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Antibacterial Activity Ethyl Acetate Extracts Of Earthworms (*Lumbricus Rubellus, Eisenia Foetida, Nereis* Sp) Toward *Staphylococcus Aureus, Enterococcus Faecalis, Salmonella Thyposa* Invitro

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Abstract

Earthworms had a mechanism of antibacterial. The research aimed to observe Lumbricus rubellus, Eisenia foetida, Nereis sp. antibacterial activity against Salmonella thyposa, Staphylococcus aureus, Enterococcus faecalis in vitro compared to ampicillin antibiotics. All the worms extracted using ethyl acetate extraction and tested their MIC. The compound of amino acids of the worms was analyzed by HPLC and nanodrop. Lumbricus rubellus was the best anti-bacteria activity followed by Eisenia foetida and Nereis sp, but these activities less than ampicillin antibiotic. Observations with SEM showed these worms extract caused cell leakage in all of these bacteria. Protein content with Nanodrop testing revealed the highest protein content was Lumbricus rubellus (21.75 ppm) followed by Eisenia foetida (21.32 ppm) and Nereis sp. (20.98 ppm), as well as for amino acids levels, there were Lumbricus rubellus (24.66%), Eisenia foetida (22.78%), Nereis sp. (18.37%). From the 15 amino acids detected, all of the worms had the same sequence of fourth the highest amino acids (Glutamate, Aspartate, Leucine, Arginine) and fourth the lowest amino acid levels (Methionine, Hystidin, Tyrosin, Glisan). It had not been tested amino acid sequences of antibacterial compounds of these worms (Lumbricin 1: Phe-Ser-Lys-Tyr-Glu-Arg in Lumbricus rubellus worms, Fetidin 1: Ala-Met-Val-Ser-Ser and Fetidin 2: Ala-Met- Val-Gly-Thr in the Eisenia foetida worm, Hemerythrin: His-Glu-Asp in Nereis sp).

1. Introduction

Nonburrowing earthworm annelids (*Lumbricus rubellus*, *Eisenia foetida*,) and sandworm (*Nereis* sp,) are known as feed fish; high nutritional value and protein content which triggering immune innate of fish. Non burrowing annelids mostly red, reddish in color, 10-15 cm length, life span not more than 28 month (Nagalakshmi, Prakash, & Aysha, n.d.). In nature, these earth worms are detritus feeder, decomposed organic matter in soil and fertilized the soil (Ansari & Ismail, 2012) as well as fungal repellent by contact with mucus secretion or coelomic

fluid(Plavšin, Velki, Ečimović, Vrandečić, & Ćosić, 2017). Earthworms antibacterial testing in vitro have been done by using the chloragocytes, a class of free coelomocytes existing only in annelids (Valembois, Roch, & Lassegues, 1988) dried worms (Prakash & Gunasekaran, 2011), mucus of the earthworms (Andleeb et al., 2016), coelomic fluid of the earthworm (Roch, Lassegues, & Valembois, 1991) (Nicholas & Hodgkin, 2002) or by innate immune defenses system challenged (Nicholas & Hodgkin, 2002);(Hancock & Diamond, 2000), injecting bacteria into coelomic of living earthworms (Lass, Roch, & Valembois, n.d.). Antibacterial of the earthworms included in non-ribosomal synthesized peptides in which peptides elaborated in bacteria (Hancock & Diamond, 2000).

Local wisdom of Java society has been used earthworm as typhus fever medication. Fresh worms are washed, disposed of stomach contents, chopped, mixed in cooking as foodstuff ingredient, given to the typhoid fever patient. Another way is dried earthworm, put in capsules and taken as medicine, while others use worm extract mixed with extracts of other natural ingredients into herbs for typhoid drugs. However, using ethyl acetate extract earthworm against *Salmonella thyposa*, bacteria causing typhoid fever, *Enterococcus faecalis* and *Streptococcus aureus* in vitro is little data. Salmonella spp.

Caused a wide spectrum of disease in humans and animals and effectively avoid killing by the host immune system (Gunn & Miller, 1996). *Staphylococcus aureus*, Gram positive bacteria, has ability into adapt to different environments and can be said as intrinsic virulence (Lowy, 2003), able to form biofilm in situ (Stewart & William Costerton, 2001), produces a wide variety of toxins, emetic or not emetic in primate (Argudín, Mendoza, & Rodicio, 2010). *Enterococcus faecalis* has the numbers of virulence factor (Stuart, Schwartz, Beeson, & Owatz, 2006). The aim of the research was to know the ability ethyl acetate extract of earthworms (*Lumbricus rubellus, Eisenia foetida, Nereis* sp) against *Salmonella thyposa, Sreptococcus aureus, Enterococcus faecalis* in vitro

2. Materials and Methods

Earthworms

All the worms were obtained alive. *Lumbricus rubellus* and *Eisenia foetida* were obtained by digging and hand sorting collecting of CV Alam Organik, Sukun, Malang, East Java, Indonesia. *Nereis* sp worms were obtained by digging and hand sorting on the coast of Pasir Putih, Situbondo East Java, Indonesia. Identification of the worms used special features morphology. *Eisenia foetida* had yellow and dark red rings along its body, a flat tail tip, and pink in dorsal part, white-reddish in ventral part, orange in tail and body length of about 7 cm, diameter of 3 mm. The length of *Lumbricus rubellus* earthworms were about 10 cm, reddish-skinned, slightly transparent and segmented skin in circular sections (annulus). The dorsal prostomium *Nereis* sp consists of 2 antennas, 2 septum, and 4 relatively large sized eyes. The samples of the research can be seen at Figure 1. All the worms were cleaned by washing in running water, drained, sliced, washed again with running water, drained, dried in an oven (Memmert, Germany produced 2015) at 50°C for 12 hours, blended and stored in a tightly closed container.

Earthworm's ethyl acetate extraction

The worm's powder were mixed with ethyl acetate (Sigma) (1:20, 12 hours, stirred with magnetic stirrer) and filtered. The filtrates were concentrated with a rotary evaporator (the IKA RV-10 produced IKA ASIA, Malaysia, in 2014) at a temperature of 50°C for 30 minutes, stored in a sterile vial bottle.

Protein examination

Testing of worm extract protein concentrations using Nano Drop (type ND 1000, serial number 9189, Thermo Fisher

Scientific, USA, 2006). Absorption solution of worms extracts at ƛ 280 nm for estimating protein concentration with correction absorption of ƛ 260 nm (presence of nucleic acid). 280/260 absorption ratio was a correction factor in the table. Protein content of mg / mL = Absorption at ƛ 280 x correction factor x dilution

Total Nitrogen of the worms

Examination of total nitrogen of the worms extracts based on total N of the samples, were carried out by the Kjeldhal method (Kjelmaster buchi k-375 Grobest Corporation Co. Ltd., Thailand, 2006). 0.5 gram sample was added to 25 mL H_2SO_4 and a catalyst into the Kjeldahl flask, heated to a clear greenish color.

Amino acids content of the worms

Amino acids of the worms extracts were determined using HPLC (HPLC RF 20 A type ICI with ODS column, Shimadzu, Kyoto, Japan, 2007)), 50 µL sample solution mixed with 50 µL buffer. 5µL of that mixture was taken and stored on an auto sampler vial between positions 1-44. Another line with the OPA reagent work solution was placed at position 45 on the auto sampler. 5µL sample was injected into an HPLC column. In order for the amino acid separation process works well, each column chromatography process always balance with the A buffer which programmed into the gradient file of the K-45 gradient programmer

Bacterial Testing

Salmonella thyposa, Streptococcus faecalis and *Enterococcus faecalis* were obtained from the collection of the Laboratory of Microbiology, Faculty of Medicine, Brawijaya University. Bacterial density was determined by Optical Density (Shimadsu, λ 600 nm) in TSB (OXOID) and then poured in Muller Hinton Agar (OXOID) to obtain a bacterial density of 10^6. This bacterial density was used to measure anti-bacterial activity of the worms extracts by disc method.

Anti-Bacterial Testing

Antibacterial activity of the worms extracts were examined using Minimum Inhibitory Concentration (MIC). Muller Hinton Agar (OXOID) media on petri dishes were planted with 10^6 densities these bacteria with poured method. The worm extracts were made in concentrations of 0 ppm, 10 ppm, 100 ppm, 1,000 ppm, 10,000 ppm, and 100,000 ppm with 10% DMSO as a control and 100 ppm amphyciline as a positive control. Sterile disc paper (Φ 6 mm, OXOID) is dipped in each test solution and placed on the media. Incubation was carried out for 24 hours at 37 ° C. antibacterial activity of the worms extracts were measured by measurement clear zone around the blank disk. All treatments were repeated 4 times

Minimum Bactericidal Concentration (MBC) Measurement

The test was performed by preparing six tubes, each of tube containing 10 ml of sterile TSB (OXOID) medium in which filled worms extracts 0 ppm, 12,500 ppm, 25,000 ppm, 50,000 ppm, 100,000 ppm and 100 ppm ampicillin as a positive control. 1 mL of the bacterial suspension was added in those tubes and mixed (vortex mixer IKA, Germany, 2015). Optical density of the worms were measured using visible UV spectrophotometer (Spectroquant Pharo 300, Germany, 2011) at λ 600 nm, incubated at 37 ° C. The measurement was repeated at the sixth hour. MIC value was determined by observe decreasing absorbance of worms extract concentration in the first time. 0.1 mL those MIC value were planted on 5 mL sterile TSB (OXOID), incubated in 37°C, 24 hours. The MBC value was determined by the absence of turbidity (transparent) of those broths at the lowest concentration

Bacterial morphology Observation

Form the tubes of MIC bacterial suspension was observed with a light microscope (Olympus CX22, Japan, 2014) and Scanning Electron Microscopy (TM 3000 Hitachi with Swift ED 3000 X-Ray Microanalysis, Japan, 2016). The bacteria were fixed in 2% glutaraldehyde solution for 3 hours at 4°C. The suspension then washed using phosphate buffer pH 7.4, followed by dehydration using ethanol concentration (20%, 50%, 70%, 96%, absolute) each of these for 15 minutes. Suspension smeared on the cover glass and continued smearing with gold-palladium as coating and observed into the SEM device.

3. Result

Examination the number of peptides using Nano drop and proteins content based on N total can be seen in Table 1. *Lumbricus rubellus* worms had more peptide bonds, followed by *Eisenia foetida* and *Nereis* sp. The same results were shown from the number of N Total with the Kjeldhal method. The amino acid profile of worms extract can be seen in Table 2. The total amino acid of *Lumbricus rubellus* worm was the highest compared to the worms *Eisenia foetida* and *Nereis* sp, as well as the levels of each amino acid detected. All worms were dominated by aspartate and glutamate acids and had small amounts of Methionine, Histidine, Tyrosine and Serine amino acid.

All worms also had the highest number of amino acids was hydrophobic amino acid (Ala, Iso, Leu, Met, Val), followed anionic amino acid (aspartic acid and glutamate acid), cationic amino acid (Arg, His, Lys) and hydrophilic uncharged amino acid (Gly, Ser, Threo, Tyr). Proline, Tryptophan, Asparagine, Glutamine, Cysteine were not detected due to lack of standard amino acids in the test laboratory. Non-polar amino acids of *Nereis* worm were 6.91%, *Lumbricus rubellus* 8.99% and *Eisenia foetida* 8.31%. The number of anionic amino acids for *Nereis* sp was 5.14%, *Lumbricus rubellus* 7.03% and *Eisenia foetida* 6.3%. The cationic amino acids *Nereis* sp worms are 3.53%, 4.2% in *Lumbricus rubellus* and 4.19% in *Eisenia foetida.*

Clear zones as a sign of anti-bacterial activity from all samples at concentrations of 0 ppm, 10 ppm, 100 ppm, 1,000 ppm, 10,000 ppm worms extract were not observed. The clear zone of worm extract only seen at worms extract 100 000 ppm, which clear zones small enough compared with the amphyciline 100 ppm. The clear zones of these worms extracts at 100 000 ppm can be seen in Table 3. From Table 3 can be observed that all worm extracts at 100 000 ppm had a better anti-bacterial activity to inhibit *Salmonella thyposa* and *Staphylococococcus aureus*. *Enterococcus faecalis* resisted from all worms extracts.

Minimum Bactericidal Concentration testing of worm extracts at concentration of 100 000 ppm on TSB media still showed turbidity but not for amphyciline 100 ppm as shown in Table 4. Observation using light microscopy (Figure 2, 4, 6) confirmed anti-bacterial activity of those extract worms as shown MBC. Antibacterial activity of those worms extracts in 100 000 ppm were weak antibacterial. However, from the three earthworms, *Lumbrecus rubellus* had the highest anti-bacterial activity. Observations using electron microscope (Figures 3, 5, 7) showed that the death of those bacteria were thought due to bacterial cell wall leakage.

Table 1. The worms extract protein content

The worms extract	Nanodrop (mg/mL)	N Total (%, db)	Amino acid (%)
Nereis sp.	20,98 ± 1.01	42 ± 2.47	18.37
Lumbricus rubellus	21,75 ± 1.11	50 ± 2.69	24.66
Eisenia foetida	21,32 ± 1.07	44 ± 2.36	22.78

A **B** **C**

Figure 1. A. *Nereis* sp; B. *Lumbricus rubellus* C. *Eisenia foetida*

Table 2. The worms extract amino acids profile

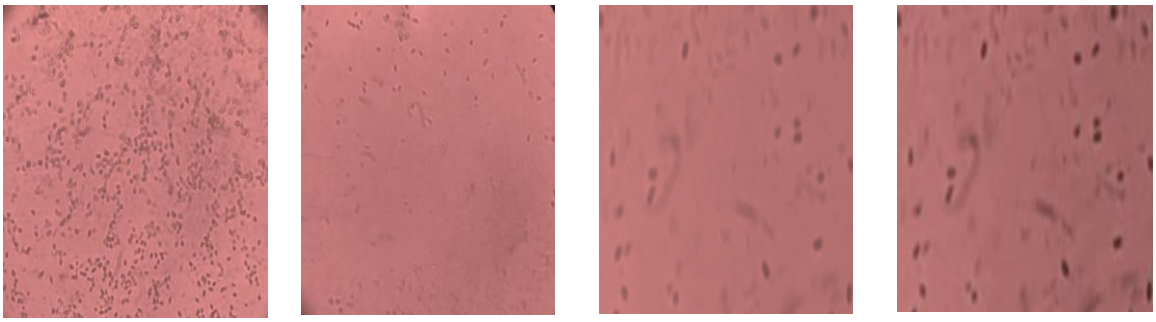
No	Amino acid	*Nereis* sp	*Lumbricus rubellus*	*Eisenia foetida*
1	Glutamate	2.95 %	4.16%	3.70%
2	Aspartic	2.19 %	2.87%	2.60%
3	Lysine	1.49%	1.65%	1.72%
4	Leucine	1.69%	2.32%	2.17%
5	Arginine	1.60%	1.85%	1.82%
6	Valine	1.12%	1.59%	1.48%
7	Alanine	1.49%	1.71%	1.51%
8	Iso-Leucine	1.30%	1.54%	1.45%
9	Phenylalanine	0.98%	1.33%	1.26%
10	Threonine	1.00%	1.34%	1.34%
11	Glycine	0.97%	1.31%	1.18%
12	Serine	0.64%	0.93%	0.80%
13	Tyrosine	0.68%	0.87%	0.77%
14	Histidine	0.44%	0.70%	0.65%
15	Methionine	0.23%	0.50%	0.44%

Table 3. Clear zone of Earthworms

Antibacterial	***Salmonella thyposa***	***Staphylococcus aureus***	***Enterococcus faecalis***
Amphyciline 100 ppm	18,39 ± 0,017 mm	18.26 ± 0.11 mm	18,644±0,051 mm
***Lumbricus rubellus* extract 100.000ppm**	3,07 ± 0,035 mm	3.16 ± 0.28mm	2,244±0,108 mm
***Eisenia foetida* extract 100.000 ppm**	2,44 ± 0,036 mm	2.51 ± 0.03 mm	1,344±0,143 mm
***Nereis* sp extract 100.000 ppm**	2,29 ± 0,065 mm	2.05 ± 0.05 mm	1,060±0,062 mm

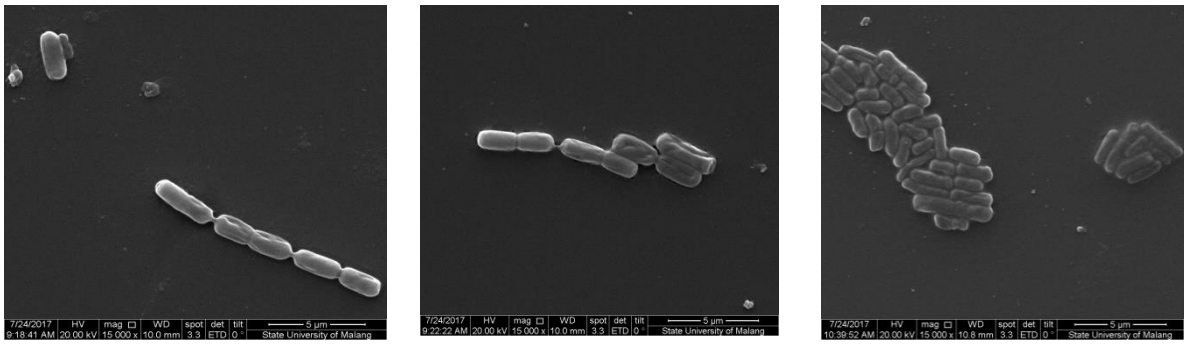
Table 4.Minimum Bactericidal Concentration of earth worms extract

Antibacterial	*Salmonella thyposa*	*Staphylococcus aureus*	*Enterococcus faecalis*
Amphyciline 100 ppm	transparent	transparent	transparent
Lumbricus rubellus extract 100.000 ppm	turbid	turbid	turbid
Eisenia foetida extract 100.000 ppm	turbid	turbid	turbid
Nereis sp extract 100.000 ppm	turbid	turbid	turbid



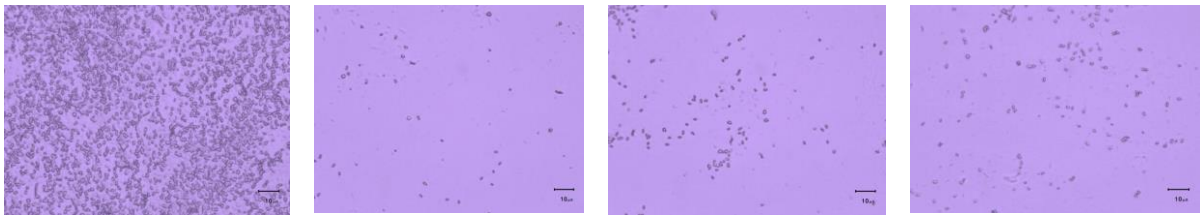
A **B** **C** **D**

Figure 2.*Salmonella tyhposa* (A) *Salmonella thyposa* in *Lumbricus rubellus* extract 100 000 ppm (B) *Salmonella thyposa* in *Eisenia foetida* extract 100 000 ppm (C) *Salmonella thyposa* in *Nereis* sp extract 100 000 ppm (D)



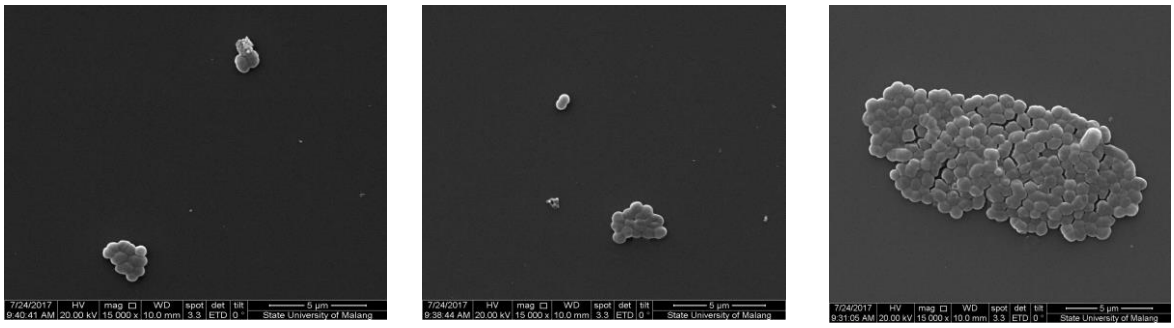
A B C

Figure 3. SEM photo *Salmonella thyposa* in *Lumbricus rubellus* extract 100 000 ppm (A) *Salmonella thyposa* in *Eisenia foetida* extract 100 000 ppm (B) *Salmonella thyposa* in *Nereis* sp extract 100 000 ppm (C)



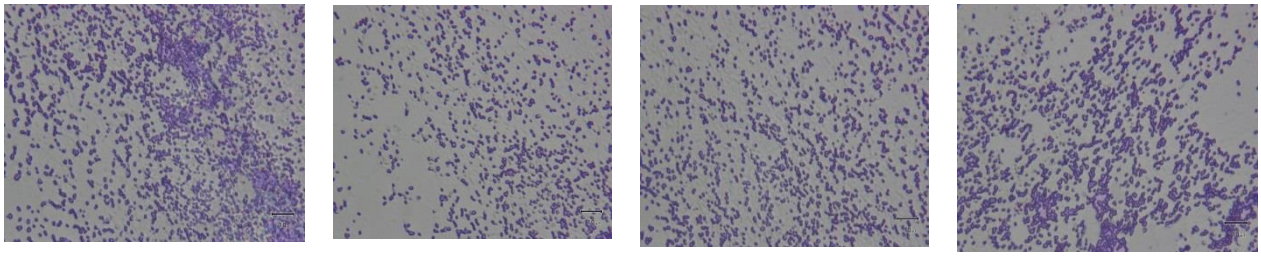
A B C D

Figure 4 *Staphylococcus aureus* (A) *Staphylococcus aureus* in *Lumbricus rubellus* extract 100 000 ppm (B) *Staphylococcus aureus* in *Eisenia foetida* extract 100 000 ppm (C) *Staphylococcus aureus* in *Nereis* sp extract 100 000 ppm (D)



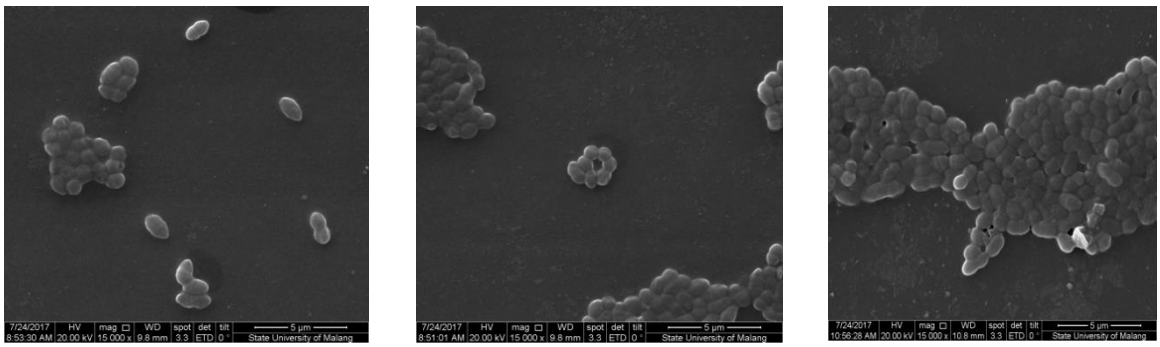
A B C

Figure 5. SEM photo *Staphylococcus aureus* in Lumbricus rubellus extract 100 000 ppm (A) *Staphylococcus aureus* in *Eisenia foetida* extract 100 000 ppm (B) *Staphylococcus aureus* in *Nereis* sp extract 100 000 ppm (C)



A **B** **C** **D**

Figure 6 .*Enterococcus faecalis* (A) *Enterococcus faecalis* in *Lumbricus rubellus* extract 100 000 ppm (B) *Enterococcus faecalis* in *Eisenia foetida* extract 100 000 ppm (C) *Enterococcus faecalis* in *Nereis* sp extract 100 000 ppm (D)



A **B** **C**

Figure 7. SEM photo *Enterococcus faecalis* in *Lumbricus rubellus* extract 100 000 ppm (A) *Enterococcus faecalis* in *Eisenia foetida* extract 100 000 ppm (B) *Enterococcus faecalis* in *Nereis* sp extract 100 000 ppm (C)

4. Discussion

Amino acid profile of the worms (Table 3) showed the biggest content was hydrophobic amino acids (6.91 % in *Nereis*, 8.99% and 8.31% in *Lumbricus rubellus* and *Eisenia foetida*). Anionic amino acid was the second amount (5.14%, 7.03%, 6.3% in *Nereris, Lumbricus rubellus* and *Eisenia foetida*), and then hydrophilic uncharged amino acid (3.29%, 4.45%, 4.09% in *Nereris, Lumbricus rubellus* and *Eisenia foetida*). The last content was cationic amino acid (3.53%, 4.2%, 4.12% in *Nereris, Lumbricus rubellus* and *Eisenia foetida*).Antibacterial peptides of these worms can be detected from amino acid residue. In this research, it did not examine so far.

Worm antibacterial was cationic peptides. Form Table 3 showed *Lumbricus rubellus* had the highest cationic amino acid. Hancock & Lehrer (Hancock & Lehrer, 1998) reviewed that characteristics of cationic peptides were excess lysine and arginine residue; Rich in proline and tryptophan; Rich in histidine residue; Amphiphatic molecules, it consist of 12-45 amino acid residue. The main site of action cationic peptide antimicrobial was in cytoplasmic membrane and carpet effect (peptide cluster at the membrane surface and cause disruption of cell permeability). In the Gram negative, the peptides initially interact with lipopolysaccharide, a highly anionic outer membrane, and glycolipid and then disrupt the membrane locally.

Researched of Yeamant & Yount (Yeaman & Yount, 2003) showed hydrophobic amino acid able to damage the permeability of bacterial cell membranes

three times stronger than hydrophilic amino acid. The ability of worms as an antimicrobial was caused by electrostatic interactions. Cationic peptides antimicrobial (Lys and Arg) bound in phosphate groups of membrane cell, caused leaking of membrane cell and then cell lysis as seen at SEM photograph in the Figure 3, 5,7 50% or more of the amino acids were hydrophobic, these will interact with bacterial membranes causing membrane damage (Hancock & Diamond, 2000) Cell death of bacteria caused by membrane pore formed by electrostatic interaction between peptide antimicrobial with phospholipid in membrane bilayer; cytoplasmic membrane dysfunction caused loss of ion and then cessation of respiration; inhibition peptidoglycan and macromolecules synthesis as well as inhibition of intracellular function (Hancock & Lehrer, 1998). Research of (Gunn & Miller, 1996) showed lipopolysaccharide of lipid A in membrane bilayer of *Salmonellas thyposa* bind with cationic antimicrobial peptides causing cell death. Bacterial cell membrane damage that causes cell death also observed by Carson (Carson, Mee, & Riley, 2002) in which tea tree oil rich in terpenoid caused *Staphylococcus aureus* membrane damage and followed by cell lysis. The same phenomenon was observed by Jung (Jung et al., 2008). The cell death of Staphylococcus *aureus* begin with membrane cell damaged due to membrane interaction with silver ion.

Nereis muscles contain abundant quantities of soluble, sarcoplasmic, high affinity Ca^{2+} binding proteins (SCBPs). SCBP is a single polypeptide chain of 174 amino acids, including single residues of glutamine and histidine, 2 tyrosine, and 3 tryptophan (Collins, Coxti, & TheibertS, n.d.). Another researcher showed Nereis contains peptide antimicrobial bromo tryptophane, in which specific for estuarine organism.(Tasiemski et al., 2007). (*Lumbricus rubellus* had Lumbricin1 antimicrobial, a proline-rich antimicrobial peptide (Cho, Park, Yoon, & Kim, n.d.);(Wang, Wang, Zhang, Qu, & Yang, n.d.). Fetidin, antimicrobial peptide composed of amino acid Gly-Thr-Lys-Thr-Leu-Ala-Ser-His-Ser-Iso obtained from ceolomic *Eisenia foetida* extraction (Lasseues, Milochau, Doignon, Pasquier, & Valembois, 1997).

Gram-positive cells, exposure to antimicrobial peptides caused water and ion flow increasingly, especially ion K^+ and osmotic disturbed (Yeaman & Yount, 2003) However, it did not apply *in Enterococcus faecalis* as seen in Figure 6 and 7. *Enterococcus faecalis* was a ubiquitous micro-organisms, grows normally in GI human and animals, high heat tolerance and had ability to growth in adverse environment (Giraffa, 2002). It had a proton pump that can maintain pH homeostasis; disrupted growth only in pH up to 11.5 (Stuart et al., 2006) .*Enterococcus faecalis* resistant to vancomycin (MICs, 32 to 64 ug/ml) but not to teicoplanin (MIC, .0.5 ug/ml) (Sahm et al., 1989). Ethyl acetate these earthworms extract were crude extracts, the main component was cationic peptide in the form unpurified so it not showed effective antibacterial against *Enterococcus faecalis*.

A little number of clear zones (Table 3) and a high number of MIC value (Table 4) indicated that all of worms ethyl acetate extract were not effective antibacterial against *Salmonella thyposa*, *Staphylococcus aureus, and Enterococcus faecalis* in vitro. The research with the same result was showed by The research of Andleeb (Andleeb et al., 2016) showed that antimicrobial of mucus extract of the worm was lower than the mucus. The same result had been shown by the search of (Plavšin et al., 2017) Coelomic extract of *Eisenia foetida* did not showed antibacterial effect against some of fungal, but it showed different result in using coelomic fluid. (Lasseues et al., 1997)and (Milochau, Lassègues, & Valembois, 1997) showed the antimicrobial of worms in ceolomic fluid. Coelomycites in coelomic fluid was the natural responses of bacterial

challenge (Procházková et al., 2011). In this research used dried of worms, so coelomic fluid was little enough. Liu *et al* (Liu, Ju, & Xia, 2014) showed induced bacterial can trigger peptide antibacterial in the tissue of earthworms, but it only produced by earthworms adult (Cho et al., n.d.).

5. Conclusion

Ethyl acetate worms extract of *Lumbricus rubellus, Eisenia foetida, Nereis* sp were antibacterial ineffective against *Salmonella thyposa, Sterptococcus aureus, Enterococcus faecalis* in vitro. From the worms, ethyl acetate extract of *Lumbricus rubellus* was the best antibacterial. *Enterococcus faecalis* was the resistant microorganism of the worm antimicrobial ethyl acetate extract.

References

Andleeb, S., Ejaz, M., Awan, U. A., Ali, S., Kiyani, A., Shafique, I., & Zafar, A. (2016). In vitro screening of mucus and solvent extracts of Eisenia foetida against human bacterial and fungal pathogens. *Pak. J. Pharm. Sci.*, 10.

Ansari, A. A., & Ismail, S. A. (2012). *Role of Earthworms in Vermitechnology*. *8*, 13.

Argudín, M. Á., Mendoza, M. C., & Rodicio, M. R. (2010). Food Poisoning and Staphylococcus aureus Enterotoxins. *Toxins*, *2*(7), 1751–1773. https://doi.org/10.3390/toxins2071751

Carson, C. F., Mee, B. J., & Riley, T. V. (2002). Mechanism of Action of Melaleuca alternifolia (Tea Tree) Oil on Staphylococcus aureus Determined by Time-Kill, Lysis, Leakage, and Salt Tolerance Assays and Electron Microscopy. *Antimicrobial Agents and Chemotherapy*, *46*(6), 1914–1920. https://doi.org/10.1128/AAC.46.6.1914-1920.2002

Cho, J. H., Park, C. B., Yoon, Y. G., & Kim, S. C. (n.d.). Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: Puri¢cation, cDNA cloning and molecular characterization. *Biochimica et Biophysica Acta*, 10.

Collins, J. H., Coxti, J. A., & TheibertS, J. L. (n.d.). *Amino Acid Sequence of a Sarcoplasmic Calcium-binding Protein from the Sandworm Nereis diversicolor*. 8.

Giraffa, G. (2002). Enterococci from foods. *FEMS Microbiology Reviews*, *26*(2), 163–171. https://doi.org/10.1111/j.1574-6976.2002.tb00608.x

Gunn, J. S., & Miller, S. I. (1996). PhoP-PhoQ activates transcription of pmrAB, encoding a two-component regulatory system involved in Salmonella typhimurium antimicrobial peptide resistance. *Journal of Bacteriology*, *178*(23), 6857–6864. https://doi.org/10.1128/jb.178.23.6857-6864.1996

Hancock, R. E. W., & Diamond, G. (2000). The role of cationic antimicrobial peptides in innate host defences. *Trends in Microbiology*, *8*(9), 402–410. https://doi.org/10.1016/S0966-842X(00)01823-0

Hancock, R. E. W., & Lehrer, R. (1998). Cationic peptides: A new source of antibiotics. *Trends in Biotechnology*, *16*(2), 82–88. https://doi.org/10.1016/S0167-7799(97)01156-6

Jung, W. K., Koo, H. C., Kim, K. W., Shin, S., Kim, S. H., & Park, Y. H. (2008). Antibacterial Activity and Mechanism of Action of the Silver Ion in Staphylococcus aureus and Escherichia coli. *Applied and Environmental Microbiology*, *74*(7),

2171–2178. https://doi.org/10.1128/AEM.02001-07

Lass, M., Roch, P., & Valembois, P. (n.d.). *Antibacterial Activity of Eisenia fetida andrei Coetomic Fluid: Evidence, Induction, and Animal Protection*. 6.

Lasseues, M., Milochau, A., Doignon, F., Pasquier, L., & Valembois, P. (1997). Sequence and Expression of an Eisenia-Fetida-Derived cDNA Clone That Encodes the 40-kDa Fetidin Antibacterial Protein. *European Journal of Biochemistry*, *246*(3), 756–762. https://doi.org/10.1111/j.1432-1033.1997.00756.x

Liu, S., Ju, J., & Xia, G. (2014). Identification of the flavonoid 3′-hydroxylase and flavonoid 3′,5′-hydroxylase genes from Antarctic moss and their regulation during abiotic stress. *Gene*, *543*(1), 145–152. https://doi.org/10.1016/j.gene.2014.03.026

Lowy, F. D. (2003). Antimicrobial resistance: The example of Staphylococcus aureus. *Journal of Clinical Investigation*, *111*(9), 1265–1273. https://doi.org/10.1172/JCI18535

Milochau, A., Lassègues, M., & Valembois, P. (1997). Purification, characterization and activities of two hemolytic and antibacterial proteins from coelomic fluid of the annelid Eisenia fetida andrei. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, *1337*(1), 123–132. https://doi.org/10.1016/S0167-4838(96)00160-4

Nagalakshmi, P. K., Prakash, M., & Aysha, O. S. (n.d.). WORMICOMPOSTING, GOLD FROM GARBAGE - A REVIEW. *World Journal of Pharmacy and Pharmaceutical Sciences*, *5*(12), 21. https://doi.org/10.20959/wjpps201612-8210

Nicholas, H. R., & Hodgkin, J. (2002). Innate Immunity: The Worm Fights Back. *Current Biology*, *12*(21), R731–R732. https://doi.org/10.1016/S0960-9822(02)01249-6

Plavšin, I., Velki, M., Ečimović, S., Vrandečić, K., & Ćosić, J. (2017). Inhibitory effect of earthworm coelomic fluid on growth of the plant parasitic fungus Fusarium oxysporum. *European Journal of Soil Biology*, *78*, 1–6. https://doi.org/10.1016/j.ejsobi.2016.11.004

Prakash, M., & Gunasekaran, G. (2011). Antibacterial Activity of the Indigenous Earthworms *Lampito mauritii* (Kinberg) and *Perionyx excavatus* (Perrier). *The Journal of Alternative and Complementary Medicine*, *17*(2), 167–170. https://doi.org/10.1089/acm.2009.0720

Procházková, P., Dvořák, J., Šilerová, M., Roubalová, R., Škanta, F., Halada, P., & Bilej, M. (2011). Molecular characterization of the iron binding protein ferritin in Eisenia andrei earthworms. *Gene*, *485*(2), 73–80. https://doi.org/10.1016/j.gene.2011.06.010

Roch, P., Lassegues, M., & Valembois, P. (1991). Antibacterial activity of Eisenia fetida andrei coelomic fluid: III—Relationship within the polymorphic hemolysins. *Developmental & Comparative Immunology*, *15*(1–2), 27–32. https://doi.org/10.1016/0145-305X(91)90044-Y

Sahm, D. F., Kissinger, J., Gilmore, M. S., Murray, P. R., Mulder, R., Solliday, J., & Clarke, B. (1989). In vitro susceptibility studies of vancomycin-resistant Enterococcus faecalis. *Antimicrobial Agents and Chemotherapy*, *33*(9), 1588–1591. https://doi.org/10.1128/AAC.33.9.1588

Stuart, C., Schwartz, S., Beeson, T., & Owatz, C. (2006). Enterococcus faecalis: Its Role in Root Canal Treatment Failure

and Current Concepts in Retreatment. *Journal of Endodontics*, *32*(2), 93–98. https://doi.org/10.1016/j.joen.2005.10.049

Tasiemski, A., Schikorski, D., Le Marrec-Croq, F., Pontoire-Van Camp, C., Boidin-Wichlacz, C., & Sautière, P.-E. (2007). Hedistin: A novel antimicrobial peptide containing bromotryptophan constitutively expressed in the NK cells-like of the marine annelid, Nereis diversicolor. *Developmental & Comparative Immunology*, *31*(8), 749–762. https://doi.org/10.1016/j.dci.2006.11.003

Wang, X., Wang, X., Zhang, Y., Qu, X., & Yang, S. (n.d.). *An antimicrobial peptide of the earthworm Pheretima tschiliensis: CDNA cloning, expression and immunolocalization*. *7*.

Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of Antimicrobial Peptide Action and Resistance. *Pharmacological Reviews*, *55*(1), 27–55. https://doi.org/10.1124/pr.55.1.2