TEST OF INHIBITORY EFFECTIVENESS OF EARTHWORM EXTRACTS LUMBRICUS RUBELLUS AND PHERETIMA SP AGAINST SALMONELLA TYPHI AND STAPHYLOCOCCUS AUREUS BACTERIA

Sri wahyuni nasution¹, Ali Napiah Nasution², Sri Lestari Ramadhani Nasution³
Faculty of Medicine Universitas Prima Indonesia, Medan Indonesia
Email: sriwahyuni_nst88@yahoo.com

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 utililized by people are Lumbricus rubellus and Pheretima sp earth worm. This research was conducted with laboratory experimental method to determine the effectiveness test of Lumbricus rubellus and Pheretima sp with a concentration of 20%, 40%, 60%, 80% and 100% to inhibit Salmonella typhi and Staphylococcus aureus and tested by using Kirby-bauer disc diffusion method. The result show that extracts of Lumbricus rubellus and Pheretima sp earth worm begin to inhibit Salmonella typhi and Staphylococcus aureus at a concentration of 20% with medium inhibitory criteria. Where as at the concentration of 100% extracts of Lumbricus rubellus and Pheretima sp earth worm were most 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 its how ed the influence of the Lumbricus rubellus and Pheretima sp extracton Salmonella typhi and Staphylococcus aureus.

Keywords: Lumbricus rubellus, Pheretima sp, Salmonella typhi, Staphylococcus aureus

1. INTRODUCTION

Infection is a disease caused by pathogenic microbes which is the cause of increasing morbidity and mortality in developing countries, especially Indonesia. Infectious diseases are closely related to environmental and individual health as well as inadequate public awareness for healthy living [1-2]. Infectious diseases that are often found are caused by the bacteria Salmonella typhi and Staphylococcus aureus. Salmonella typhi is a pathogenic bacterium that causes typhoid fever which causes an acute infection in 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 enter the stomach are destroyed and some that enter the intestine will multiply. Clinical manifestations that appear in the first week are fever, gastrointestinal disturbances, headache, muscle aches, anorexia, cough and epistaxis [3-4].

Based on data from the World Health Organization (WHO) in 2009, there were 17 million cases of typhoid fever per year worldwide with a mortality rate of around 600,000 and 70% from Asia. In Indonesia, the number of patients with typhoid fever is around 81% per 100,000 [5]. In 2010 around 41,081 cases of typhoid fever occurred in hospitals and ranked 3rd out of the 10 most hospitalized diseases, with a mortality rate of 274 people and a case fatality rate of 0.67% based on the 2011 Indonesian Health Profile [6].

The highest incidence of typhoid fever was found at the age of 5-14 years (1.9%), the age of 1-4 years (1.6%), the age of 15-24 years (1.5%) and the age of <1 year (0.8%). In North Sumatra, typhoid fever was detected with a percentage of 0.9% and 0.2~0.3% spread across all districts or cities. The highest percentage of typhoid fever was found in South Nias Regency at 3.3% [7].

Staphylococcus aureus is also the most common cause of infection. Staphylococcus aureus is a typical gram-positive facultative anaerobic bacterium, cocci-shaped and the main pathogen in humans. Staphylococcus aureus
is coagulase-positive and its colonies are gray to dark yellow-brown. In humans, 20-50% of Staphylococcus aureus is found in the nose. Some people have experienced Staphylococcus aureus infections, but the severity varies, from food poisoning, mild or severe skin infections, pneumonia, arthritis, meningitis, endocarditis and sepsis with suppurative in various organs [8].

The history of immunity and the child's age have a strong relationship with the microbes that cause sepsis. Children whose immunity is compromised will suffer from sepsis caused by a variety of bacteria. The most common trigger in neonates is Staphylococcus aureus bacteria [9].

Based on data from the World Health Organization (WHO), there are around 5 million neonatal deaths with the death rate reaching 34 per 1000 live births, of which 98% of deaths are found in developing countries. In developed countries, cases of sepsis in neonates reach 1-4 per 1000 live births, and the mortality rate is 10.3% lower than in developing countries, which is 10-50 per 1000 live births and the mortality rate is 12-68%[ 10].

Treatment of infectious diseases can be done medically through antibiotic or traditional therapy. However, uncontrolled use of antibiotics can lead to resistance to the antibiotics given. With the emergence of this problem, efforts are needed to develop natural traditional medicines so that they are considered safer and have better tolerance than antibiotic therapy [11].

One of the traditional medicines that can be used by the community is the type of earthworms Lumbricus rubellus and Pheretima sp. Lumbricus rubellus worm contains 64-76% protein, 7-10% fat, 1% phosphorus, and 0.55% calcium. Earthworm extract contains antipurine, antidote, vitamins, arachidonic acid and antipyrastic substances containing ascorbic acid to reduce the increase in body temperature due to infection. There are also enzymes, namely lumbrokinase, catalase, cellulose, phosphatase, peroxidase, glucuronidase, and lysozyme which act as antimicrobials by destroying the cell walls of bacteria, especially gram-positive, inhibiting pathogenic bacteria by producing extracellular products that are cytotoxic, antibacterial and affect the phagocytosis process [12].

Earthworm Pheretima sp contains 76% protein, 3.9% glutamic acid, 3.73% tyrosine, 1.13% lysine, 19.04% hydroxyproline, 4.15% aspartic acid, 3% fat and 75-100% water. In the earthworm extract there are also enzymes, namely lumbrokinase, cellulose, peroxidase and there are compounds of arachidonic acid, antipurine, antitoxin, and vitamin K which function as antipyretic and antibacterial [13]

Lumbricus rubellus and Pheretima sp contain the bioactive compound Lumbricin 1 which is a broad-spectrum antimicrobial peptide group so that it can inhibit gram-positive and gram-negative bacteria. Earthworms change the mechanism of membrane permeability by creating pores in the bacterial cell wall, so that the activity in the bacterial cell is disrupted due to the loss of cell metabolites and the cytoplasm is exposed to the outside environment which results in cell lysis [14].

Based on the above background, the researcher wanted to know the effectiveness of the inhibition of the extracts of earthworms Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus bacteria.

II. METHODS

This research is a laboratory experimental study using Salmonella typhi and Staphylococcus aureus bacteria by testing the effectiveness of the inhibitory power of the earthworm extracts Lumbricus rubellus and Pheretima sp. This research was carried out at the Biology Laboratory for Microbiology, Faculty of Pharmacy, University of North Sumatra in August-September 2019. The samples used were earthworms Lumbricus rubellus and Pheretima sp obtained from Medan Worm Cultivation. The data collection method used purposive sampling. The tools used are autoclave, stirring rod, beaker glass, blender, porcelain dish, petri dish, erlenmeyer, measuring cup, hot plate, incubator, caliper, ose needle, filter paper, parchment paper, laminar Air Flow Cabinet, lamp bunsen, refrigerator, drying cabinet, micropipette, water bath, tweezers, plastic wrapping, oven, test tube rack, rotary evaporator, spoon, spatula, test tube, analytical balance, vial, vortex.

The materials used were aquadest, 96% ethanol, earthworms Lumbricus rubellus and Pheretima sp, nutrient agar, nutrient broth, chloramphenicol, 6 mm diameter paper scraps, dimethylsulfoxide (DMSO). The bacteria used were Salmonella typhi ATCC 14028 and Staphylococcus aureus ATCC 25923.
III. RESULTS

Based on the data that has been collected and analyzed, it was found that the antibacterial activity of the extracts of the earthworms Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus were tested using the Kirby-bauer disc diffusion method. By using the sensitivity test, an inhibition zone was obtained around the disc paper that had been dripped with several concentrations with three repetitions, namely repetition 1 (P1), repetition 2 (P2), and repetition 3 (P3). The inhibition zone was measured using a caliper and the inhibition zone was obtained as shown in the table below.

Inhibitory Zone Diameter of Lumbricus rubellus Earthworm Extract Against Salmonella typhi and Staphylococcus aureus

![Figure 1](image1.png)

Figure 1. Graph of Inhibitory Zone Results for Lumbricus rubellus Earthworm Extract Against Salmonella typhi Bakteri

![Figure 2](image2.png)

Figure 2. Graph of Inhibitory Zone Results for Lumbricus rubellus Earthworm Extract Against Staphylococcus aureus

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter of The Bland Zone (mm) of Lumbricus rubellus Earthworm Extract Against Salmonella typhi</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Salmonellatyphi</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>20%</td>
<td>7,5</td>
<td>7,7</td>
</tr>
<tr>
<td>40%</td>
<td>9,4</td>
<td>9,1</td>
</tr>
<tr>
<td>60%</td>
<td>10,0</td>
<td>9,7</td>
</tr>
<tr>
<td>80%</td>
<td>11,2</td>
<td>11,4</td>
</tr>
<tr>
<td>100%</td>
<td>13,0</td>
<td>12,0</td>
</tr>
</tbody>
</table>

Table 1. Result of Inhibitory Zone Diameter of Lumbricus rubellus Earthworm Extract against Salmonella typhid and Staphylococcus aureus
Figure 4. Average Inhibition Zone of *Lumbricus rubellus* Earthworm Extract Against *Salmonella typhi* and *Staphylococcus aureus*

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

Inhibitory Zone Diameter of Pheretima sp. Earthworm Extract Against *Salmonella typhi* and *Staphylococcus aureus*

Inhibitory Zone Diameter of Pheretima sp. Earthworm Extract Against *Salmonella typhi* and *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter of The Bland Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>Pheretima sp.</td>
</tr>
<tr>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Result of Inhibitory Zone Diameter of Pheretima sp. Earthworm Extract Against *Salmonella typhi* and *Staphylococcus aureus***

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In the test against Salmonella typhi the average inhibition zone was sequential, namely at a concentration of 20% was 7.70 mm, a concentration of 40% was 8.17 mm, a concentration of 60% was 9.33 mm, a concentration of 80% was 10.23 mm, and concentration of 100% is 11.70 mm. The lowest diameter of the inhibition zone was at a concentration of 20% and the highest diameter of the inhibition zone was found at a concentration of 100%. In the test against Staphylococcus aureus the average inhibition zone was sequential, namely at a concentration of 20% was 7.07 mm, a concentration of 40% was 8.00 mm, a concentration of 60% was 9.60 mm, a concentration of 80% was 11.20 mm, and the concentration of 100% is 13.20 mm. The lowest diameter of the inhibition zone was at a concentration of 20% and the highest diameter of the inhibition zone was found at a concentration of 100%.

Diameter of Inhibition Zone Control (+) and Control (-) Against Salmonella typhi and Staphylococcus aureus

Figure 6. Average Inhibition Zone of Pheretima sp. Earthworm Extract Against Salmonella typhi and Staphylococcus aureus

Figure 7. Graph of Chloramphenicol Inhibitory Zone Results Against Salmonella typhi

Figure 7. Graph of Chloramphenicol Inhibitory Zone Results Against Staphylococcus aureus
Table 3. Results of Chloramphenicol Inhibitory Zone Diameter Against Salmonella typhi and Staphylococcus aureus

<table>
<thead>
<tr>
<th>Control Concentration (+)</th>
<th>Diameter of The Bland Zone (mm) Chloramphenicol</th>
<th>Salmonella typhi</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
<td>P3</td>
</tr>
<tr>
<td>0.5%</td>
<td>27.0</td>
<td>27.5</td>
<td>27.1</td>
</tr>
<tr>
<td>1%</td>
<td>29.0</td>
<td>29.5</td>
<td>29.1</td>
</tr>
<tr>
<td>2%</td>
<td>32.0</td>
<td>32.4</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Based on the tables and figures, the negative control test using DMSO solution showed no effect on the inhibitory power and antibacterial test. In the chloramphenicol test against Salmonella typhi the average inhibition zone was 27.20 mm at a concentration of 0.5%, a concentration of 1% was 29.20 mm, and a concentration of 2% was 32.17 mm. In the chloramphenicol test against Staphylococcus aureus the average inhibition zone was 31.90 mm at a concentration of 0.5%, a concentration of 1% was 34.03 mm, and a concentration of 2% had an average of 36.00 mm.

From the results of the effectiveness test of Lumbricus rubellus and Pheretima sp extracts against Salmonella typhi and Staphylococcus aureus bacteria, it is known that:

Hₐ = There is a difference in the effectiveness of the inhibitory power of the extracts of the earthworms Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus bacteria.

H₀ = There is no difference in the effectiveness of the inhibitory power of the earthworm extracts Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus bacteria.

From the results of the One Way ANOVA test, the value of P = 0.000 means that p < where < 0.05 then Hₐ is accepted and H₀ is rejected, there are differences in the effectiveness of the extracts of the earthworms Lumbricus rubellus and Pheretima sp against Salmonella typhi and Staphylococcus aureus. Then to find out the difference between the treatments, a post hoc test was further analyzed with Tukey's choice.

After the Post Hoc Test was carried out, it was found that there was a significant difference from each treatment given with a 95% confidence index. In the Post Hoc Tukey test, the most effective extract against Salmonella typhi was Lumbricus rubellus extract at a concentration of 100% compared to Pheretima sp extract at a concentration of 100%, with diameters respectively 12.5mm and 11.7mm. Meanwhile, the most effective extract against Staphylococcus aureus was Lumbricus rubellus at a concentration of 100% compared to Pheretima sp extract at a concentration of 100%, with diameters of 15.1mm and 13.2mm respectively. As for the positive control, 2% chloramphenicol was most effective in inhibiting Salmonella typhi and Staphylococcus aureus bacteria with diameters of 32.1mm and 36mm respectively.

Based on the results of the study, it was found that the extracts of the earthworms Lumbricus rubellus and Pheretima sp had the effectiveness of inhibition against Salmonella typhi and Staphylococcus aureus bacteria, namely by the formation of an inhibitory zone around the paper disc.

Table 4. Classification of Inhibitory Zone Diameter According to Davis and Stout
Table 5. Measurement of Inhibitory Zone Diameter of Earthworm Extracts Lumbricus rubellus and Pheretima sp According to Davis and Stout

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Average Diameter of The Bland Zone and The Criteria of Resistor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lumbricus rubellus</td>
</tr>
<tr>
<td></td>
<td>Salmonella typhi</td>
</tr>
<tr>
<td>20%</td>
<td>7.60 = Medium</td>
</tr>
<tr>
<td>40%</td>
<td>9.17 = Medium</td>
</tr>
<tr>
<td>60%</td>
<td>9.83 = Medium</td>
</tr>
<tr>
<td>80%</td>
<td>11.23 = Strong</td>
</tr>
<tr>
<td>100%</td>
<td>12.50 = Strong</td>
</tr>
</tbody>
</table>

Based on table 3.5, earthworm extracts Lumbricus rubellus and Pheretima sp began to inhibit Salmonella typhi and Staphylococcus aureus bacteria at a concentration of 20% with moderate inhibition criteria, while earthworm extract Lumbricus rubellus at 100% concentration against Staphylococcus aureus bacteria had the most effective inhibition criteria compared to extract earthworm Pheretima sp. In positive control, chloramphenicol has the most effective zone of inhibition in inhibiting the growth of Salmonella typhi and Staphylococcus aureus bacteria with a very strong inhibitory response. 25%, 50%, 75% and 100%. Where the earthworm extract Lumbricus rubellus began to inhibit the growth of Salmonella typhi and Staphylococcus aureus bacteria at a concentration of 50%–100%. Meanwhile, research conducted by Liliis (2010) stated that the extract of the earthworm Lumbricus rubellus had a bactericidal effect against Staphylococcus aureus bacteria [12][15].

This shows that the higher the concentration of the extract, the more antibacterial activity is indicated by an increase in the inhibition zone because the more antibacterial compounds contained in the extract. The thing that causes the formation of an inhibition zone is because in the earthworms Lumbricus rubellus and Pheretima sp there is a bioactive compound Lumbricin 1 which is an antimicrobial peptide group with a broad spectrum so that it can inhibit gram-positive and gram-negative bacteria. Earthworms change the mechanism of membrane permeability by creating pores in the bacterial cell wall, so that the activity in the bacterial cell is disrupted due to the loss of cell metabolites and the cytoplasm is exposed to the outside environment which results in cell lysis [14].

IV. CONCLUSION

1. Extracts of earthworms Lumbricus rubellus and Pheretima sp have effective inhibition against Salmonella typhi and Staphylococcus aureus bacteria.

2. Based on the inhibition zone classification, the extracts of the earthworms Lumbricus rubellus and Pheretima sp began to inhibit the growth of Salmonella typhi and Staphylococcus aureus bacteria at a concentration of 20% with a moderate inhibitory response.

3. Based on the inhibition zone classification, the extracts of earthworms Lumbricus rubellus and Pheretima spp. were the most effective in inhibiting the growth of Salmonella typhi and Staphylococcus aureus bacteria at a concentration of 100% with a strong inhibitory response.

V. SUGGESTION

1. Further research is needed to determine the content of active substances in earthworm extracts in inhibiting bacterial growth.

2. It is necessary to conduct further research related to the factors that cause greater differences in the effectiveness of the inhibition zone in the earthworm Lumbricus rubellus compared to Pheretima sp.

3. Further research is needed to determine the effectiveness of earthworm extract in vivo so that it can be applied as an alternative treatment and the concentration used can be clinically tested.
Further research is needed to determine the antibacterial effectiveness of earthworm extract against other pathogenic bacteria.

REFERENCES