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The Potential of Sea Worm *Perinereis aibuhitensis* Extract as Anti-microbe toward Bacteria *Salmonella typhi* and Fungus *Candida albicans*

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Abstract

The research about the potential of sea worm *P. aibuhitensis* extract as anti-microbe toward bacteria *S. typhi* and fungus *C. albicans* had been conducted. The aim this research is to know the extract's concentration of sea worm *P. aibuhitensis* which is effective to inhibit the growth of bacteria *S. typhi* and fungus *C. albicans.* The result of research that was obtained shows that sea worm's extract which used ethanol solution 96% and was needed in the culture of bacteria *S. typhi* with the concentration of sea worm's extract 7.5%, 15%, 30%, and 60% involved the zone of inhibition's form at the medium with duration incubation 1x24 hours and 2x24 hours. Based on the research which was conducted, it can be concluded that the sea worm *P. aibuhitensis* extract had the potential as anti-microbe toward bacteria *S. typhi* because can inhibit the growth of that bacteria, but it had no effect as anti-microbe toward fungus *C. albicans.*

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Keyword
Perinereis aibuhitensis,
Anti-microbe,
Salmonella typhi,
Candida albicans.

Introduction

One of the sea worms has the potential to be cultivated at Makassar city that is sea worm *P. aibuhitensis,* this sea worm was founded in great quantity at Reklamasi area of Losari beach, Makassar city. This worm is only used as fish bait. The potential of this worm is not yet utilized properly because the lack of scientific information about that worm. Various scientific researches about sea worm *P. aibuhitensis* need to be conducted to know the potential of one of that sea biota and its further utilization. Activity test as anti-microbe is also not yet conducted, therefore the research about activity test of sea worm *P. aibuhitensis* extract as anti-microbe needs to be conducted.

Sea worm contents the high protein but it is not yet utilized optimally in Indonesia. Sea worm has quite complete nutrition thus can be used as alternative food. The contents of nutrition in the sea worm are; protein, fat, carbohydrate, ashes, fat acid and amino acid, vitamins A, B1, B6, B12, E and chemical elements P, I_2, Ca, Mg, C which are almost equal to the nutrition contents of fish (Silaban 2012). The extract of sea worm *S. austral* had proven in containing 56.35% protein, 15.08% ashes concentration, 9.82% fat concentration, and 5.06%

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carbohydrate concentration (Nurhikma, et al, 2017). There were also various amino acids in sea worm where amino acid is very important to support the various physiological activities of body. One of amino acids in the sea worm is glutamate acid which has the important role in the metabolism of sugar and fat, in addition, glutamate acid in the animal or plant can be used as medical treatment substance in solving epilepsy disease, mental retardation, dystrophy, abscess, hypoglycemic coma, and also the side effect of insulin treatment for diabetes. Based on the information above and the lack of information about the ability of sea worm *P. aibuhitensis* as anti-microbe, then activity test of sea worm *P. aibuhitensis* extract as anti-microbe toward bacteria *S. typhi* and fungus *C. albicans* is important to be done.

Materials and Methods

Materials

The materials which were used –sea worm *P. aibuhitensis* which was obtained at Reklamasi area of Losari beach and the materials for extracting were absolute ethanol, culture of bacteria *S. typhi*, chloramphenicol, fungus *C. albicans,* $MgCl_2$ 72%, Potato Dextrose Agar (PDA), aquades, NaCMC and griseofulvin.

The extraction of sea worm *P. aibuhitensis*

The material is the worm *P. aibuhitensis.* The worm *P. aibuhitensis* were taken around 1000 gram. Furthermore, the samples were washed by using flow water. The clean sample of worms were dried, after that pounded by using blender. The pounded worm *P. aibuhitensis* were used for extraction process (Purwaningsih et al. 2008). Extraction process was conducted by using 24 hour maseration method. The pounded worm were soaked by using ethanol solution with ratio 1:4 (b:v), were conducted maseration during 24 hours by using orbital shaker 24 hours at room temperature, then were distillated by using filter paper whatman number 42. Maseration was conducted in 3x24 hours. The resulted filtrates were separated with its solvent by using rotary vacuum evaporator at temperature 40℃ during 6 hours. Then, the resulted extracts were weighted.

Activity test of anti-microbe from sea worm *P. aibuhitensis* toward bacteria *S. typhi*

The used method for activity test was conducted through measuring the zone of inhibition's form. NA medium which had been sterilized was poured sufficiently into petri cup. After it is dense, the suspense bacteria were scratched into all surfaces of medium with swab technique by using sterilized cotton bud. After that, it was included paper disk which had been soaked by the extract of sea worm *P. aibuhitensis* in various concentration, those were 7.5%, 15%, 30%, and 60%, Chloramphenicol as positive control and NaCMC as negative control. Petri cup was labeled, then was incubated in incubator at temperature 37℃ during 24 hours, and then was observed and measured its zone of inhibition. Incubation was continued during 48 hours and was measured again its zone of inhibition which was formed.

Activity test of anti-microbe from sea worm *P. aibuhitensis* toward fungus *C. albicans*

Medium of Potato Dextrose Agar (PDA) which had been cooled was poured into petri cup and was waited until dense. After that, the suspense of fungus was taken by using cotton bud, then was stretched into medium Potato Dextrose Agar (PDA) by using swab method. After that, it was included paper disk which had been soaked by the extract of sea worm *P. aibuhitensis* in various concentration, those were 60%, 30%, 15% and 7.5%, positive control

(griseofulvin) and negative control (NaCMC). Petri cup was labeled, then was incubated in incubator at temperature 37℃ during 24 hours and 48 hours, then was observed and measured the transparent zone which was formed around paper disk by using ruler.

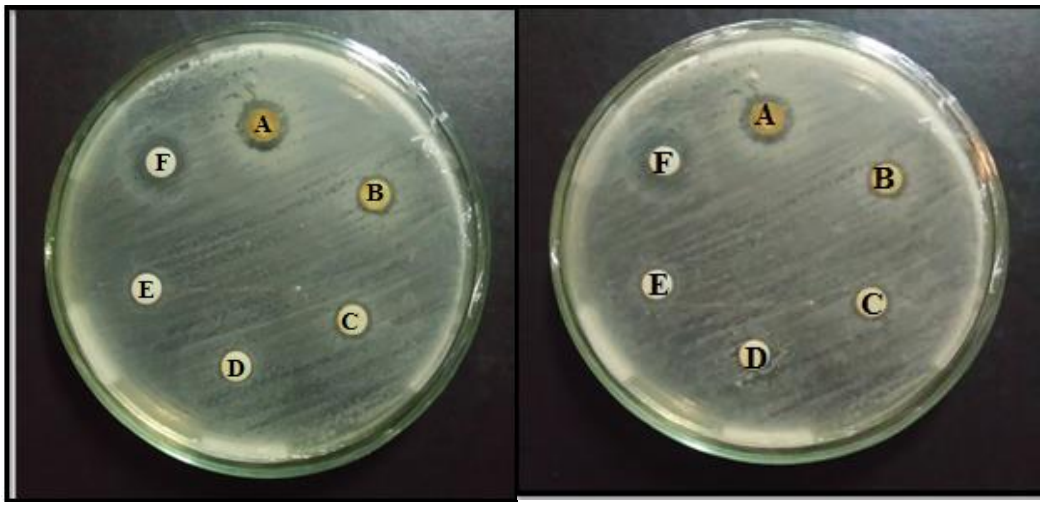
Data Analysis

Data which was gained during the observation of resistibility test was analyzed descriptively which was showed in form of figures and histogram.

Results and Discussion

Activity test of anti-microbe from sea worm *P. aibuhitensis* toward bacteria *S. typhi*

The observation toward bacteria *S. typhi* by using four kinds of concentration showed the form of various transparent zones at the time of incubation 1x24 hours. At the concentration 7.5% sea worm *P. aibuhitensis* extract did not form the zone of inhibition or transparent zone, concentration 15% formed the zone of inhibition or transparent zone around 9 mm which was included on medium category, concentration 30% formed the zone of inhibition or transparent zone around 10 mm which was also included on medium category, and concentration 60% formed the zone of inhibition or transparent zone around 12 mm which was included on strong category, while positive control chloramphenicol which formed the zone of inhibition or transparent zone 14 mm was included on strong category and negative control NaCMC did not form zone of inhibition or transparent zone. At the incubation 2x24 hours, the fourth concentrations which were used also showed the existence of the formed transparent zone, those were at the concentration 7.5% did not form the zone of inhibition or transparent zone, concentration 15% (8.5 mm) included on medium category, concentration 30% (10 mm) included also on medium category, concentration 60% (12 mm) included on strong category., while positive control (14 mm) included on strong category and negative control was not formed transparent zone. Figure 1 show the zone of inhibition or transparent zone which was formed at incubation 1x24 hours and 2x24 hours.



(a) 1x 24 hours (b) 2x24 hours

Figure 1. The test of resistibility of sea worm *P. aibuhitensis* extract in inhibiting the growth of bacteria *S. typhi*

Note:

A : Concentration 60%	C : Concentration 15 %	E : Negative Control
B : Concentration 30%	D : Concentration 7.5%	F : Positive Control

Histogram of average diameter's measurement result of zone of inhibition or transparent zone at incubation 1x24 hours and 2x24 hours can be seen in figure 2.

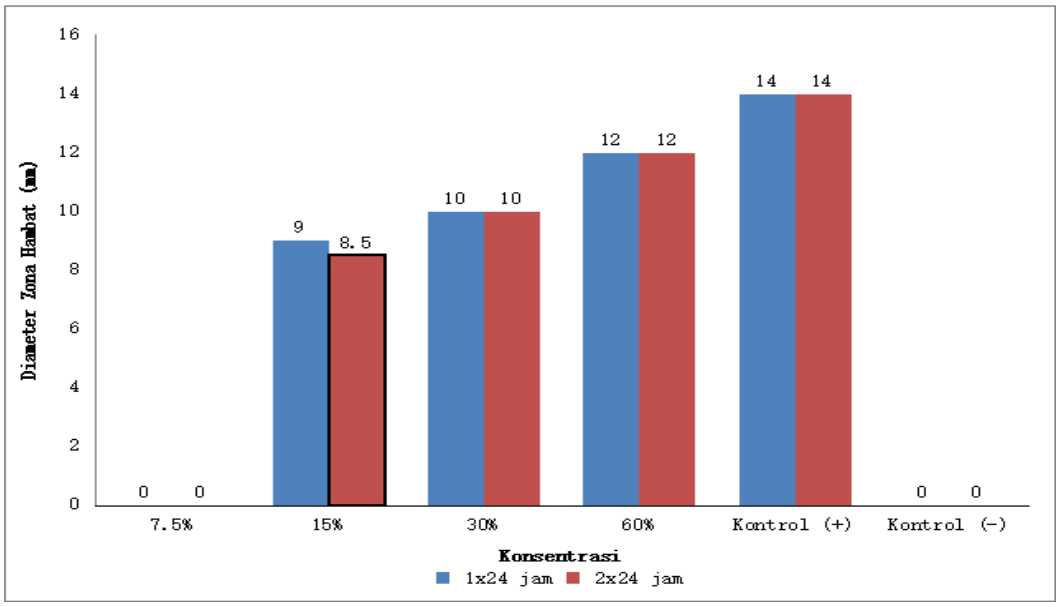
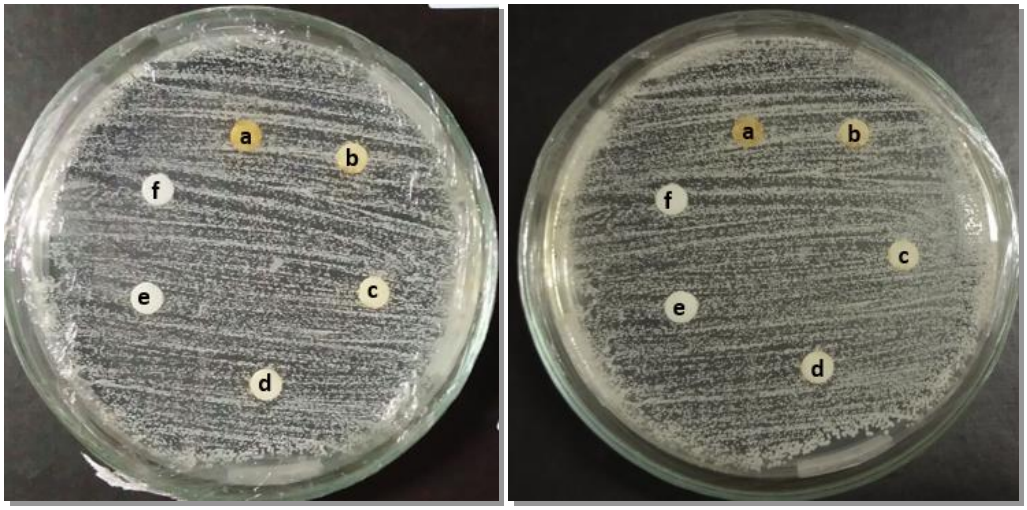


Figure 2. The comparison histogram of average diameter's measurement result of inhibition (mm) sea worm *P. aibuhitensis* extract in inhibiting the growth of *S. typhi* at incubation 1x24 hours and 2x24 hours

On the resistibility test, concentration 60% of sea worm extract had the higher zone of inhibition rather than other concentrations. It was caused because of the higher concentration of anti-microbial substance which was given then the more excessive anti-microbial substance on the worm extract thus had the bigger effect. Ningtyas (2010) explained that the higher concentration of extract than the more excessive active substance of its anti-bacteria. The addition of anti-bacterial substance's concentration expected can increase the penetration of anti-bacterial substance into microbe's cell which will damage metabolism system of cell and can cause the death of cell. Most of bacteria's growth will more decreased along with the increasing of additional anti-bacterial concentration. The higher concentration of extract then the number of detached anti-bacterial substances are bigger, thus make easier the penetration of those substances into cell (Maleki et al., 2008). Nevertheless, if it was compared to positive control with the gain 14 mm showed positive control more effective in inhibiting the growth of bacteria *S. typhi.* The lysis of bacteria cell was caused by the disability of cell wall to maintain the form and to protect the bacteria which has the high inside osmotic pressure (Ajizah et al., 2007). Without cell wall, bacteria cannot survive toward the outside influence and will die.

Activity test of anti-microbe from sea worm *P. aibuhitensis* toward fungus *C. albicans*

The observation result of zone of inhibition toward fungus *C. albicans* after incubation during 24 hours until 2x24 hours can be seen in figure 3.



(a) 24 hours (b) 2x24 hours

Figure 3. The test of resistibility of sea worm *P. aibuhitensis* extract in inhibiting the growth of fungus *C. albicans*

Note:

A : Concentration 60%	C : Concentration 15 %	E : Negative Control
B : Concentration 30%	D : Concentration 7.5%	F : Positive Control

On the concentration 60%, 30%, 15%, 7.5% and also positive control griseofulvin were not formed transparent zone. Thus can be concluded that sea worm *P. aibuhitensis* extract and griseofulvin cannot inhibit the growth of fungus *C. albicans.*

Resistance of anti-fungus is defined as adaptation or adjustment of fungus cell which is stable because of anti-fungus medicines, thus causes the sensitivity toward that anti-fungus decreased rather than the normal condition. Generally, fungus can be resistance intrinsically toward anti-fungus medicines (primary resistance) or resistance can occur as the response toward anti-fungus medicine during the treatment (secondary resistance). Fungoustatic medicine will more accelerate the resistance rather than fungousidal medicine (Apsari and Adiguna, 2013)

Candida as fungus can play the role as commensal or pathogen, where this fungus can change its phenotype randomly and reversible. The change of this phenotype supports adaptation mechanism of *C. albicans* toward the change from host or all factors which are on the human body that can influence its emergence and also on the way of disease which can be caused by the use of anti-fungus, immune response or physiological change. The effects are morphological appearance, form of cell, virulence and anti-genetic of *Candida* can change (Yugo and Ridhawati, 2013). According to Apsari and Adiguna (2013), some of funguses have also biofilm that can cause that fungus susceptible toward anti-fungus medicine.

Based on Nobile and Mitchell (2005), the ability of microorganism to affect its environment is based on its ability to form a community. *C. albicans* forms its community by binding the colony which is called biofilm. According to Mukherjee, et al. (2005) biofilm is microbes' colony (usually the cause of a disease) which forms organic polymer matrix that can be used as the sign of microbes' growth. That biofilm can be functioned as the protection, thus microbe which formed biofilm usually has the resistance toward the usual anti-microbe or avoid the immunity system of host's cell. The development of biofilm usually along with the increasing of clinical infection at host's cell, so this biofilm can be one of the factor of virulence and resistance (Kusumaningtyas, 2009)

According to Pramana et. al. (2006), some of researches mentioned that *C. albicans* had been resistance toward some kinds of anti-fungus, they are; flukonazol, ketonazol, nistatin, and amfoterisin B (Hastuti *et* al, 2013).

Conclusions

The effective concentration of sea worm *P. aibuhitensis* extract in inhibiting the growth of bacteria *S. typhi* was on the concentration 60% with forming the diameter of inhibiting zone 12 mm. But, sea worm *P. aibuhitensis* extract cannot inhibit the growth of fungus *C. albicans* which was signed by no forming of zone of inhibition.

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