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Analyze Effectiveness Extract of Worm Lumbricus Rubellus and Pheretima Based on Bacteria Salmonella Typhi and Staphylococcus Aureus

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Abstract— *Infections disease is a main cause of high morbidity and mortality in Indonesia that usually caused by Salmonella typhi and Staphylococcus aureus bacteria. Infectious disease treatment with uncontrolled antibiotic can cause resistance. Therefore, one of traditional medicines that can be utilized by people are Lumbricus rubellus and Pheretima sp earthworm. This Paper described effective in inhibiting Salmonella typhi and Staphylococcus aureus with a strong inhibitory response. The result were analyzed by using One Way ANOVA test, and were obtained the value of p= 0,000<0,05. So that it showed the influence of the Lumbricus rubellus and Pheretima sp extract on Salmonella typhi and Staphylococcus aureus.*

Keywords— *Lumbricus rubellus, Pheretima sp, Salmonella typhi, Staphylococcus aureus.*

I. Introduction

Infection is a disease caused by pathogenic microbes that cause increased morbidity and mortality in developing countries, especially Indonesia. Infectious diseases are closely related to environmental and personal health as well as inadequate public awareness for healthy living (Darmadi, 2008).

Infectious diseases that are often found are caused by the bacteria *Salmonella typhi (st)* and *Staphylococcus aureus (sa)*. *St bacteria* is a pathogenic bacterium that causes typhoid fever which causes acute infections of the digestive tract. Typhoid fever can be transmitted through 5F, namely (food, feces, fomitus, finger, fly) caused by the entry of *Salmonella typhi* bacteria from contaminated food. Bacteria that travel to the stomach are destroyed and some that enter the intestine will multiply. Clinical manifestations that appear in the first week are fever, gastrointestinal disorders, headaches, muscle aches, anorexia, coughing and epistaxis (Wibisono E, 2014).

Based on data from the World Health Organization (WHO) in 2009, 17 million occurrences of typhoid fever per year throughout the world with a mortality of around 600,000 and 70% from Asia. In Indonesia, typhoid fever sufferers are around 81% per 100,000. (Sudoyo, 2014)

In 2010 around 41,081 cases of typhoid fever that occurred in hospitals and was ranked 3rd of the 10 most inpatient cases, with mortality reaching 274 people based on Indonesia's Health Profile in 2011.

Sa bacteria is also the most common cause of infection. *Sa bacteria* is a typical Gram-positive facultative anaerobic bacterium, in the form of cocci and a major pathogen in humans. *Sa bacteria* has coagulase-positive properties and its colonies are gray or dark brownish yellow. In humans 20-50% of *Sa bacteria* is found in the nose. Some people have experienced *Sa bacteria* infection, but the severity varies, from food poisoning, mild or severe skin infections, pneumonia, arthritis, meningitis, endocarditis and sepsis with suppuration in various organs. (RI, 2011)

A child's history of immunity and age have a strong relationship with the microbes that cause sepsis. Children whose immunity is impaired will suffer from sepsis caused by a variety of bacteria. The most common trigger for neonates is the bacterium *Sa bacteria* (Soedarmo SSP, 2010)

Based on data from WHO, neonatal mortality is around 5 million with a mortality rate of 34 per 1000 live births, from 98% of deaths found in developing countries. In developed countries, the number of sepsis cases in neonates reaches 1-4 per 1000 live births, and the mortality rate is 10.3% less than in developing countries, which is 10-50 per 1000 live births and the mortality rate is 12-68% .

One of the traditional medicines that can be utilized by the community is earthworms *Lumbricus rubellus (Lr)* and *Pheretima sp*. The *Lr* worm contains 64-76% protein, 7-10% fat, 1% phosphorus, and 0.55% calcium. In earthworm extracts found antipurin, antidotes, vitamins, arachidonic acid and antipyretics containing ascorbic acid to reduce the rise in body temperature due to infection. There are also enzymes namely lumbrokinase, catalase, cellulose, phosphatase, peroxidase, glucoronidase, and lysozim which act as antimicrobials by damaging bacterial cell walls, especially gram-positive. Hyalin compounds, granular amoebocytes, and chloragocytes contained in *Lr Worms* are useful as a mechanism of immunity in inhibiting pathogenic bacteria by producing cytotoxic and antibacterial substances from extracellular products.

Pheretima sp earthworms contain 76% protein, glutamic acid 3.9%, tyrosine 3.73%, lysine 1.13%, hydroxyproline 19.04%, aspartic acid 4.15%, 3% fat and 75-100% water. In earthworm extracts also found enzymes, namely lumbrokinase, cellulose, peroxidase and arachidonic acid compounds, antipoisons, antipurines, and vitamin K which act as antibacterial and antipyretic (EK, 2013).

In *Lr worm* and *Pheretima sp* there is a bioactive compound Lumbricin 1 which is a broad spectrum antimicrobial peptide group that can inhibit gram-negative and gram-positive bacteria. Earthworms change the permeability mechanism of the membrane by making pores in the bacterial cell wall, so that the activity in the bacterial cell is disrupted due to loss of cell metabolites and cytoplasm exposed to the outside environment that causes cell lysis.

II. Literature Review

2.1. Classification and Identification of Earthworms

2.1.1. Lumbricus rubellus

Lumbricus rubellus worm belong to a group of invertebrate animals that are not vertebrate and its body is composed of ring-shaped segments (annulus) so that it belongs to the phylum Annelida. Lumbricus rubellus belongs to the Oligochaeta class because it has very few setae in the segment marked by the presence of short hard hairs. This segmentation occurs outside and inside, including muscles, nerves, circulation, excretion and reproduction. The classification of *Lr Worms* is as follows:

Kingdom : Animalia
Phylum : Annelida
Class : Clitellata
Sub Class : Oligochaeta
Order : Haplotaxida
Family : Lumbricidae
Genus : Lumbricus
Species : Lumbricus rubellus

Figures 1. *Lumbricus Rubellus.*

Earthworms *Lr* grow in the soil in the tropics and have bilateral symmetrical body shapes covered with thin cuticles. Earthworm's body at the top (dorsal) is pink to dark red and round in shape while at the bottom (ventral) is younger in color and flat. There is mucus in the body that is the fluid produced by the epidermis gland to lubricate the body, facilitate movement in the soil, and help breathing. The size of the earthworm's body is relatively small with a length of 4-7cm and has 100 to 180 segments, and each segment has several setae that function as a grip on where the earthworm is located. This worm has no eyes, with the prostomium as a sensory nerve organ, will make it more sensitive to the material around it. In tanag worms the digestive part is in the form of a prostomium that is shaped like the lips, pharynx, esophagus, cache, stomach muscle (empela), intestine and anus. The earthworm's respiration system is supported by the skin as a tool for the exchange of oxygen and carbon dioxide and passes through capillary vessels throughout the cuticle tissue found in the upper layers of the skin.

The cuticle is responsible for maintaining the moisture with mucus produced by the epidermis. Oxygen in the blood vessels is then distributed to all parts of the body through the blood circulation. The earthworm's reproductive system is hemaprodit, that is, it has female and male reproductive organs in one body, but these animals are unable to reproduce on their own. Female reproductive organs include a pair of ovaries that are in the 13th segment in the front and a pair of infundibulums, each of which empties into an egg bag in the 14th segment. Male reproductive organs have two pairs of testes (each pair of testes is located in segment 10 and segment 11), and two testes.

Earthworms (Lumbricus rubellus) contain high protein that is 64-76%, 7-10% fat, 1% phosphorus, 1.08% crude fiber and 0.55% calcium. The protein contained consists of nine types of amino acids and four types of non-essential amino acids. The nine types of essential amino acids include isoleucine, methionine, arginine, histidine, leucine, lysine, phenylalanine, valine, and threonine. Whereas the four types of non-essential amino acids are cysteine, glycine, serine, and tyrosine. In earthworm extracts found antipurin, antidotes, vitamins, arachidonic acid and antipyretics containing ascorbic acid to reduce the increase in body temperature due to infection. There are also enzymes such as peroxidase, catalase, lumbrokinase, cellulose, phosphatase, glucoronidase, and lysozim which act as antimicrobials that can damage gram-positive cell walls and protect against pathogenic microbial habitats. In earthworms there is a bioactive compound Lumbricin 1 which is a broad spectrum antimicrobial peptide group so that it can inhibit gram negative and positive bacteria. Earthworms change the membrane permeability mechanism by building burrows in bacterial cell walls, so that bacterial cell activity is disrupted because the cytoplasm is exposed to the outside environment and results in cells becoming lysis.

2.1.2 Pheretima Sp

Earthworms (Pheretima sp) are most commonly found in Indonesia. These earthworms belong to the group of invertebrate animals, which are not vertebrate and soft body. The whole body is composed of short segments (seta), ring-shaped and the segments have hard hair so that it belongs to the phylum Annelida. The classification of Pheretima sp earthworms is as follows:

Kingdom : Animalia
Phylum : Annelida
Class : Chaetopoda
Order : Oligochaeta
Family : Megascolecoidae
Genus : Pheretima
Species : Pheretima sp

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Figures 2. *Pheretima sp* worm

2.2. Chemical Content

Pheretima sp earthworms contain 76% protein, glutamic acid 3.9%, tyrosine 3.73%, lysine 1.13%, hydroxyproline 19.04%, aspartic acid 4.15%, 3% fat and 75-100% water. The contents contained in the earthworm extract are enzymes in the form of peroxidase, lumbrokinase and cellulose, and there are antipyretic substances namely antipurin, arachidonic acid, antiroxides and vitamin K which function to reduce body temperature and inhibit bacterial growth. In earthworms contained nine essential amino acids namely isoleucine, methionine, arginine, histidine, leucine, lysine, phenylalanine, valine, and threonine as well as four types of non-essential amino acids namely cysteine, glycine, serine, and tyrosine. When the process of purifying the protein of earthworm extract was found 7 protein bands, in the 6th protein band there was a zone of inhibition that was almost as large as the chloramphenicol antibiotics.

In earthworms there is a bioactive compound Lumbricin 1 which is a broad spectrum antimicrobial peptide group that is a form of natural defense against pathogenic microbes that will inhibit gram-negative and gram-positive microbes. Earthworms change the permeability mechanism of membranes by building pores in bacterial cell walls, so that bacterial cell activity is disrupted due to loss of cell metabolites and cytoplasm exposed to the outside environment which causes lysis of cells. In the body of earthworms there are microbes that can produce antibacterial namely Streptomyces. Streptomyces genus belongs to the actinomycetes group which is closely related to bacteria so that it prevents the growth of gram-negative bacteria such as Salmonella typhi. Streptomyces can produce antimicrobial compounds which are often used in the fields of medicine such as streptomycin, aureomycin, chloramisetin, teramisin, erythromycin, and magnamycin.

III. Method of Research

Data from the results of the study were analyzed using the SPSS program with the One way ANOVA test with the Post Hoc Test Tukey. Among them the work methods carried out

1. Sterilizer Tool

All tools are sterilized before use. The glassware is sterilized in the oven for 1-2 hours at 170 ° C. The media was sterilized in an autoclave for 15 minutes at 121 ° C.

2. Making Nutrient Agar and Nutrient Broth Media

A total of 28 g of agar nutrient and 13 g of nutrient broth were weighed and put into an erlenmeyer, then suspended in 1000 ml distilled water and heated until dissolved, then sterilized in an autoclave for 15 minutes at 121 ° C.

3. Making Tilting

A total of 3 ml of diluted nutrient media was put into a test tube, then sterilized using an autoclave at 121°C. Placed at an angle of 30°C and allowed to solidify.

4. Bacteria Breeding

a) Making bacterial culture stock and bacterial rejuvenation

Performed to multiply the bacteria by inoculating 1 ose of pure bacterial culture by etching technique into the nutrient to tilt, then incubated for 18-24 hours at 37 ° C in the incubator.

b) Inoculum preparation

Using a sterile loop, take each bacterial colony from the culture stock, then suspend it to a test tube containing 10 ml of sterile nutrient broth, and incubate at 33-37 ° C for 18-24 hours.

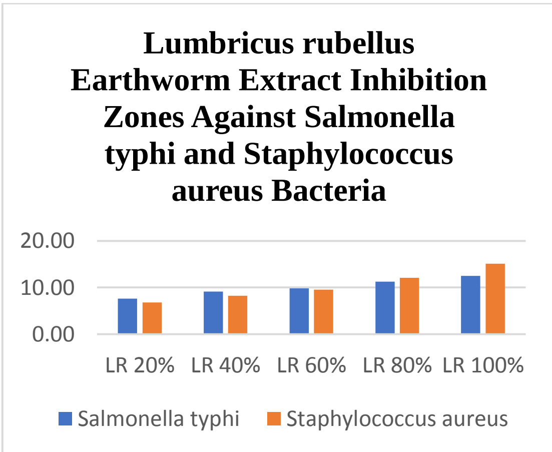
5. Antibacterial Activity Test

On sterile petri dishes dripped 0.1 ml inoculum (10^6 CFU / ml), then nutrient media to be poured as much as 15 ml at 40 ° C. In order to keep the bacterial and media suspension evenly mixed, homogeneate the petri dish on the table surface and leave it until the media solidifies.

Antibacterial activity was tested using the agar diffusion method. Place the paper disk soaked at each concentration and let it sit for 15 minutes, then incubate in an incubator with a temperature of 35-39°C, then the inhibition zone is measured with the calipers.

IV. Analyze And Result

Based on data collected and analyzed, it was found that the antibacterial power obtained from earthworm extracts of Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus bacteria were tested using the Kirby-Bauer disk diffusion method. By using a sensitivity test obtained inhibitory zones around paper discs that have dropped several concentrations. The Inhibition Zone is measured using a calipers and the inhibition zone is obtained as shown in the chart below.



Figures 3. Average of Lumbricus Earthworm Extract Inhibition Zones Rubellus Against Salmonella typhi and Bacteria Staphylococcus aureus

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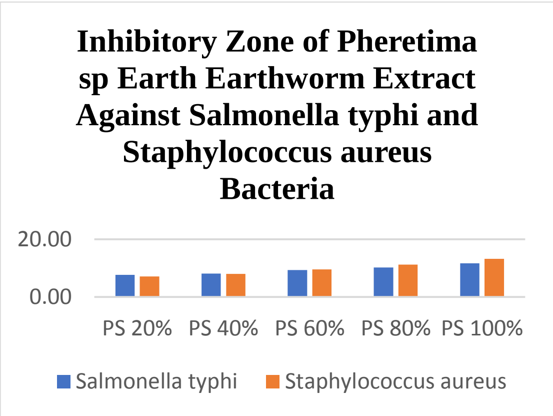
In the test of Salmonella typhi bacteria, the average inhibitory zone in a sequence that is at a concentration of 20% is 7.60 mm, a concentration of 40% is 9.17 mm, a concentration of 60% is 9.83 mm, a concentration of 80% is 11.23 mm , and the concentration of 100% is 12.50 mm. The diameter of the lowest inhibition zone is at a concentration of 20% and the highest diameter of the inhibitory zone is at a concentration of 100%. In the test of the Staphylococcus aureus bacteria, the average inhibitory zone in a sequence that is at a concentration of 20% is 6.80 mm, a concentration of 40% is 8.27 mm, a concentration of 60% is 9.53 mm, a concentration of 80% is 12.10 mm , and at a concentration of 100% is 15.10 mm. The diameter of the lowest inhibition zone is at a concentration of 20% and the highest diameter of the inhibitory zone is at a concentration of 100%.



Figures 4. Average inhibition zone of Pheretima sp earth extracts against Salmonella typhi and Staphylococcus aureus bacteria

In the test of Salmonella typhi bacteria, an average inhibitory zone in succession is at a concentration of 20% is 7.70 mm, a concentration of 40% is 8.17 mm, a concentration of 60% is 9.33 mm, a concentration of 80% is 10.23 mm , and the concentration of 100% is 11.70 mm. The diameter of the lowest inhibition zone is at a concentration of 20% and the highest diameter of the inhibitory zone is at a concentration of 100%. In the test of Staphylococcus aureus bacteria, the average inhibitory zone in a sequence that is at a concentration of 20% is 7.07 mm, a concentration of 40% is 8.00 mm, a concentration of 60% is 9.60 mm, a concentration of 80% is 11.20 mm , and the concentration of 100% is 13.20 mm. The diameter of the lowest inhibition zone is at a concentration of 20% and the highest diameter of the inhibitory zone is at a concentration of 100%.

TABLE 1. Classification of Inhibitory Zones according to Davis and Stout

Diameter (mm) Criteria for inhibition zones	**Diameter (mm) Criteria for inhibition zones**
> 20 Very strong	> 20 Very strong
10-19 Strong	10-19 Strong
5-9 Medium	5-9 Medium
<5 Weak	<5 Weak

Average Inhibition Zone Diameter and Inhibition Criteria Lumbricus rubellus Pheretima sp

Salmonella typhi Staphylococcus aureus Salmonella typhi Staphylococcus aureus
20% 7.60 = Medium 6.80 = Medium 7.70 = Medium 7.07 = Medium
40% 9.17 = Medium 8.27 = Medium 8.17 = Medium 8.00 = Medium
60% 9.83 = Medium 9.53 = Medium 9.33 = Medium 9.60 = Medium
80% 11.23 = Strong 12.10 = Strong 10.23 = Strong 11.20 = Strong
100% 12.50 = Strong 15.10 = Strong 11.70 = Strong 13.20 = Strong

TABLE 2. Measurement of Inhibitory Zones of Lumbricus rubellus and Pheretima sp. Earthworm Extract According to Davis and Stout

Concentration	**Average Inhibition Zone Diameter and Inhibition Criteria**			
	Lumbricus rubellus		*Pheretima sp*	
	Salmonella typhi	*Staphylococcus aureus*	*Salmonella typhi*	*Staphylococcus aureus*
20%	7,60 = Medium	6,80 = Medium	7,70 = Medium	7,07 = Medium
40%	9,17 = Medium	8,27 = Medium	8,17 = Medium	8,00 = Medium
60%	9,83 = Medium	9,53 = Medium	9,33 = Medium	9,60 = Medium
80%	11,23 = Strong	12,10 = Hard	10,23 = Hard	11,20 = Hard
100%	12,50 = Strong	15,10 = Hard	11,70 = Hard	13,20 = Hard

This study is in line with research conducted by Fitria (2017) using Lumbricus rubellus earthworm extracts with concentrations of 10%, 25%, 50%, 75% and 100%. Where earthworm extract Lumbricus rubellus began to inhibit the growth of bacteria Salmonella typhi and Staphylococcus aureus at concentrations of 50% - 100%. In the research of Lilis (2010) stated that earthworm extract Lumbricus rubellus has a bactericidal effect on Staphylococcus aureus. In the Zohra study using Pheretima sp chloroform extract against Staphylococcus aureus bacteria using agar diffusion method. In the MHA media (Muller Hinton Agar) the inhibition zone was obtained at 14.3 mm and in the phytochemical test Pheretima sp contained alkaloids.

V. Conclusion

Earthworm extracts of Lumbricus rubellus and Pheretima sp had effective inhibitory properties against the bacteria Salmonella typhi and Staphylococcus aureus. In the inhibition classification, earthworm of Lumbricus rubellus and Pheretima sp extracts began to inhibit a growth of Salmonella typhi and Staphylococcus aureus bacteria at a concentration of 20% with a moderate inhibitory response. Based on inhibition zone classification, earthworm extracts of Lumbricus rubellus and Pheretima sp were most effective in inhibiting the growth of Salmonella typhi and Staphylococcus aureus bacteria at 100% concentrations with strong inhibitory responses. This shows that the higher the concentration of the extract, the more antibacterial activity is characterized by an increase in the inhibitory zone because of the greater content of antibacterial compounds contained in the extract. The reason for the

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formation of inhibitory zones is because of the earthworms Lumbricus rubellus and Pheretima sp have Lumbricin 1 bioactive compounds which are antimicrobials of the broad-spectrum peptide group so that they can inhibit gram-positive and gram-negative bacteria. Earthworms change the permeability mechanism of the membrane by making pores in the bacterial cell wall, so that the activity in the bacterial cell is disrupted due to loss of cell metabolites and cytoplasm exposed to the external environment that causes cell lysis

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