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# *Lumbricus rubellus* earthworm as an antibacterial: A systematic review

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**ABSTRACT**

Earthworms, *Lumbricus rubellus,* have a long history of medicinal use as adjuvant therapy or alternative medicine in a range of diseases including typhoid fever, infection of *Staphylococcus*, and dermatitis. The anti-bacterial effect of *L. rubellus* has been proven in many scientific studies, and some of the traditional applications of earthworms as a home remedy have been confirmed in humans. In this systematic review, we summarized the current evidence on the antimicrobial effects of *L. rubellus* against bacteria. Electronic databases including Science Direct, Wiley Online Library, Academic Search Complete, PubMed, Directory of Open Access Journal, Cochrane Library, and Indonesian Publication Index were searched for relevant studies published until October 2022, as well as direct contact with specific researchers reporting the effect of *L. rubellus* as an antibacterial. This review identified 17 studies that met the inclusion criteria. Fifteen studies show the antibacterial effect of *L. rubellus* has broad-spectrum antimicrobial peptides against Gram-negative and positive bacteria. The findings suggest that *L. rubellus* can be used as an alternative to antibiotics. Further high-quality clinical trials should be conducted to provide information about the long-term effectiveness and ability of antibacterial agents of *L. rubellus.*

## INTRODUCTION

The excessive use of antibiotics and intrinsic changes of (gene expression changes) in bacteria has increased antimicrobial resistance (AMR). In 2019, The World Health Organization released that AMR is 1 of 10 warnings to global health. It can affect clinical, economic, and death losses, especially in developing countries (Aslam *et al*., 2018; Foekh *et al*., 2019). Infection with AMR causes severe illnesses, prolonged length of stay in the hospital, increases in healthcare costs, and the higher cost of second-line drugs and treatment failures. Centers for disease control and prevention released that AMR adds a 20 billion dollar surplus in health costs in the United States and caused 23,000 deaths per year and 2.5 months extra hospital days, while in Europe, more than 9 billion euros per year spent on AMR and caused more than 25,000 deaths per year and 2.5 months additional hospital days (Dadgostar, 2019).

AMR has triggered researchers to find new alternative drug ingredients that can act as antibiotics from nature. One of the natural materials that could be used as an antibiotic is earthworms. Earthworms have been used as a traditional medicine for 100 years. Only a few species can be used as medicine. *Lumbricus rubellus* is one of them. *Lumbricus rubellus* extract comprises antibacterial characteristics and could inhibit Gram-positive and Gram-negative bacteria (Foekh *et al*., 2019; Sun, 2015). *Lumbricus rubellus* has an antimicrobial peptide (AMP) called lumbricin-I, which plays a vital role in natural defense against pathogenic microbes. Lumbricin-I was a proline-rich AMP of 62 amino acids. Lumbricin-I represented antimicrobial action *in vitro*

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against a broad spectrum of microorganisms and fungi without hemolytic side effects. This peptide leads to the establishment of multimeric pores in the bacterial cell wall. It causes the cytoplasm of bacterial cells to be exposed to the outside environment causing bacterial death. Lumbricin-I found in adult *L. rubellus* (Cho *et al*., 1998). Several studies on the use of *L. rubellus* extract as an alternative drug to treat infectious diseases such as typhoid fever (Lestari *et al*., 2019; Purwitanto *et al*., 2013), periapical infection (Andayani *et al*., 2016) periodontitis (Dharmawati *et al*., 2019), and *pullorum* disease in poultry (Damayanti *et al*., 2009). There has been no systematic review to date to evaluate the evidence of the antibiotic property of earthworm extract. We, therefore, conducted a systematic review of the available literature on *L. rubellus* as an antibacterial. We analyzed the effect of *L. rubellus* as an antibacterial on a comprehensive and heterogenous range of bacteria to evaluate its effectiveness and safety.

## MATERIALS AND METHODS

### Objectives

The objective of this study was to assess the evidence from previous studies on the use of *L. rubellus* as an antibacterial agent.

### Protocol

We followed the preferred reporting items for systematic reviews and meta-analyses guidelines (Liberati *et al*., 2009).

### Data sources and search strategy

Searches were performed in Science Direct (1998–2022), Wiley Online Library (1892–2022), Academic Search Complete (1983–2022), PubMed (1973–2022), Directory of Open Access Journal (1990–2022), Cochrane Library (2018-2022), Indonesian Publication Index (2012–2022), and direct contact the specific researcher with keywords "*Lumbricus rubellus*". We included all research types (*in vitro* study, animal study or clinical trial) published in English and Indonesian up to October 2022. A selection of relevant studies based on title and abstract, then the selected article is downloaded and reviewed by the author. The available data were extracted and tabled (Table 1). We excluded literature on *L. rubellus* as other agents such as antipyretic, antithrombotic, antiaging, and duplicate records.

### Outcome

Our primary outcome of this systematic review was the effect of *L. rubellus* as an antibacterial.

### Risk of bias assessment

The risk of bias was assessed by the Cochrane Risk of Bias tools. There was only one clinical trial evaluation performed.

## RESULTS

The literature searches identified 2,567 studies, of which 57 articles were excluded as duplicates between databases. The title and abstract were reviewed and yielded 23 articles that were reviewed in full text; of these, six were excluded, and 17 articles met the inclusion criteria (Fig. 1).

Table 1 describes the microorganisms tested, the type of the study, the methodologies used, and the study's outcome. Among all the eligible studies, one article was a clinical trial (Purwitanto *et al*., 2013), four articles were animal studies (Damayanti *et al*., 2009; Lestari *et al*., 2019; Muchtaromah *et al*., 2019; Ulhaq *et al*., 2021) while the remaining 13 studies were *in vitro* studies (Andayani *et al*., 2016; Ayuwardani and Susilowati, 2019; Cho *et al*., 1998; Damayanti *et al*., 2009; Dharmawati *et al*., 2019; Ekasari *et al*., 2012; Indrawati *et al*., 2013; Indriati *et al*., 2012; Istiqomah *et al*., 2012; Julendra and Sofyan, 2007; Mulyatno and Melinda, 2017; Rinanda *et al*., 2014; Soedjoto, 2016). Eleven studies used *L. rubellus* extract as an active ingredient (Andayani *et al*., 2016; Cho *et al*., 1998; Dharmawati *et al*., 2019; Ekasari *et al*., 2012; Istiqomah *et al*., 2012; Julendra and Sofyan, 2007; Lestari *et al*., 2019; Muchtaromah *et al*., 2019; Rinanda *et al*., 2014; Soedjoto, 2016; Ulhaq *et al*., 2021). Two studies used *L. rubellus* boiling water (Indrawati *et al*., 2013; Indriati *et al*., 2012). *Lumbricus rubellus* as an additive in poultry feed (Damayanti *et al*., 2009), combination therapy with ciprofloxacin (Purwitanto *et al*., 2013), and combination with turmeric rhizome extract (Ayuwardani and Susilowati, 2019), and combination with *Pheretima asiatica* earthworm (Indrawati *et al*., 2013).

The tested microorganisms varied: Gram-negative, Gram-positive, and fungi. Five studies determine antibacterial activity of *L. rubellus* in *Salmonella typhi* (Ayuwardani and Susilowati, 2019; Muchtaromah *et al*., 2019; Mulyatno and Melinda, 2017; Purwitanto *et al*., 2013; Soedjoto, 2016), four studies against *Staphylococcus aureus* (Cho *et al*., 1998; Indrawati *et al*., 2013; Istiqomah *et al*., 2012; Mulyatno and Melinda, 2017), four studies against *Escherichia coli* (Cho *et al*., 1998; Indriati *et al*., 2012; Istiqomah *et al*., 2012; Julendra and Sofyan, 2007), three studies against *Pseudomonas aeruginosa* (Indrawati *et al*., 2013; Istiqomah *et al*., 2012; Rinanda *et al*., 2014), two examinations against *Salmonela pullorum* (Damayanti *et al*., 2009; Istiqomah *et al*., 2012), two studies against methicillin resistant *S. aureus* (MRSA) (Rinanda *et al*., 2014; Ulhaq *et al*., 2021) and Fluconazole resistant *Candida albicans* (Rinanda, *et al*., 2014), one study against *Vibrio harveyi* (Ekasari *et al*., 2012), one study against *Enterococcus faecalis* (Andayani *et al*., 2016), and one study against *Porphyromonas gingivalis* (Dharmawati *et al*., 2019).

The concentration and dose of *L. rubellus* vary depending on the dosage form used. Five studies used *L. rubellus* extract as an active ingredient with concentrations of 0% to 100% (Damayanti *et al*., 2009; Ekasari *et al*., 2012; Julendra and Sofyan, 2007; Mulyatno and Melinda, 2017; Soedjoto, 2016), two studies used *L. rubellus* extract as an active ingredient with dose 500 and 100 mg/kg, respectively (Lestari *et al*., 2019; Purwitanto *et al*., 2013), two studies used *L. rubellus* powder with dose 100 till 600 mg (Andayani *et al*., 2016; Rinanda *et al*., 2014). Among 14 studies, 9 studies concluded that the greater the dose or concentration of *L. rubellus* used, the greater the antibacterial effect found (Andayani *et al*., 2016; Damayanti *et al*., 2009; Dharmawati *et al*., 2019; Indrawati *et al*., 2013; Istiqomah *et al*., 2012; Mulyatno and Melinda, 2017; Rinanda *et al*., 2014; Soedjoto, 2016; Ulhaq *et al*., 2021)

Only one clinical trial established the effect of *L. rubellus* extract in typhoid fever patients treated with ciprofloxacin. It was a double-blind study with a pretest-posttest with the control group. The treatment group consisted of 26 samples treated with 500 mg

**Table 1.** Main characteristics from the included studies.

| No | Author, year | Research type | Population | Intervention and comparison | Outcome |
|---|---|---|---|---|---|
| **1.** | Cho *et al.*(1998) | *In vitro* study | • Gram-positive (*Bacillus subtilis, S. aureus, Streptococcus. mutans*)<br>• Gram-negative (*E. coli, Pseudomonas putida, Serratia* sp.) | 1. *Lumbricus rubellus* (200 g) were homogenized in an acidic medium consisting of 1% (*v/v*) TFA, 1 M HCl, 5% (*v/v*) formic acid, 1% (*w/v*) NaCl and pepstatin A 1 µg/ml, centrifuged and supernatant was collected. The concentration of 0.1, 0.5, and 1 M. All the fractions were assayed for antimicrobial activity of lumbricin I, lumbricin I (6–34), and magainin 2.<br>2. A cell suspension containing $1 \times 10^6$ bacterial CFUs was added to 6 ml of underlayer agar broth and the mixture was poured into a petri dish. Incubated overnight at 37°C. Antimicrobial activity was examined by minimal inhibitory concentrations. | Lumbricin I was approximately three times more potent against tested bacteria. Lumbricin I (6–34) showed stronger antimicrobial activity than lumbricin I. |
| **2.** | Julendra and Sofyan (2007) | *In vitro* study | *Escherichia coli* | *Escherichia coli* inoculated into 20 ml NA media. *Lumbricus rubellus* powder with different concentrations (0%, 25%, 50%, 75%, 100% *w/v*) was placed on the surface of the media, and incubated for 24 hours at 37°C. | *Lumbricus rubellus* is capable to inhibit *E. coli* at optimum level of 50% and significantly higher than 25%, 75%, and 100% concentration. |
| **3.** | Damayanti *et al.* (2009) | *In vitro* and animal study | 1. *Salmonela pullorum*<br>2. Broiler chickens | 1. *Salmonela pullorum* inoculated into 30 ml liquid NA media. After the NA solid, *L. rubellus* powder with different concentrations (0%, 25%, 50%, 75%, and 100%) was inserted in a hole with a diameter of 9 mm, incubated for 48 hours at $37^oC$.<br>2. Administration of *L. rubellus* powder and filler with different concentrations (0%, 25%, 50%, 75%, 100%) for 80 broiler chickens infected *S. pullorum.* Then conducted serological test, isolation, and identification of *S. pullorum.* | *Lumbricus rubellus* can inhibit *S. pullorum* at 75% concentration. The use of *L. rubellus* powder 25%–100% in feed additives can inhibit *S. pullorum* infection. |
| **4.** | Istiqomah *et al.* (2012) | *In vitro* study | *Escherichia coli, S. aureus, S. pullorum, P. aeruginosa* | *Lumbricus rubellus* extract and *L. rubellus* encapsulated with concentrations of 0%, 0.26%, 0.52%, 0.78%, and 1.04% (g/vol) were plated into petri dish containing NA Oxoid media for *E. coli, S. aureus, S. pullorum, P. aeruginosa,* incubated for 24 hours at 37°C. | Concentration of *L. rubellus* started to inhibit:<br>0.26% for *P. aeruginosa* and *S. aureus*<br>0.52% for *E. coli* and *S. pullorum* along the increase in concentration level.<br>Concentration of *L. rubellus* for 50% lethal dose:<br>0.52% for *S. aureus*<br>1.04% for *E. coli* and *P. aeruginosa*<br>The results showed that *S. aureus* was the most sensitive bacteria to *L. rubellus* extract. |
| **5.** | Indriati *et al.* (2012) | *In vitro* study | *Escherichia coli* | 20 g dry *L. rubellus* was boiled in 25 ml of distilled water at 72°C for 15 seconds. Diluted with different concentrations of 20%, 40%, 60%, and 80%. Dip the disc paper in boiled water and put it in petri dish containing of *E. coli.*<br>Positive control: amoxicillin 10%<br>Negative control: boiling water without *L. rubellus* | The biggest inhibition area at 20% concentration and the smallest *L. rubellus* boiling water can inhibit *E. coli* growth at 80% concentration. |

*Continued*

| No | Author, year | Research type | Population | Intervention and comparison | Outcome |
|---|---|---|---|---|---|
| **6.** | Ekasari *et al.* (2012) | *In vitro* study | *Vibrio harveyi* | *Vibrio harveyi* inoculated into NA media. The paper disc containing *L. rubellus* with different concentrations (0%, 25%, 50%, 75%, and 100%) were placed on agar surface for 15–20 minutes, incubated for 24 hours at 37°C.<br>Positive control: cloramphenicol 30 µg | *Lumbricus rubellus* powder from the lowest concentration (25% *w/v*) to the highest concentration (100% *w/v*) were not able to inhibit *V. harveyi* growth. |
| **7.** | Indrawati *et al.* (2013) | *In vitro* study | *Staphylococcus aureus, S. pyogenes, P. aeruginosa* | Administration of *L. rubellus* boiling water and *P. asiatica* with concentration 0.625%; 1.25%; 2.5%; 5%; 10%; 20%; 40%; and 80%.<br>Positive control: penicilin G, basitracin, chloramphenicole,and gentamicin. | The bacteria were sensitive to *L. rubellus* boiling water and *P. asiatica* at different concentrations. The highest sensitivity showed by *S. aureus* to *L. rubellus* boiling water at 80% concentration. The higher concentration of worm boiled water, the greater the inhibition area. |
| **8.** | Purwitanto *et al.* (2013) | Clinical trial | 52 patients with typhoid fever | 1. Treatment group: 26 samples treated with 500 mg *L. rubellus* extract thrice daily and 400 mg ciprofloxacin twice daily<br>2. Control group: 26 samples treated by 400 mg ciprofloxacin twice daily and placebo thrice daily for 7 days. | The addition of *L. rubellus* extract on ciprofloxacin no effect on the long fever, antipyretic administration, gastrointestinal disorders, hemoglobin, leukocyte count, platelet count, SGOT, SGPT, and does not adversely affect the side effects of treatment. |
| **9.** | Rinanda *et al.* (2014) | *In vitro* study | Multidrug resistant (MDR) *P. aeruginosa*,<br>MRSA and Fluconazole resistant *C. albicans* | *Lumbricus rubellus* powder with different doses (100, 200, 300, 400, and 500 mg) added with 2 ml of 50% acetic acid became earthworm powder solution. The MHA medium was soaked with earthworm powder solution of different concentrations for 30 minutes. The tested microbes were inoculated to MHA medium. Incubated for 24 hours at 37°C for bacteria and 48 hours for fungi.<br>Negative control: MHA medium soaked in sterile water. | The *L. rubellus* powder at any tested concentration showed broad spectrum antimicrobial activity against MDR *P. aeruginosa*, MRSA and Fluconazole-resistant *C. albicans*. |
| **10.** | Soedjoto (2016) | *In vitro* study | *Salmonella thypi* | Administration of *L. rubellus* extracts with different concentrations (10%–100%) into MC media containing *S.typhi.* Incubated for 24 hours at 37°C. | *Lumbricus rubellus* extract can inhibit the growth of *S. thypi* start from 30% concentration. |
| **11.** | Andayani *et al.* (2016) | *In vitro* study | *Enterococcus faecalis* | *Enterococcus faecalis* swabbed on MHA media for 5 minutes. The disc paper that has been immersed in 1 ml solution of *L. rubellus* with different doses (300–600 mg) for 30 minutes was placed on MHA media, and incubated for 24 hours at 37°C.<br>Positive control: CHX 2%.<br>Negative control: acetic acid 1%. | *Lumbricus rubellus* possessed strong antibacterial activity toward the growth of *E. faecalis.* |
| **12.** | Mulyatno and Melinda (2017) | *In vitro* study | *Salmonella thypi* and *S. aureus* | Administration of *L. rubellus* ethanol extract with concentrations of 10%, 25%, 50%, 75%, and 100%.<br>Positive control: amoxicillin and chloramphenicol.<br>Negative control: aquadest. | *Lumbricus rubellus* etanol extract proved to have the ability to inhibit the growth of bacteria *S. typhi* at 50%–100% concentration and *S. aureus* at 25%–100% concentration. |

*Continued*

| No | Author, year | Research type | Population | Intervention and comparison | Outcome |
|---|---|---|---|---|---|
| **13.** | Ayuwardani and Susilowati (2019) | *In vitro* study | *Salmonella typhi* | *Salmonella thypi* were transferred into NA media. The disc paper containing *a* combination of *L. rubellus* and turmeric rhizome extract (*Curcuma Longa L*) with concentrations of earthworm and turmeri*c* were 40%:60%; 50%:50%; and 60%:40% placed in media, incubated for 24 hours.<br>Control: solely earthworm extract, solely turmeric rhizome extract.<br>Control positive: chloramphenicol 30 µg. | Combination of earthworm extract and turmeric rhizome extract at 50%:50% concentration could inhibit *S. typhii* growth optimally compared with the other groups. |
| **14.** | Lestari *et al.* (2019) | Animal study | Male Wistar rats infected with *Salmonella thypimurium* | Administration extract of earthworm *L. rubellus* 100 mg/kg which was followed by 28 male Wistar rats divided into four groups:<br>1. Only placebo<br>2. Positive control (infected with *S. thypimurium*)<br>3. Treatment group 1 (infected with *S. thypimurium* on 1st day and given *L. rubellus* until the 18th day)<br>Treatment group 2 (giving *L. rubellus* in the 1st week then infected with *S. thypimurium* on the 8th day, followed by extract until the 18th day) | *Lumbricus rubellus* has antibacterial properties by significantly reducing bacterial colonies of *S. thypimurium* in male Wistar rats. |
| **15.** | Muchtaromah *et al*. (2019) | Animal study | Small intestine and kidney of white rats infected with *S. thypi* | Administration of *L. rubellus* powder with various concentrations (32%, 48%, and 60%) and administration (7 and 14 days) of earthworm powder. | The worm powder affects improving the histological picture of the small intestine and kidneys of white rats (*R. norvegicus*). The effective treatment was found at a concentration of 60%, with a duration of 14 days. |
| **16.** | Dharmawati *et al*. (2019) | *In vitro* study | *Phorphyromonas gingivalis* | The *P. gingivalis* was swabbed on the surface of media agar. The paper discs containing different concentrations of *L. rubellus* (50%; 25%; 12.5%; and 6.25%) were placed on the agar surface, then incubated at 37°C for 24 hours. | *Lumbricus rubellus* can inhibit the growth of *P. gingivalis* bacteria from 12.5% concentration and 50% has the largest diameter of the inhibitory zone on the growth of the *P. gingivalis* bacteria. |
| **17.** | Ulhaq *et al*. (2021) | Animal study | Female mice were injected with hydrocortisone subcutaneously and intranasal infections of *MRSA* | Thirty female mice were randomly divided into six groups:<br>1. Negative control<br>2. Positive control<br>3. Infected mice treated with 2% mupirocin 125 mg diluted in 240 ml saline<br>4. Three different doses of 40-kDa protein of *L. rubellus* diluted in saline (1:10, 1:50, 1:100) | The high dose of 40-kDa protein of *L. rubellus* was able to eradicate MRSA colonization as effectively as mupirocin. |

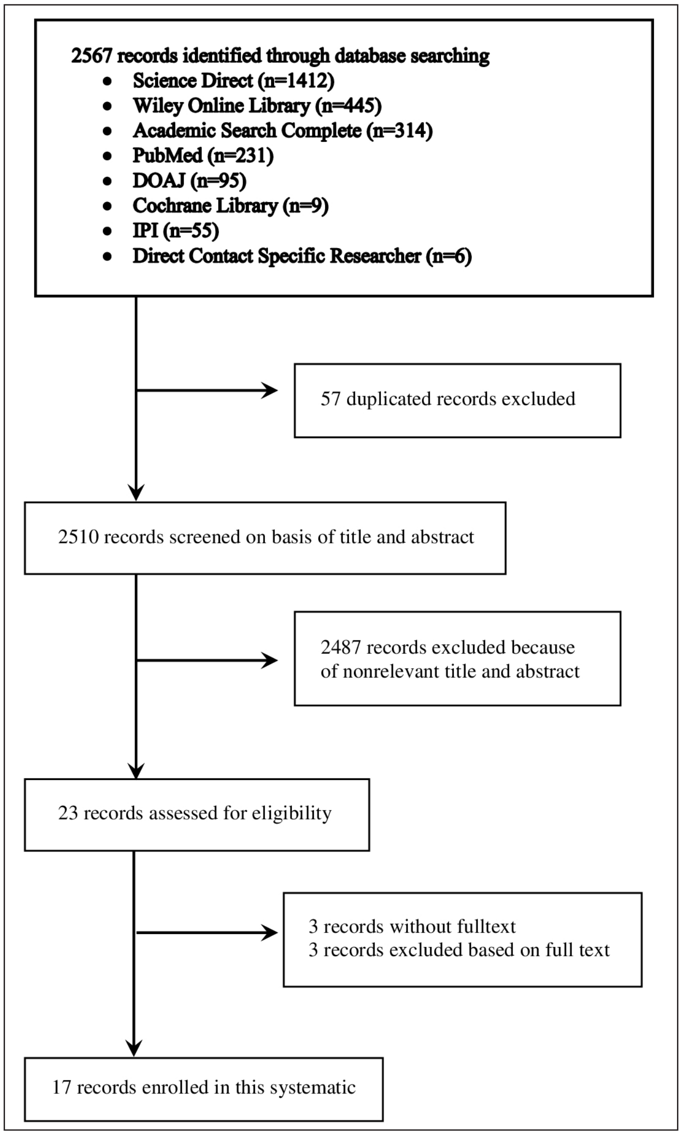


**Figure 1.** Flow diagram.

*L. rubellus* extract thrice daily and 400 mg ciprofloxacin twice daily. In the control group, 26 samples were treated with 400 mg of ciprofloxacin twice daily and a placebo thrice daily. In both groups treated for 7 days, there was no significant difference in loss of fever ($p$ = 0.896), using antipyretic ($p$ = 0.159), amount of leukocytes ($p$ = 0.484), amount of hemoglobin ($p$ = 0.984), and the total of thrombocytes ($p$ = 0.657) (Purwitanto *et al*., 2013).

## DISCUSSION

This study aimed to review the antibacterial effect of *L. rubellus* and to gain further and specific conclusions about the mechanism of action of *L. rubellus* as an antibacterial agent. Most researchers have reported promising results for *L. rubellus* as an antibacterial agent from a literature review. However, we still found two studies that did not support this finding (Ekasari *et al*., 2012; Purwitanto *et al*., 2013).

Eathworm of *L. rubellus* contains peptides with board spectrum antimicrobial activity known as lumbricin-I (formed by 62 amino acids and having a measurement of 7,231 Da). It has a proline that includes a conformational structure that influences the secondary system and then represents the mechanism of action. Besides lumbricin-I, certain AMPs have the same design with proline-rich, such as apidaecins, drosocin, metchikowin, bactenecins, and PR-39 but show various mechanisms of action. The mechanism of action of lumbricin-I in inhibiting pathogens remains unclear. However, there was one hypothesis that presents a different tool from other proline-rich AMPs (Cho *et al*., 1998). The mechanism of action of AMPs begins with the first interaction between the peptide and the target cell (bacteria or fungi) due to the impact of electrostatic power. Cationic is an essential factor that plays a vital role in electrostatic interactions between AMPs and the negatively charged phospholipid membrane of bacteria. The permeability of the cell membrane is assumed can be influenced by the exchange of cation (AMP) and anions (bacteria and fungi cell surface) and cause cell damage so that lumbricin-I can pierce the cytoplasmic membrane (Epand and Vogel, 1999; Jenssen *et al*., 2006). AMP cationic has a positive correlation with antimicrobial activity. The higher cationicity strongly influences antimicrobial activity (Yeaman and Yount, 2003). Another effectiveness of lumbricin-I as an antimicrobial is evidenced by the interaction between the hydrophobic surface and the hydrophilic surface of the bacterial cell membrane. It causes an increase in membrane permeability so that the lumbricin-I can enter the hydrophilic lipid layer. The entry of lumbricin-I into the intracellular cell membrane causes intracellular instability and inhibits bacterial growth (Pasupuleti *et al*., 2009).

Overall, the majority of the studies have reported antibacterial properties of *L. rubellus* in the form of extract, powder, or boiling water against Gram-negative bacteria, Gram-positive bacteria, and fungi (Andayani *et al*., 2016; Ayuwardani and Susilowati, 2019; Cho *et al*., 1998; Damayanti *et al*., 2009; Dharmawati *et al*., 2019; Indrawati *et al*., 2013; Indriati *et al*., 2012; Istiqomah *et al*., 2012; Julendra and Sofyan, 2007; Lestari *et al*., 2019; Muchtaromah *et al*., 2019; Mulyatno and Melinda, 2017; Rinanda *et al*., 2014; Soedjoto, 2016; Ulhaq *et al*., 2021). Only two studies did not report this finding (Ekasari *et al*., 2012; Purwitanto *et al*., 2013). *Lumbricus rubellus* is unable to inhibit the growth of *V. harveyi* bacteria because *V. harveyi is* resistant to the active ingredients in earthworms through several mechanisms: 1) bacteria produce proteolytic enzymes that can degrade AMPs; 2) proline in earthworms was used by *V. harveyi*; 3) positive charge of lumbricin-I is only +1, which is very low to interaction through electrostatic force, while the bacterial cell membrane of *V. harveyi* is negatively charged. That mechanism of action that causes damage to bacterial membrane cells cannot occur (Cho *et al*., 1998; Ekasari *et al*., 2012).

The study by Purwitanto *et al*. (2013) reported that the addition of *L. rubellus* extract on ciprofloxacin had no effect on leukocyte count in typhoid fever patient ($p$ = 0.484). This study did not assess the effect of pure *L. rubellus* extract as antibacterial but was seen from the addition of *L. rubellus* extract in the primary therapy, ciprofloxacin. The antibacterial activity of *L. rubellus* can cause a negative result from this study against *S. typhi*, not synergy with ciprofloxacin, and the dose of *L. rubellus* maybe not be the optimal dose because an optimal dose of *L. rubellus* remains unclear. *Lumbricus rubellus* has antithrombotic and thrombolytic effects in several studies. In this study, there were no reports of bleeding manifestations in the treatment group and no significant changes in hemoglobin and thrombocyte levels. The addition of earthworm extract to antibiotic treatment did not affect the duration of fever, gastrointestinal disturbances, leucocytes, liver function in typhoid patients, and incidence of side effects of treatment. The risk of bias in this clinical trial study is high because the sample is not entirely homogenous between the treatment and control groups. It can affect the results (Purwitanto *et al*., 2013).

Additionally, in this review, we found that *S. aureus* was the most sensitive bacteria in *L. rubellus* extract (Indrawati *et al*., 2013; Istiqomah *et al*., 2012; Mulyatno and Melinda, 2017). *Staphylococcus aureus* is a Gram-positive bacteria and more sensitive to bacterial compounds than Gram-negative bacteria due to the structure of the bacteria's cell walls. Gram-positive bacteria have a single layer of peptidoglycan arranged by tissue with many pores, so the lumbricin-I easy to enter the intracellular cell membrane and causes intracellular instability and inhibits bacterial growth (Istiqomah *et al*., 2012).

Finally, although the broad-spectrum antibacterial activity demonstrated by *L. rubellus* shows that lumbricin-I can be possibility developed as a potent antimicrobial agent. Still, this review cannot make definitive conclusions about the compelling form of *L. rubellus* nor the safety of *L. rubellus*.

## CONCLUSION

Studies show the potential use of *L. rubellus* for various antibacterial agents. However, investigations that determine the effect of *L. rubellus* as an antibacterial are still limited. Future clinical studies are required to examine the impact of *L. rubellus* as an antibacterial agent.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included in this research article.

## PUBLISHER'S NOTE

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