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Effectivity of Earthworm (*Lumbricus rubellus*) Extract as Antidiarrhea Caused by *Enteropathogenic Escherichia coli :* A Narrative Review

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ABSTRACT

Background: Diarrhea still becomes a health problem in Indonesia with a high mortality rate among children under five years old. Antibiotics are used in the management of diarrhea caused by *Enteropathogenic Escherichia coli*, but it can change the intestinal microbiota in children and can cause resistance. Therefore, raw material is needed as an antibacterial.
Methods: The sources of the narrative review come from Google Scholar, PubMed, and ResearchGate with keywords such as earthworm, *Lumbricus rubellus*, earthworm extract, antidiarrhea, and Escherichia coli
Result: Earthworms (*Lumbricus rubellus*) have some active compounds that are useful for fibrinolytic, thrombolytic, antithrombotic, anti-inflammatory, antioxidant, and broad-spectrum antimicrobial properties. The antimicrobial activity of earthworms is due to the presence of Lumbricin-1 content where its activity against *Escherichia coli* has been proven by various studies.
Conclusion : Earthworm (*Lumbricus rubellus*) extract can inhibit the growth of Escherichia coli bacteria so it has the potential to become an antidiarrhea

Keywords: Antidiarrheal; earthworm; *Lumbricus rubellus*; Lumbricin-1; *Enteropathogenic Escherichia coli*

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Introduction

Diarrhea is a condition of increasing frequency of bowel movements, changes in the shape and consistency of stool that can be caused by various organisms, such as bacteria, viruses, and parasite.[1,2] Diarrhea which can causes dehydration and sepsis contributes 9% of death and it's the second leading cause of death in children under five years old in the world. Diarrhea is still a problem in developing countries, including Indonesia because of poor sanitation and inadequate management. Indonesia is in the top 15 countries with the highest number of deaths due to diarrhea among children under five in 2015 which reached 8,600 deaths.[3]

The cause of infant and children under age five mortality in Bali is also still dominated by diarrhea which is 3,2% and 2,1% in 2018.[4] The incidence of diarrhea in children in 2017 at Sanglah General Hospital was mostly at the age of three to five years. This is because the child's immune system and intestines are not yet fully developed and are not able to maintain cleanliness.[5] Enteropathogenic *Escherichia coli* (EPEC) is a pathotype of E. coli bacteria that causes persistent diarrhea that ranges from 5-15 days to weeks, and is most common in children less than two years old.[6] EPEC has the ability to adhere specifically to the intestinal epithelium which can cause subclinical to fatal infections and can lead to malabsorption of nutrients.[7]

Management of diarrhea is generally carried out by rehydration, nutrition, zinc supplementation, selective antibiotics, and parent education. Even though there are guidelines, it can be said that implementation is still not suitable.[5,8] Diagnosis and identification of specific causes of diarrhea also require special tests so that it requires a long time of treatment and requires more expensive costs.[7,9]

Treatment of diarrhea due to EPEC infection is carried out by rehydration and administration of antibiotics which is trimethoprim-sulfamethoxazole (TMP-SMX) intravenously or orally for five days. However, several studies in developing countries have found that diarrheagenic E. coli strains are generally resistant to antibiotics, such as trimethoprim-sulfamethoxazole (TMP-SMX), so that specific antibiotic therapy for diarrheagenic E. coli requires accurate diagnosis and the use of antibiotics must be considered carefully.[10] The formation of resistant organisms creates new problems in the medical world, especially in terms of treatment. This has led many researchers to start researching alternative ingredients that are effective as antibacterial agents.[9]

Several studies have also shown that antibiotic treatment can change the gut microbiota, particularly in early life for children whose gut microbiota is still vulnerable and unstable before the age of three. Changes in the gut microbiota will have health implications in the future and change the developmental pathways of the immune system, adipose growth, muscle and bone tissue.[11] Because of these reasons, current diarrhea management only aims to prevent and treat dehydration, shorten the duration of illness, and prevent diarrhea from becoming severe.[5]

Earthworms (*Lumbricus rubellus*) have a long history in Hindu, Chinese, Japanese, Vietnamese, Korean and Arabic cultures as a source of food and medicine for various diseases.[1] Earthworms have high protein and complete amino acids, one of which is Lumbricin I which functions as a natural defense against microbes by damaging the plasma membrane of pathogenic bacteria.[12] The potency of earthworm extract with a concentration of 1.04% as antibacterial E. coli has been proven in vitro based on the research of Istiqomah et al., In 2012. Thus, the writing of this paper is expected to contribute to a new modality in the form of worm extract. soil that can be used as an anti-diarrheal medicine in the future.[13]

Methods

The sources that are used in this narrative review are searched systematically through Google Scholar, PubMed, and ResearchGate. After searching, we found 53 journals that match the topic. The keywords used are earthworms, *Lumbricus rubellus*, earthworm extract, anti-diarrhea, *Escherichia coli*.

Result

Enteropathogenic Escherichia coli as a cause of diarrhea

Diarrhea is a condition in which a person defecates with a soft consistency, even in the form of water with a frequent frequency (3 or more times) in one day. The causes of diarrhea can be grouped into six major groups, namely infection (bacteria, viruses, or parasites), malabsorption, allergies, poisoning, immunodeficiency, and other causes.[14] One of the bacteria that can cause diarrhea, namely *Escherichia coli*.[7] *Escherichia coli* (*E.coli*) is a gram-negative, facultative anaerobic bacteria, and belongs to the Enterobacteriaceae family. Normally, E. coli is a normal flora in the human intestine but some strains can cause disease.[15] There are six main groups of E. coli that can cause diarrhea which are Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), Shiga Toxin-producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC or EggEC), and Diffusely Adherent *E. coli* (DAEC).[10]

Enteropathogenic *E. coli* (EPEC) is the main bacteria that causes acute, prolonged, and persistent diarrhea in children under 2 years of age in developing countries.[10] EPEC transmission can occur fecal-orally from water, food, or food equipment contaminated with feces with EPEC bacteria. Lack of good environmental sanitation, low hygiene, and population density greatly affect the process of EPEC contamination into the human body. In addition, consuming food that is not cooked according to the procedure is also a significant role.[16] Diarrhea of a watery consistency, accompanied by vomiting, abdominal pain, and fever are symptoms of an infection caused by EPEC. Biopsy samples from the jejunum or rectal mucosa show moderate to severe damage, irregular surface epithelial atrophy, and epithelial crypt vacuolization.[17,18]

Pathogenesis begins with the attachment of EPEC to small intestinal enterocytes with "bundle-forming" villi, then the lesion progresses to local brush border degeneration, loss of microvilli and changes in cell morphology. The combination of these actions is called an attachment and effacing (A/E) lesion. The steps that are involved in the formation of A/E lesions are genetically controlled in PAI (pathogenicity islands), which includes genes for the main EPEC protein attachment, intimin, and injection secretion system (type III). The secretion system injects more than 30 *E. coli* secretion proteins (Esps) into the cytoplasm of the host cell including surface receptors (Tir) for intimin that migrates to the surface after injection. Other *E. coli* secretes proteins interfere with intracellular signal transduction pathways, one of the effects of which is the induction of modification in the enterocyte cytoskeleton proteins (actin, talin). furthermore the cytoskeleton accumulates under adhering bacteria to form a bed and completes the actin-rich A/E lesions. Then Esps causes mitochondrial damage and induces apoptosis. The relationship between A/E morphological changes and diarrhea is not known, however, the injected protein secretion causes changes in electrolyte transport across the lumen membrane.[19]

Earthworms (Lumbricus rubellus)

Earthworms are invertebrates that have a tube shaped, soft and segmented body. Earthworms *(Lumbricus rubellus)* originate from Europe and have medium size body, which is about 10-15 cm.[20] *Lumbricus rubellus* has 90-195 rings that surrounds its body and has a thickening on the body called the clitelum in segment 27-23. The adult age of this worm is 179 days and can reach until 2.5 years.[21] Earthworms play a very important role in the soil ecosystem in recycling dead plant material therefore can improve the structure and aeration of the soil, which will increase soil fertility so that nutrients can be well absorbed by plants.[22,23] Several countries, including Indonesia, have cultivated and used earthworms as medicinal materials, especially in the West Java area.[24]
The classification of earthworms (*Lumbricus rubellus*) can be seen in Table 1.

Table 1. *Lumbricus rubellus* classification[25]

	Lumbricus rubellus
Kingdom	Animalia
Division	Annelida
Class	Clitella
Order	Crassiclitellata
Family	Lumbricidae
Genus	Lumbricus
Species	*Lumbricus rubellus*

Earthworms (Lumbricus rubellus) active ingredients

Earthworms contain high protein and amino acids. Some of the bioactive components found in earthworms *(Lumbricus rubellus)* are non-essential amino acids, such as aspartic acid, glutamic acid, serine, glycine, alanine, proline, and essential amino acids, such as valine, methionine, phenylalanine, lysine, isoleucine, threonine, histidine, arginine, leucine, cysteine, tyrosine.[26] Crude protein content in the earthworm *Lumbricus rubellus* is 65.63% with high proline content, which is about 15% of the total 62 acids amino.[27,28]

Earthworms *(Lumbricus rubellus)* have an enzyme called lumbrokinase which can be extracted from the digestive tract of earthworms.[29] Lumbrokinase is a class of proteolytic enzymes and have molecular weight of 25-32 kDa consisting of six proteases, namely LrPI-0, LrPI-1, LrPI-2, LrP-II, LrP-III-1, and LrPIII-2.[30,31] In addition, earthworms have a bioactive protein fraction called DLBS1033 in which there is also a group of serine proteases.[32] Lumbrokinase can activate plasminogen to plasmin and can hydrolyze fibrin and fibrinogen to become fibrin/ fibrinogen degradation product.[29] This ability is obtained from inhibition of the intrinsic coagulation pathways and activation of fibrinolytic pathways by increasing tissue plasminogen activity activator (tPA).[33] Lumbrokinase is also suspected can inhibit the action of the cyclooxygenase-1 (COX-1) enzyme so that it does not form thromboxane A_2.[34]

Earthworms also contain antimicrobial peptides. Antimicrobial peptides is a natural antibiotic produced by all living organisms rich in a protein called Lumbricin-1 which is a defense natural mechanism against pathogenic microbes in the environment. Lumbricin-1 expressed by the adult worm *Lumbricus rubellus* and has broad-spectrum antimicrobials properties against gram-positive and negative bacteria as well as fungi.[12,35,36]

The ability to enter the cell membrane affects activity of antibacterial peptides. Lumbricin-1 contains high proline amino acid where the proline is particularly useful for changing the shape of the peptide chain, -so it is not recognized as an antigen by bacteria. As a result, the peptide will not be recognized as foreign and will not be attacked by bacterial cells when it is entering the membrane cell. Apart from that, Lumbricin-1 also has a hydrophobic surface which is formed from hydrophobic amino acids. The lipid bilayer of the bacterial cell membrane has a hydrophilic surface as well hydrophobic. The hydrophobic surface of the peptide interacts with the surface hydrophilic of the cell membrane resulting in increased permeability of the cell membrane. Therefore, Lumbricin-1 can enter the hydrophilic layer.[37,38] After successfully entering the cell, peptides will inhibit the synthesis of macromolecules and DNA from bacterial cells so this causes the bacteria to experience death.[39]

Benefits and uses of earthworm (Lumbricus rubellus)

Various research has explored the activity and potential of earthworms in the health sector. Earthworm *(Lumbricus rubellus)* is reported to have antimicrobial activity against several pathogens, antioxidant and anti-inflammatory agent, anti-ischemic, antiplatelet, fibrinolytic, thrombolytic, and antithrombotic.[1,29-33,40-43]

The lumbrokinase enzyme is reported to have the potential to become fibrinolytic and oral antithrombotic agents indicated for secondary prevention after acute thrombosis, such as myocardial infarction and stroke. The lumbrokinase mechanism as fibrinolytic agents is to activate plasminogen to plasmin and degrade fibrin and fibrinogen. The working mechanism of earthworm can be seen in Table 2.

Table 2. Mechanism of action of earthworm *(Lumbricus rubellus)*

Activity	Mechanism of Action
Fibrinolytic, thrombolytic, antithrombotic	Activates plasminogen and fibrinogen degradation
Anti-inflammatory	Reducing the activation of inflammatory mediators NFkB and TNF-α
Antioxidants	Form H-O bonds from the reaction of hydrogen cations with peroxyl radicals
Antimicrobial	Increase the permeability of cell membrane and inhibit the synthesis of macromolecules and bacterial cell DNA

As an antithrombotic agent, lumbrokinase has a mechanism similar to aspirin, which inhibits the action of the cyclooxygenase-1 (COX-1) enzyme, which causes thromboxane A2 not to form.[31] DLBS1033, proven to have a fibrinogenolytic activity on fibrinogen, specifically α, β, and γ chains, could reduce thrombocyte aggregation and prolong clotting time on in vitro research conducted by Trisina et al. in 2011 and clinical trials in 14 healthy adults by Gayatri et al. in 2018.[31,42]

The anti-inflammatory activity of earthworms *(Lumbricus rubellus)* was proven in Foekh's research in 2019. Earthworm Extracts with a dose of 100 mg/kg body weight could reduce the activation of Nuclear Factor Kappa Beta (NFkB) and Tumor Necrotic Factor (TNF-α), which are inflammatory mediators in Rattus norvegicus rats induced by lipopolysaccharide. Furthermore, coelomic fluids from earthworms *(Lumbricus rubellus)* was reported to increase the anti-proliferative effect 5-fluorouracil by reducing the expression of focal adhesion kinase and interleukin-1β on colorectal cancer in the in vivo studies.[44]

The antioxidant activity of earthworms is directly attributed to phenolic compounds that play a role in neutralizing free radicals because phenolic has a hydroxyl group.[45] Phenolic hydroxyl groups are good hydroxyl donors where phenolic compounds will transfer hydrogen cations and react with peroxyl radicals and form H-O bonds.[46,47] Studies by Aldarraji et al., in 2013, with the Folin-Ciocalteu method, the total phenolic content of *Lumbricus rubellus* extract at 75% methanol was 237.73 mg/L, and at 80% methanol was 226 mg/L.[48]

The antimicrobial activity of earthworms has proven in numerous studies. Earthworm *(Lumbricus rubellus*) flour was able to inhibit the growth of C*andida albicans* in vitro, which shows inhibitory activity in a concentration of 100 mg/ 5 ml-500 mg/ 5 ml, and the most optimum activity occurs at a concentration of 200 mg/ 5 ml.[49] In addition to inhibiting fungi, the earthworm *(Lumbricus rubellus)* flour has an antimicrobial activity of earthworms against Multidrug-Resistant (MDR) *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus aureus* (MRSA).[12,50] Antibacterial activity on Multidrug-Resistant (MDR) *Pseudomonas aeruginosa* was also proven by Oktavia in 2013, where earthworm flour with a concentration of 600mg/5ml gave the most optimum inhibitory response with an inhibition zone of 15 mm. According to Tambekar and Dahikar (2010), this inhibitory response is moderate.[51,52]

Moreover, the antibacterial activity of earthworms was also proven by Andayani et al. in 2016. This study tested the antibacterial activity of earthworm *(Lumbricus rubellus)* flour against *Enterococcus faecalis* in vitro. It was found from this research, that 300 mg earthworm flour dissolved in 1% acetic acid could inhibit the growth of *Enterococcus faecalis* bacteria with an average inhibition zone diameter of 11,25 mm. Whereas, earthworm flour solution with a concentration of 400 mg/5ml, 500 mg/5ml, and 600mg/5ml resulted in an inhibition zone with an average diameter of 13 mm, 12.25 m, 11.75 mm.[39]

The potential of earthworm extract as anti-diarrhea caused by EPEC

The potential of earthworm extract as an inhibitor of *E.coli* has proven from various validated studies. The ability of earthworms to inhibit *E. coli* bacteria comes from the active ingredients of earthworms, that is Lumbricin-1.

The evidence of research results on the effectiveness of earthworm is as follows.

Based on the research by Istiqomah et al., in 2012, the earthworm extract could inhibit the growth of *E. coli* bacteria in vitro. The results obtained by Istiqomah et al. stated that the earthworm extract with a concentration of 0.52% began to have an inhibitory effect on E.coli and the maximum concentration was 1.04%. Also, boiled earthworm water could inhibit the growth of *E. coli*. Where at a concentration of 20% shows the largest average inhibition zone area is 14.49 mm and at a concentration of 80% shows the smallest average inhibition area, that is 7.92 mm. The positive control used 10% amoxicillin and produced an average diameter of 12.7 mm. On the contrary, the negative control without using boiled earthworm water showed no inhibition.[13,21]

The potential of earthworms as an anti-diarrhea, also supported by the research conducted by Julendra and Sofyan in 2007. This study used earthworm flour with a treatment of 25%, 50%, 75%, 100%, where inhibition zones formed when compared to the control. Found from the five days of observation, *Lumbricus rubellus* can inhibit the growth of E.coli bacteria in vitro with the highest inhibitory power at the use level of 50%.[53]

Conclusions

There are a lot of health benefits in Lumbricus rubellus such as antioxidants, anti-inflammatory, anti-ischemia, antiplatelet, fibrinolytic, thrombolytic, anti-thrombotic and also antimicrobial. The antimicrobial activity of earthworms is due to the presence of an antimicrobial peptide called lumbricin-1 so that earthworms (Lumbricus rubellus) have the potential to be an anti-diarrheal therapy caused by enteropathogenic Escherichia coli (EPEC). Research related to the effectiveness of earthworms as an anti-diarrheal caused by E.coli has been researched, but its effect in enteropathogenic E.coli strains has not been studied. Also, there aren't studies on the pharmacokinetics of earthworms, that is why further research and reviews on this modality are very much needed.

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