Antibacterial Activity of Earthworm Extract (*Lumbricus rubellus*) from Organic Farmland in Bali Agaist *Streptococcus pyogenes* Bacteria

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ABSTRACT

Introduction: Bacterial infections are still a global health problem. Severe bacterial infections can lead to morbidity and mortality. *Streptococcus pyogenes* (*S.pyogenes*) is one of the bacteria that causes health problems including pharyngitis, necrotizing fasciitis, and even toxic shock syndrome. Antibiotics are used to treat bacterial infections, but the incidence of antibiotic resistance is currently very worrying. Earthworm extract (*Lumbricus rubellus*) contains various substances that have the potential to be antibacterial, one of which is *lumbricin-1*. Aim: This study aims to test the antibacterial activity of earthworm extract (*Lumbricus rubellus*) from organic farmland in Bali against *S. pyogenes* bacteria. Method: This study is an experimental study. Twenty-five samples were divided into 5 groups, namely the negative control group, positive control, and 3 extract treatment groups (50%, 75%, and 100%). The method used was the disc diffusion test method. Results: The results of the observation found the inhibitory power of earthworm extract (*Lumbricus rubellus*) from organic farmland in Bali against S. pyogenes bacteria at various extract concentrations ($p < 0.05$). The inhibitory ability of earthworm extract (*Lumbricus rubellus*) from organic farmland in Bali against *S. pyogenes* is in the strong category. Conclusion: Earthworm extract (*Lumbricus rubellus*) from organic farmland in Bali has strong inhibitory ability against *S. pyogenes* bacteria and the effect is found to be dose dependent. Contribution: This research is expected to be a reference and basis for finding new sources of antibiotics from nature and developing earthworms from organic farmland in Bali towards phytopharmaceuticals.

Keywords: Earthworms, Inhibitory power, S. pyogenes

INTRODUCTION

Streptococcus pyogenes (S. pyogenes), a member of Group A Streptococcus (GAS), is a pathogen that causes several illnesses in humans, such as pharyngitis, toxic shock syndrome, and necrotizing fasciitis. One of its virulence factors is Streptococcus pyogenes nuclease (SpnA), which can attach to human cell walls and evade the immune system (Chalmers et al., 2020). Some diseases caused by S. pyogenes can be fatal if not treated properly. Pharyngitis even has a high incidence in Indonesia. Based on basic health research data in Indonesia, the prevalence of ARI patients in Indonesia in 2018 was recorded at 9.3%, and 5-40% were caused by bacteria (Ministry of Health of the Republic of Indonesia, 2019).

Antibiotics are used to treat diseases caused by bacterial infections, but currently there is a lot of resistance, one of which is antibiotic resistance against the bacteria Staphylococcus aureus (S. aureus) known as Methicillin-Resistant Staphylococcus aureus (MRSA), this resistance can also occur in other bacteria (Adelaide et al., 2025). Resistance is one reason to find new antibiotic candidates. Natural materials in the form of plants, microorganisms, and animals may be candidates for the development of new drugs, considering that the first antibiotic discovered, penicillin, came from the fungus Penicillium notatum (Katzung et al., 2020).

One natural ingredient, earthworms (Lumbricus rubellus), have been used empirically to treat infectious diseases. Earthworms are known to contain the compound lumbricin-I, which has

antibacterial activity (Parwanto et al., 2016). This content allows earthworms to be used as antibacterial candidates. Earthworms from organic farms in Bali are earthworms cultivated in organic farms to obtain good quality earthworms. This study aims to test the antibacterial activity of earthworm extracts (Lumbricus rubellus) from organic farms in Bali against S. pyogenes bacteria.

METHODS

This research is an experimental research conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine, Udayana University. The tools used include analytical scales, petri dishes, ose, autoclaves, test tubes and racks, incubators, petri dishes, calipers, blue tape filters, rotary evaporators, jars, measuring cups, stirring spoons, spirit lamps, elemeyer flasks, and tweezers. The materials used include earthworm flour, ethanol, aquabidest, filter paper, S. pyogenes bacterial culture, Vancomycin, and physiological solution. Extraction of earthworm flour was carried out by maceration of earthworm flour cultivated in organic agricultural land in Bali in 80% ethanol solvent, after which it was evaporated to form a thick extract. The thick extract obtained was then diluted with 80% ethanol to concentrations of 50%, 75%, and 100%.

The method used in this study is the disc diffusion test method or agar diffusion using caram paper. Petri dishes containing Mueller Hinton Agar (MHA) medium were prepared, then the S. pyogenes bacterial suspension was evenly planted on the MHA using a sterile cotton bud pressed on the inner wall of the tube until no more liquid dripped. A total of 25 disc papers were divided into 5 groups. The negative control group was given 80% ethanol, the positive control group was given Vancomycin antibiotics, and 3 treatment groups were each given earthworm extract with concentrations of 50%, 75%, and 100%. The disc paper was then placed on the surface of the medium containing the S. pyogenes bacterial culture and then pressed with tweezers so that the disc paper really sticks to the bacterial culture and the media. One petri dish contains 5 disc papers from each group. The petri dish was then incubated for 24 hours in an incubator at 37 oC. An inhibition zone will form after 24 hours, observe and measure the inhibition zone. The size of the sample inhibition zone was observed by measuring the inhibition zone, namely the clear area on the MHA surface around the disc paper using a vernier caliper. The strength of bacterial inhibition was categorized based on Davis and Stout (1971).

Table 1. Categories of bacterial growth inhibition response based on the diameter of the inhibition zone (mm)

Inhibition Zone Diameter	Barrier Response
≥20 mm	Very Strong
11-19 mm	Strong
5-10 mm	Moderate
< 5 mm	Weak

RESULTS AND DISCUSSION

The results of measuring the diameter of the inhibition zone of earthworm extract from organic agricultural land in Bali can be seen in table 2. Table 2 shows that the average diameter of the highest extract inhibition zone is at a concentration of 100%, which is 11 mm with a strong category. The average lowest extract inhibition zone is at a concentration of 50%, which is 7 mm, which is in the moderate category. The Kruskal-Wallis test showed a significant difference in the diameter of the inhibition zone between the positive control, negative control, extract concentrations of 50%, 75%, and 100%, but between doses of 75% and 100% did not show a significant difference.

Table 2. Diameter of Inhibition Zone of Earthworm Extract

Group	Median (Min-Maks) (mm)	p
C+	31 (28-33)	
C-	0 (0-0)	
ECT 50%	7 (6-9)	0,001
ECT 75%	8 (7-9)	
ECT 100%	11 (9-11)	

Figure 1 shows the inhibitory activity of earthworm extract and the inhibition zone formed, a clear area indicates the formation of an inhibition zone. The positive control was placed in the center of the culture and appeared to have the largest inhibition zone diameter. No inhibition was found in the negative control, as evidenced by the absence of a clear zone around the paper. Based on the diameter of the inhibition zone, earthworm extract at concentrations of 50% and 75% fell into the moderate category, while the extract with a concentration of 100% fell into the strong category.

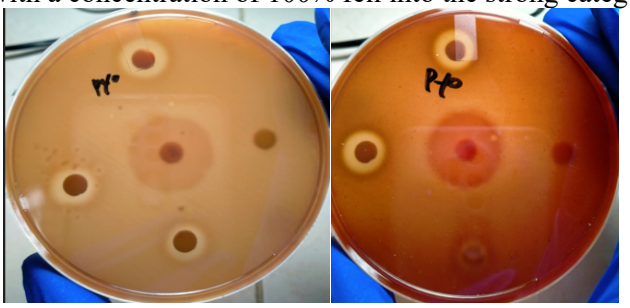

Figure 1. Inhibitory Activity of Earthworm Extract

Negative controls used the same solvent as the extract to ensure unbiased results. No inhibition zones were found in the negative controls. Vancomycin, a broad-spectrum antibiotic, exhibited the highest inhibition zone compared to other samples. Vancomycin is an antibiotic frequently used for gram-positive bacterial infections such as Staphylococcus and Streptococcus. Vancomycin has a bactericidal effect, acting by inhibiting peptidoglycan polymerization in bacterial cell walls, specifically by binding to D-alanine, which inhibits glucosyltransferase (peptidoglycan synthase) and the P-phospholipid carrier, thus preventing the synthesis and polymerization of NAM and NAG within the peptidoglycan layer. This inhibition weakens the bacterial cell wall and ultimately leads to leakage of intracellular components, resulting in bacterial cell death (Patel et al., 2024).

The research results also found that the inhibition of S. pyogenes bacterial growth by earthworm extract was directly proportional to the concentration of the extract, the greater the concentration of the extract, the greater the inhibition zone. Earthworm extract contains several compounds that have antibacterial activity, including lumbricin-1, OEP3121, PP-1, Lumbricin-PG, and alkaloids (Zhu et al., 2024). Alkaloids contain nitrogen atoms that are bacteriostatic (Nabani et al., 2022). Lumbricin-1 is formed from complete amino acids, especially proline, which is able to inhibit bacterial growth by creating pores in the bacterial cell wall, resulting in cellular leakage and death (Sara et al., 2023). Lumbricin-PG, OEP3121, and PP-1 are types of antimicrobial peptides that have a mechanism of action that is almost similar to lumbricin-1 (Zhu et al., 2024).

Several similar studies of earthworm extract on gram-positive bacteria have shown similar results. One such study, conducted by Busman et al. in 2018, found that earthworm extract had strong inhibitory activity against S. aureus (Busman et al., 2018). Earthworm extract also had antibacterial activity against S. beta hemolyticus, even greater than against S. aureus (Suryani, 2010). Earthworm decoction also had moderate antibacterial activity against S. aureus (Juariah and Subahan, 2024).

CONCLUSION

Based on the results and discussion, it can be concluded that earthworm (*Lumbricus rubellus*) extract from organic farmland in Bali has antibacterial activity against S. pyogenes bacteria. The strongest antibacterial activity was found at an extract concentration of 100%. Judging by its diameter, the diameter of the extract's inhibitory power at a concentration of 100% falls into the strong inhibitory category. Its inhibitory power depends on the extract concentration; the higher the concentration, the stronger the inhibitory power (dose-dependent).

REFERENCES

Adelaide, L.N.A., Waworuntu, O.A., and Rares, F.E.S. (2025). Identification of Methicillin-Resistant Staphylococcus aureus in Healthcare Workers in the Intensive Care Unit and Emergency Room at GMIM Pancaran Kasih General Hospital, Manado. Syntax Dmiration Juornal, 6(1): 698-705.

Busman, Alamsyah, Y., and Saputri, N. (2018). Antibacterial Activity Test of Soil Ccaing Extract (Lumbricus rubellus) Against the Growth of Staphylococcus aureus Bacteria. Menara Ilmu Journal, 12(80), 1-6.

Chalmers, C., Khemlani, A.H.J., Sohn, C.R., Loh, J.M.S., Tsai, C., and Proft, T. (2020). Streptococcus pyogenes nuclease A (SpnA) medited virulence does not exclusively depend on nuclease activity: 53, 42-48.

Davis, W.W and Stout, T.R. (1971). Disc Plate Methods of Microbiological Antibiotic Assay. Microbiolgi, 22(4): 659-665.

Juariah, S. dan Subahan, F. (2024). Uji Daya Hambat Air Rebusan Cacing Tanah (Lumbricus rubellus) terhadap Pertumbuhan Staphylococcus aureus SSecara in Vitro. Sains and Teknology Elektro journal, 14(2): 212-219.

Katzung, B.G., Masters, S.B., and Trevor, A.J. 2020. *Basic and Cinical Pharmacology*. 14 Edition. Jakarta: EGC Medical Book Publisher.

Kemenkes RI. (2019). *2018 National Riskesdas Report*. Publishing Institute of Health Research and Development Agency.

Nabani, A., Kanang, I.L.D, Gayatri, S.W., Mangarengi, Y., and Murfat. (2022). Testing the Effectiveness of Earthworm Extract (Lumbricus rubellus) against Salmonella Typhy Bacteria, the Cause of Typhoid Fever. Fakumi Medica Journal, 2(12): 863-868

Parwanto, M.L.E., Mahyunis, H.Senjaya, H.J.Edy, and Syamsurizal. 2016. Fractionation and Characterization of Protein in Lumbricus rubellus Powders. International Journal of Farmaceutical and Clinical Reseach: 8(1):15-21.

Patel, S., Preuss, C.V., and Bernice, F. (2024). Vancomycin. USA: StatPearls Publishing LLC National Library of Medicine

Sara, M., Ilyas, F., Hasballah, K., Nurjannah, N., Harapan, H., and Mudatsir, M. (2023). Lumbricus rubellus earthworm as an antibacterial: A systematic review. Jurnal of Applied Pharmaceutical Science, 13(12): 79-86.

Suryani, L. (2010). The Antibacterial Activity of Earthworm (Lumbricus Sp) Extract agaist Several Pathogen Bacteri In Vitro. Mutiara Medica Journal: 10(1): 16-21

Zhu, Z. Deng, X., Xie, W., Li, H., Li, Y., and Deng, Z. (2024). Pharmacological Effect of Bioactive Agent in Earthworm Extract: A Comprehensive Review. *Wiley online journal*, 7: 653-672.