis niloticus*) from Loa Kulu Village of Kutai Kartanegara Regency, East Kalimantan Province. The bacteria were grown in BHI (Brain Heart Infusion Broth, DIFCO®) and

BHIA (Brain Heart Infusion Agar, DIFCO®) media for 24 h at 30°C and the density of bacteria was 10^{10} CFU/mL.

Antibacterial activity

A modified method according to Carson et al. (2002) and Limsuwan and Voravuthikunchai (2013) method was used to determine the bacteriolytic activity of the extract. The suspensions of bacteria *A. hydrophila* was 10^{10} CFU/mL in 0.45 % normal saline solution and mixed with the plant extract at concentrations 100-6000 ppm of *B. pandurata* and 25-1000 ppm of *Z. zerumbet*, respectively. In addition, the extract of *S. ferox* 100-6000 ppm was mixed with *Pseudomonas* sp. bacteria. Further the optical density (OD) was measured at 540 nm after 24 h of incubation. Bacterial cell lyses was indicated by a decrease of bacteria activity indicated by the changed medium color. Corresponding dilutions of the extract were used as blanks.

Bacteriolysis

Each bacterium was injected to 30 tilapia (15 g) every treatment through intramuscular and then the third day was injected with each extract (1 mL/fish) by intra peritoneal.

In this research, three different combinations of mix extracts and bacteria were applied to evaluate the effective dosage gifted to inhibit growth of *A. hydrophila* and *Pseudomonas* sp. in Tilapia fish. For the *A. hydrophila* inhibition, 30 fish samples were injected with 1010 CFU/mL *A. hydrophila* and 600 ppm of *B. pandurata* (1)*;* 1010 CFU/mL *A. hydrophila* and 200 ppm of *Z. zerumbet* (2). Then, for the *Pseudomonas* sp. inhibition, 1010 CFU/mL *Pseudomonas* sp. and 900 ppm of *S. ferox* was injected (3). The density of bacteria in fish was calculated using TPC (Total Plate Count) method in BHIA medium and evaluated every single week since fish sample was injected with pathogen bacteria.

RESULTS AND DISCUSSION

Antibacterial activity of extract

Finger Root (Boesenbergia pandurata)

Boesenbergia pandurata in vitro test showed that all concentration of ethanol extract have antibacterial activity against *A. hydrophila* (Figure 1). However generally the concentrations tested could inhibit the growth of bacteria. Concentrations of 600, 900 and 1000 ppm and a concentration of more than 4500 ppm are the best concentration to inhibit the bacteria growth and likely to be used as antibacterial and immunostimulant in fish.

Chahyadi et al. (2014) indicated that Rhizome of *B. pandurata* contains essential oils and many flavonoid compounds that showed many interesting pharmacological activities, such as antifungal, antibacterial, antioxidant and *B. pandurata* extract has powerful in vitro activity against *Streptococcus pyogenes*. The ability of *B. pandurata* extract to lyses the bacterial cells suggests that the mechanism of action may be associated with cell wall and cell membrane damage (Limsuwan and Voravuthikunchai 2013).

Mawaeng (Solanum ferox)

Extract of *S. ferox* indicated that the ethanol extract effectively suppressed *Pseudomonas* sp. pathogen bacteria than *A. hydrophila* (Figure 2). Concentration of extract at 300, 400, 500 ppm can inhibit the bacteria growth as well as 800-1000 ppm. Some data showed that any genus of solanum such as *S. torvum* has evoked antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Streptococcus aureus* (Wiart et al. 2004), whereas *Streptococcus nigrum* inhibit the *Salmonella typhi* (Rani and Khullar 2004). Species of *Streptococcus trilobatum* can reduce the bacteria pathogen in fish aquaculture system (Citarasu et al,. 2003) and *Streptococcus incanum* can decrease *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Enterobacter aerogenes, Enterobacter cloacae, Micrococcus kristinae* and *Streptococcus aureus* growth (Kambizi and Afolayan 2001).

Zerumbet Ginger (Zingiber zerumbet)

Results of testing multiple concentrations of the extract *Z. zerumbet* can be seen in Figure 3. The antibacterial activity of extracts of *Z. zerumbet* was not better than *B. pandurata* against *A. hydrophila*. Most *Z. zerumbet* concentrations used (of 25-10000 ppm) ability to inhibit the bacteria. The concentrations that could be used to combat *A. hydrophila* infection in tilapia are 200 and 2000 ppm. Diversity of concentrate showed that the extracts of the herb plant is still very natural, unlike synthetic antibacterial ingredients or chemicals that tend to be more constant. Furthermore, Kader et al. (2011) claimed that ethanol extract of 400 µg/disc *Z. zerumbet* effective to inhibit fungal and bacteria such as *Vibrio parahemolitycus* and Bharate et al. (2007) and Singh et al. (2014) found that the oil of *Z. zerumbet* has a good activity against *Cryptococcus neoformans* ATCC 90113 with IC50 value of 8 µg/mL.

Bacteria growth of *A. hydrophila* decrease in fish body that given with *B. pandurata* and *Z. zerumbet* extract, not as in control fish were not given the extract. Similarly, the bacterium *Pseudomonas* sp. infection showed a decrease in fish body. This suggests that the *B. pandurata, Z. zerumbet* and *S. ferox* extract can suppress the growth of *A. hydrophila* and *Pseudomonas* sp. bacteria. This activity caused *B. pandurata* and *Z. zerumbet* have antibacterial compound such as alkaloid and flavonoid. Whereas *S. ferox* has alkaloid as antibacterial compound (Figures 4 and 5). Chahyadi et al. (2014) reported that ethanol extract of *B. pandurata* has antibacterial compound such as flavonoid against food borne pathogenic bacteria among others are *Listeria monocytogenes* (5 strains), *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus*.

Conclusion from this research are *B. pandurata* and *Z. zerumbet* effectively suppress the growth *A. hydrophila* bacteria and *S. ferox* has antibacterial activity to against *Pseudomonas* sp. bacteria pathogen in tilapia.

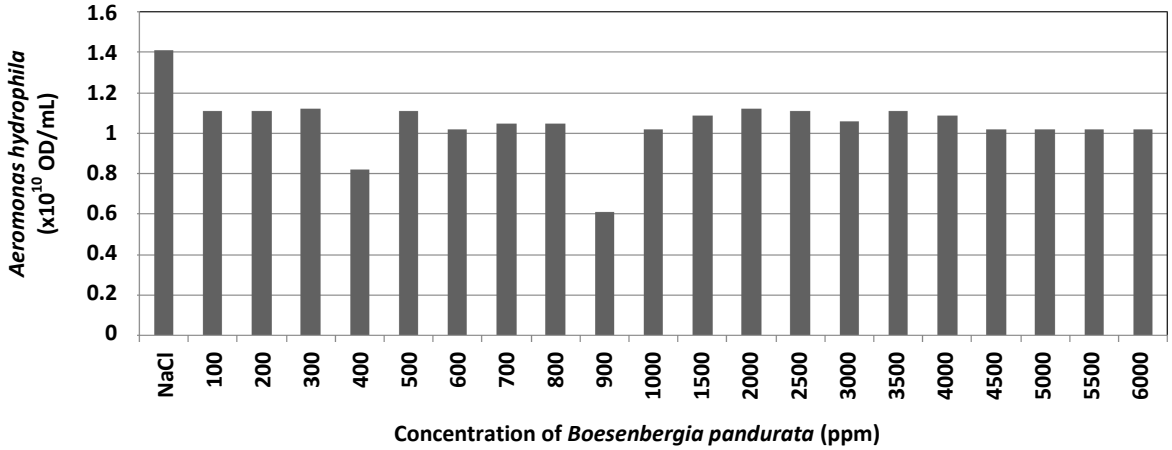

Figure 1. Antibacterial activity of *Boesenbergia pandurata* extract against *Aeromonas hydrophila* on 24 h incubation

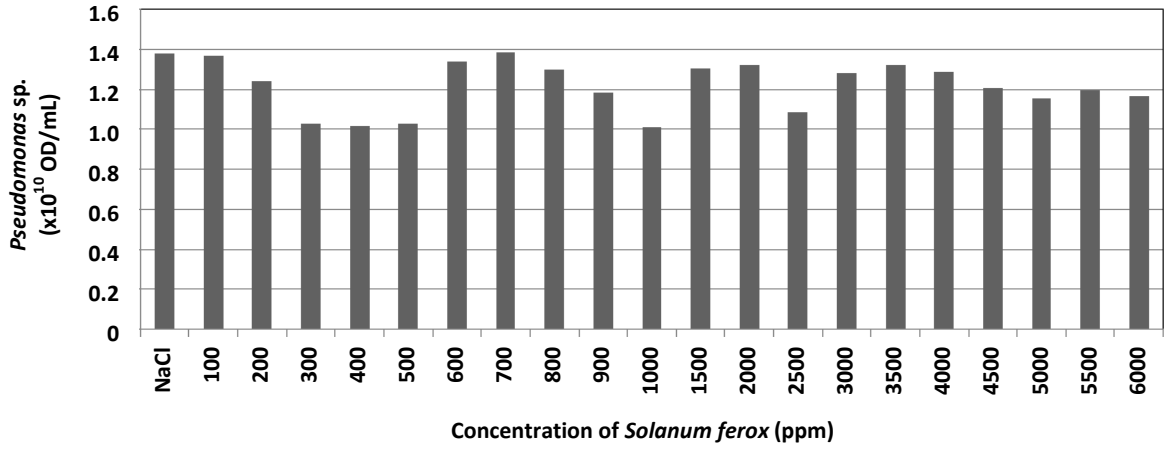

Figure 2. Antibacterial activity of *Solanum ferox* extract against *Pseudomonas* sp. on 24 h incubation

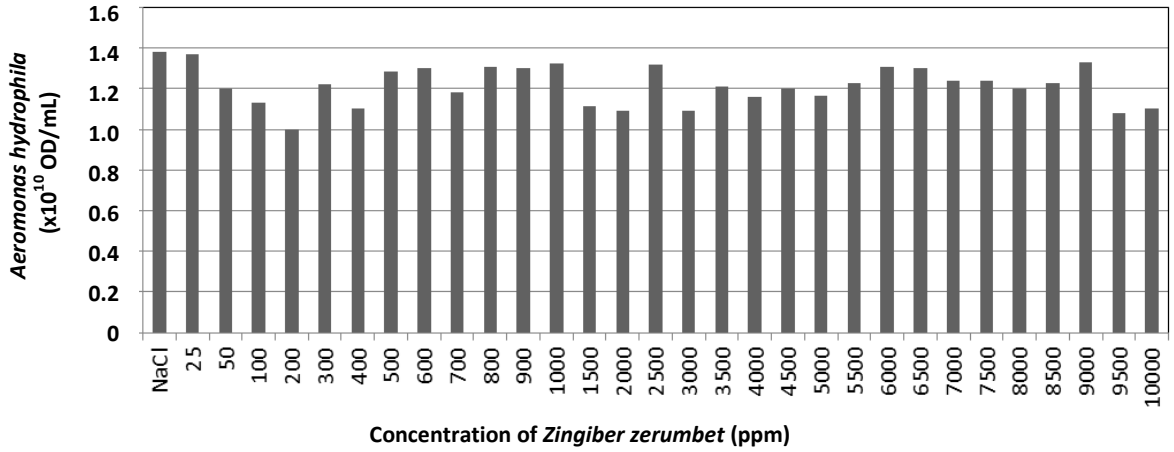

Figure 3. Antibacterial activity of *Zingiber zerumbet* extract against *Aeromonas hydropila* on 24 h incubation

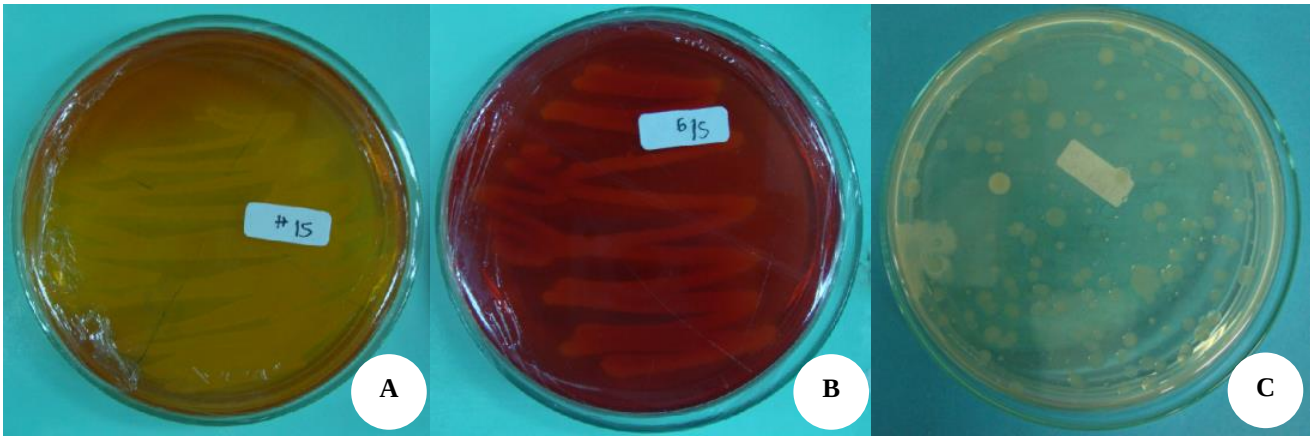

Figure 5. Colony bacteria of (A) *A. hydrophila* and (B) *Pseudomonas* sp. and (C) colony bacteria in bacteriolysis test using Total Plate Count Method

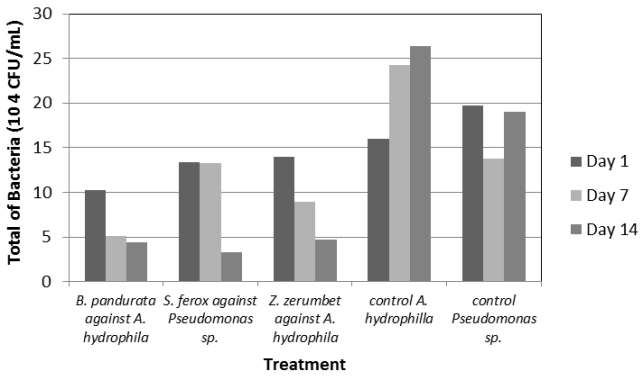

Figure 4. The bacteria density of *Aeromonas hydropila* and *Pseudomonas* sp. after administration with *Boesenbergia pandurata*, *Zingiber zerumbet* and *Solanum ferox* in vivo test

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