Aquacultura Indonesiana (2020) 21(2): 82-86
ISSN : 2477-6939

http://dx.doi.org/10.21534/ai.v21i2.202

Inhibition Activity In Vitro Test of Temu Kunci Extract (*Boesenbergia Pandurata*) againts *Streptococcu*s sp. and *Aerococcus* sp.

Wey Ninta Carina[1], Esti Handayani Hardi[2], Henny Pagoray[3]

[1]Study Program of Aquaculture, Faculty of Fisheries, University of Mulawarman Samarinda
[2,3]Departement of Aquaculture, Faculty of Fisheries, University of Mulawarman Samarinda, Indonesia

Corresponding email : weyninta1606015002@gmail.com

Received : 13 July 2020 , Accepted : 30 December 2020

Abstract

Wey Ninta Carina, Esti Handayani Hardi, and Henny Pagoray. **2020. Inhibition Activity In Vitro Test of Temu Kunci Extract (Boesenbergia Pandurata) againts Streptococcus sp. and Aerococcus sp. . *Aquacultura Indonesiana,* 21 (2): 82-86.** This study examines the effective concentration of temu kunci extract (*Bosenbergia pandurata*) as an antibacterial inhibition zone against 3 *Aerococcus* sp. isolates. and 2 *Streptococus* sp. isolates. The method used is the ADD (Agar Diffusion Disc) method and bacterial culture together with the bacterial count using the TPC (Total Plate Count) method. Extract concentrations used were concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm, and 1000 ppm. Positive controls used were amoxicillin concentration of 500 ppm and negative controls using sterile aquades. Bacteria were grown on BHIB media and then as much as 1 ml were spread on BHIA media on petri dishes, then 20 µl drops were dropped on an antibacterial disc measuring 6 mm, then incubated at 30ºC for 48 hours and carried out also for 24 hours and 48 hours. Furthermore, in the second experiment carried out by culture together the extract concentrations used were 600 and 800 ppm, the results showed that the concentration of 800 ppm was greater than the growth of bacterial destruction by the ADD method and the TPC method and the zone of inhibition of the extract against the test bacteria 10.61- 13.47 mm.

Keywords: Temu Kunci (*B.pandurata*), *Aerococcus* sp., *Streptococcus* sp., Zone of Inhibition

Introduction

Enlarging aquaculture activities can be decreased by various problems. One of the problems in fish farming is the coming bacterial diseases. Based on the research results done Hardi et al in 2018, it was found 37 types of bacteria being capable of infecting cultured fish. fourteen of these bacteria are suspected to be pathogenic to both humans and aquaculture animals. Two bacteria are called *Aerococcus* sp. and *Streptococcus* sp.

Aerococcus sp. is a bacterial pathogen which is one of its varieties, named *Aerococcus viridans* var. Homaries. It causes disease agents of gaffchemia and systemic homarid lobsters (*Homarus americanus* and *H. gammarus*). They are found in coastal waters of North America, Korea, Europe (Wood, 1999). The effect of gaffkaemia infection on lobsters is that the lobsters appear lethargic, drooping tails, and pale pinks on the sides of the stomach, lobsters swim sideways, and can lose balance when swimming (Lewbart, 2006).

Bacteria *Streptococcus* sp. is bacteria causing infectious diseases in several types of freshwater fish and sea fish. The disease known as Streptococcosis is also a main problem in tilapia farming. The difference in appearing symptoms can be related to the target organ of *S. agalactiae* (eyes, brain and kidney). The presence of bacteria in the eye organ can produce changes in the eye (purulence, opacity, exoptalmia and shrinkage of the eye). The presence of bacteria in the brain organs can cause fish swim abnormally (gasping, swimming sideways even whirling) while the presence of bacteria in fish kidneys can cause discoloration (Hardi et al., 2011)

Antibiotics have an important role in the world of health, antibiotics are expected to kill the bacteria causing infection (Jeffrey, 2006). The use of antibiotics must consider several important factors, including the selection of types and dosages of antibiotics, because the wrong use of can lead to new problem called resistance (Erviani, 2013). plant antibiotics can be used as an alternative for the resistence (Jaksa, 2009)

Boesenbergia pandurata extract contains essential oils. Essential oil or etheric oil (essential oil, volatile) is deriving from the

result of plant metabolism consisting of aromatic compounds. Components in essential oil compounds can react with damaging bacterial cell wall components. The content of compounds are benzaldehyde, linalol, simen, borneol and ossimens. They can inhibit bacterial growth (Miksusanti et al., 2008). Research done by (Chahyadi et al., 2014), shows that *B. pandurata* essential oils have antibacterial activity in *Escherichia coli bacteria, Bacillus cereus, Listeria monocytogenes* (Chahyadi *et a*l., 2014)

Based on the description above, the researcher wants to examine the extract of *B. pandurata* has antibacterial activity to inhibit the *Aerococcus* sp. and *Streptococcus* sp. in vitro.

Reserch Method

The research was carried out in September - November 2019. The research was conducted at the Laboratory of Aquatic Microbiology, Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, East Kalimantan.

The preparation Bacteria Aerococcus

Two of isolates *Aerococcus urinae*, one isolate *A. viridans*, and two of *Streptococcus inae* bacteria. Each bacteria (0.5 ml) were cultured into BHIB media and then incubated for 24 hours at 30 ^{o}C with the scatter method using L glass.

Preparation *Boesenbergia Pandurata* Extract

Boesenbergia pandurata extract concentration using in this research were 100 until 1000 ppm, and there were 10 concentration of *B. pandurata*, the extract from the Laboratory of Aquatic Microbiology, Faculty Fisheries and Marine Science, Mulawarman University.

Antibacterial activity uses the Agar Disc Diffusion (ADD) method

The antibacterial test was performed following method Dulger & Gonuz (2004). The each extracts concentration (25 μm) were dropped on Weidman sterile paper, then placed on a media that already contained cultured bacteria on BHIA medium (Brain Hearth Infusion Agar), then incubated for 24 h at 30^{o}C. The inhibition zone was observed 24 and 48 h after inoculation.

Antibacterial activity with the calculation of the Total Plate Count (TPC) method

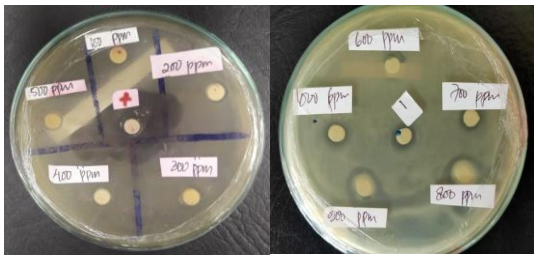
In this step was the continuous from the ADD test before. Two of the best concentration of *B. pandurata* in ADD was continuous with the MIC test, to evaluated the decreased of bacterial which using the extract. The suspensions of each bacetria Aerococcus and Streptococcus was 10^5, 10^6, 10^7 CFU/mL in normal saline solution and mixed with the plant extract at two of the best concentrations. A 1 ml sample (bacteria mix extract) was cultured in BHIA medium for 48 hours at 30 °C and observed at 24 and 48 hours,

Data analysis

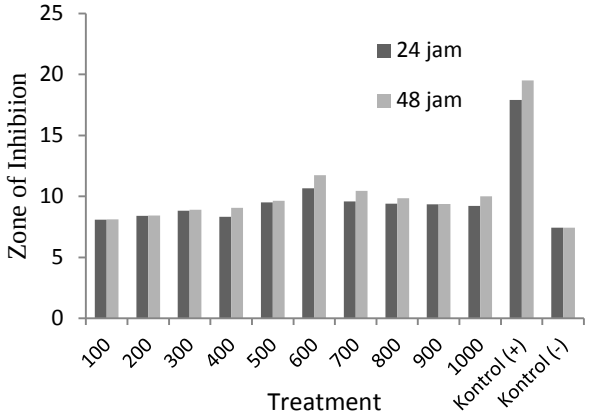
The data obtained are in the form of inhibition zone diameters in the form of graphs and tables**.**

Result

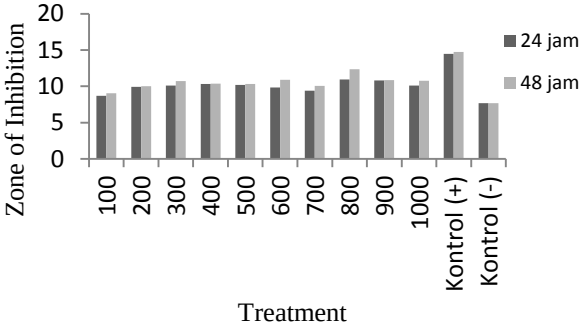
Aerocococcus sp. is a gram-positive catalase negative bacterial, facultative anaerobic, that grows in NaCl. the shape of the colony is irregular and milky white. Colony of Streptococcus sp. is clear white or pale yellow and small round. It is a gram positive bacteria, negative catalase, facultative anaerobic, and grows in NaCl (Hardi *et al* 2018.



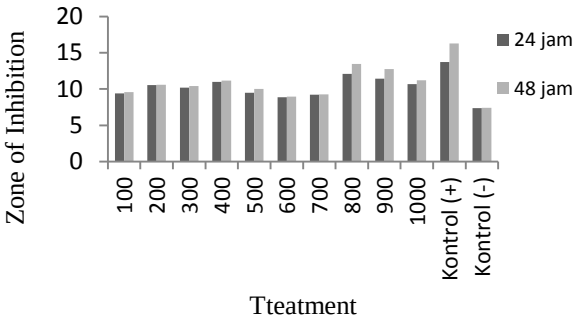
Picture 1. Zone of Inhibition on the antibacterial activity of the extract in the test bacteria.



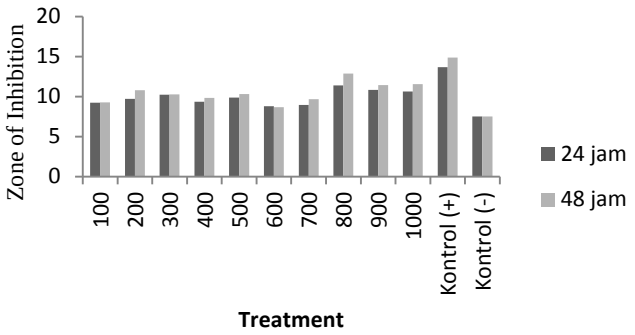
Picture 2. Result of Inhibitory Test of Temu Kunci (B.pandurata) Extracts against Bacteria A. urinae



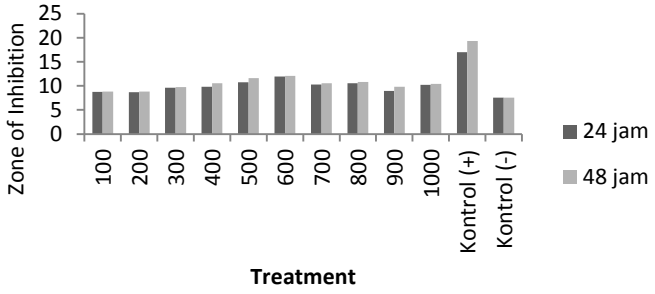
Picture 3. Result of Inhibitory Test of Temu Kunci (B.pandurata) Extracts against Bacteria A. Urinae 2



Picture 4. Result of Inhibitory Test of Temu Kunci (B.pandurata) Extracts against Bacteria *A.viridans*



Picture 5. Result of Inhibitory Test of Temu Kunci (B.pandurata) Extracts against Bacteria *S. inae*1



Picture 6. Result of Inhibitory Test of Temu Kunci (B.pandurata) Extracts against Bacteria Sreptococcus sp.

Table 7. Result of Bacteria Culture Test with Extracts using Total Plate Count Method

Bacteria	Numbers of Bacteria (TPC CFU/mL)			
	before treatment	With additional extract and antibiotics		
	10^9	600 ppm ($x10^9$)	800 ppm ($x10^9$cfu/ml)	Amoxicillin ($x10^9$cfu/ml)
***A. urinae*1**	89	0,59	0,45	0,34
***A. urinae* 2**	69	0,33	0,31	0,50
A. viridans	38	0,91	0,21	0,29
***S. inae* 1**	43	0,59	0,53	0,24
***S. inae* 2**	44	0,13	0,01	0,14

Analysis

Based on the research, it shows that in the time of observation, size of the diameter increases. this is caused by the influence of the incubation period and *B. pandurata* extract which can inhibit the growth of *Aerooccus* sp. and *Streptococcus* sp. with 600 and 800 ppm of concentration. These are the best inhibitory zones and belong to the intermediate (sensitive) category**.**

Culturbacteric test applied to Temu Kunci's (*B. Pandurata*) extract is aimed to prove that Temu Kunci's (*B. Pandurata*)

extract can inhibit the *Aerococcus* sp. and *Streptococcus* sp. in the inhibition test (ADD). Observation of bacterial content in the bacterial culture method with the extract was carried out by the TPC (Total Plate Count) method. Observation of bacterial content by the TPC method showed the ability of key methyl (*B. pandurata*) extracts to inhibit bacterial growth at concentrations of 600 ppm and 800 ppm. Temu Kunci's extract is effective in inhibiting the bacterium *Aerococcus* sp. and *Streptococcus* sp. The bacterial content in the total plate count method with concentrations of 600 ppm, and 800 ppm shows different levels. The highest antibacterial activity was shown at concentration of 800 ppm with a number of 0.01 cfu / ml colonies in *Streptoccoccus inae* 2. and 0.21 cfu / ml number of bacterial colonies in *Aerococcus viridans*. In positive control Amoxicillin, the growth of *Streptococcus iniae* bacteria is more effective than *Aerococcus* sp..

Activity of *B. Pandurata* extract shows that the ability of *B. Pandurata* extract has active ingredients. According to Hardi et al (2016) the results of phytochemical analysis of B. pandurata indicate that B. pandurata contains of alkaloids, flavonoids that can inhibit bacterial growth.

The ability of B. pandurata extract can lyse bacterial cells, this shows that *B. pandurata* extract can damage the cell wall and bacterial cell membrane (Zuhud et al., 2001). Based on this study, the antibacterial activity of *B. pandurata* extract at concentrations of 600 and 800 is the best concentration because it can inhibit bacteria greater than other concentrations.

Both concentrations are included in the category of intermediates which can be developed as antibacterial agents against bacterial attack on fish. (Saptiani et al., 2012)

Conclusion

B. pandurata extract with concentration of 600 and 800 ppm can inhibit the bacteria *Aerococcus* sp. and *Streptococcus* sp. compared to other concentrations in vitro. Bacterial culture test with extract concentration of 800 ppm is better in inhibiting the growth of *Aerococcus* sp. and *Streptococcus* sp

Bibliography

Chahyadi, A, Hartati, R., Wirasutisna, K. R., & Elfahmi. 2014. Boesenbergiapandurata Roxb, An Indonesian Medicinal Plant : Phytochemistry, Biological Activity, Plant Biotechnology.Procedia Chemistry, 13-37

Erviani, A. E. 2013. Analisi Multidrug Resistensi terhadap Antibiotik pada *Salmonella typhi* dengan Teknik Mulplex PCR.biogenesis, 51-60

Fardiaz. 2004. Analisa Mikrobiologi Pangan. PT. Raja Grafindo Persada : Jakarta.

Hardi, E.H, Sukenda, E. Harris, dan A.M Lusiastuti. 2011. Karakteristik dan Patogenisitas *Streptococcus Agalactiae* Tipe β β β β β-hemolitik dan Non-hemolitik pada Ikan Nila. Jurnal Veteriner. Vol. 12 No. 2: 152-164

Hardi, E.H, W.K Irawan, Suwinarti W, Agustina, dan A.N Rudi. 2016. Antibacterial activity of *Boesenbergia pandurata*, *Zingiber zerumbet* and *Solanum ferox* extracts againts *Aeromonas hydrophilla* and *Pseudomonas* sp. Jurnal Veteriner. 8 (No.)1:18-21

Hardi, E.H, G. Septiani, R.A. Nugroho, R. Sarinah dan M. Agriandini. 2018. Identification of Potential Pathogenic Bacteria from Tilapia (*Oreochromis niloticus*) and Channel Catfish (*Clarias batracus*) Culture in Samarinda East Kalimantan. Biodiversitas, Inpress.

Jaksa, S. 2009. Minyak Atsiri Dari Beberapa Tanaman Obat.Jurnal Kedokterandan Kesehatan.

Lewbart. *2006. "Crustaceans"*. Invertebrate Medicine. Wiley-Blackwell. *pp. 179–194*. ISBN 978-0-8138-1844-3.

Miksusanti, Jennie, B. S., Ponco, B., & Trimulyadi, G. 2008. Kerusakan Dinding Sel *Eschericia coli* kl.l oleh Minyak Atsiri Temu Kunci (*Kaempferiapandurata*). Berita Biologi Jurnal Ilmiah Nasional, 1-8.

Saptiani,G., S. B. Prayitno dan S. Anggoro. 2012. Efektifitas Ekstrak Daun Jeruju (Acanthus ilicifolius) Melindungi Udang Windu (Penaeus monodon F.) dari Infeksi Vibrio harveyi. J. of Coastal Development, 15(2): 217–224

Uderwood J.C.E . 1999.Karakteristik, Klasifikasi dan insiden Penyakit , patologi umum dan sistemik, edisi 2. buku kedokteran: Jakarta Waluyo, Lud, 2005. Mikrobiologi Umum. Malang : UMM Press.

Zuhud, EAM., Rahayu, WP., Wijaya, CH., dan Sari, PP. 2001. Aktivitas Antimikroba Ekstrak Kedawung (Parkia roxburghii G. Don) terhadap Bakteri Patogen.Jurnal Teknologi dan Industri