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Review Article

Integrative Analysis of the Pharmacological Activities of *Lumbricus rubellus*: Evidence from Preclinical and Clinical Research

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Abstract

Lumbricus rubellus, commonly known as the red earthworm, has long been used in traditional medicine and contains diverse bioactive compounds with therapeutic potential. However, comprehensive and systematic evaluation of its pharmacological mechanisms remains limited. This review systematically analyzes the pharmacological activities of *L. rubellus* based on *in vivo* and clinical trial evidence to provide an integrated scientific understanding of its therapeutic potential. A systematic literature search was performed in PubMed, ScienceDirect, Wiley Online Library, Cochrane Library, and Google Scholar for studies published between 2013 and 2022. The review followed PRISMA 2020 guidelines, applying the PICOS framework for eligibility determination. Study quality was assessed using the ARRIVE checklist for *in vivo* studies and the JADAD scale for clinical trials. Twelve studies met the inclusion criteria, comprising nine *in vivo* and three clinical trials. Lumbricus rubellus demonstrated multiple pharmacological effects, including fibrinolytic, antibacterial, hepatoprotective, neuroprotective, and anticancer activities. These effects are mainly attributed to proteins such as lumbrokinase and coelomic fluid metabolites that exhibit antithrombotic, anti-inflammatory, and cytoprotective actions. This review highlights strong evidence supporting the diverse pharmacological activities of *L. rubellus* and its potential as a natural source for developing novel therapeutic agents. Further standardized clinical investigations are required to confirm its efficacy and safety.

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INTRODUCTION

The red earthworm, *Lumbricus rubellus*, is a terrestrial annelid belonging to the family Lumbricidae[1]. Originally native to Europe and the northern Palaearctic regions, its distribution has expanded globally, and it is now common across North America, Australia, and Asia[2]. Beyond its crucial ecological function in soil regeneration, *L. rubellus* has historical significance in traditional medicine as a rich source of bioactive compounds. Molecular studies, including the mapping of its mitochondrial genome, which comprises 15,464 base pairs and contains 13 protein-coding genes, 22 tRNAs, and two rRNAs, have further elucidated its biochemical and phylogenetic profile[3,4].

Recent scientific investigations have confirmed that *L. rubellus* exhibits a broad spectrum of pharmacological properties. Extracts, particularly those containing peptides and proteins like lumbrokinase and coelomic fluid metabolites, have demonstrated significant antimicrobial, antioxidant, and wound-healing effects[5-8]. These findings strongly suggest its potential as a natural therapeutic agent. However, despite this growing interest and numerous individual reports describing its specific effects, such as antibacterial, fibrinolytic, and anticancer activities[9,10], the existing literature remains fragmented.

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There is a critical lack of systematic integration between *in vivo* studies and clinical trial evidence. Consequently, the comprehensive understanding of *L. rubellus*'s overall therapeutic mechanisms is currently limited and scattered across diverse study types.

Given the increasing global health challenges posed by conditions like antibiotic resistance, ischemic stroke, and cancer, a structured and integrated evaluation of the pharmacological evidence for *L. rubellus* is essential. Therefore, this systematic review aims to summarize and critically analyze the confirmed pharmacological activities of *L. rubellus*, with a specific focus on *in vivo* and clinical trial data. Employing the PRISMA framework, this review seeks to integrate the available biomedical evidence, elucidate the bioactive mechanisms, and clearly define the relevance of *L. rubellus* to modern pharmacotherapy.

MATERIALS AND METHODS

Materials

This systematic review was conducted in strict adherence to the PRISMA reporting guidelines[11]. Two independent researchers executed comprehensive literature searches across major electronic databases, including ScienceDirect, PubMed, the Wiley Online Library, and the Cochrane Library. Google Scholar was also utilized as an auxiliary source to mitigate potential publication bias and capture relevant unpublished data or literature not indexed in the primary databases. The English-language search strategy employed key phrases and keywords, primarily focusing on the organism and its function. The core search concept was: (((*Lumbricus rubellus*) AND ("Molecular Mechanisms of Pharmacological Action"[MeSH])) NOT (toxicology activities)) NOT (questionnaires). Filters were applied to restrict the results to the last 10 years (specifically, articles published between January 2013 and January 2022). A separate, focused search within the Cochrane Library using the phrase ("*Lumbricus rubellus*"): ti, ab, kw AND ("Pharmacological Activities"): ti, ab, kw yielded zero results. In contrast, the general search returned nine records within the specified time frame, ensuring a rigorous and systematic screening process.

Methods

Eligibility criteria

The eligibility criteria for this systematic review were meticulously defined using the PICOS (Population, Intervention, Comparison, Outcomes, and Study Design) framework to ensure methodological rigor, transparency, and reproducibility[12]. The criteria were established to focus exclusively on studies providing robust and directly relevant evidence concerning the pharmacological activities of *L. rubellus*. Inclusion criteria mandated the selection of original *in vivo* and clinical trial studies investigating the pharmacological or therapeutic effects of *L. rubellus* extracts or their bioactive derivatives[13,14]. Furthermore, eligible studies had to be indexed in reputable international databases, including PubMed, ScienceDirect, Wiley Online Library, Cochrane Library, or Scopus[15]. Crucially, the studies were required to present measurable pharmacological outcomes, such as fibrinolytic, antibacterial, hepatoprotective, neuroprotective, or anticancer effects, supported by clearly defined methodologies and validated analytical or biological procedures.

Conversely, exclusion criteria were applied to minimize bias and uphold the scientific quality of the review[16]. Studies were promptly excluded if they consisted solely of *in vitro*, computational, or molecular docking analyses without supporting *in vivo* or clinical validation. Other excluded document types included review papers, editorials, letters, conference proceedings, and gray literature, as well as articles that focused exclusively on toxicological or non-therapeutic activities. Finally, any study lacking sufficient methodological detail, critical outcome data, or demonstrable peer-review certification was omitted from the final synthesis.

Study selection and screening

Following the database search, all retrieved references were systematically imported into Mendeley Reference Manager for efficient management and to identify and remove duplicates. The screening process was conducted in two independent phases. Initially, two reviewers independently assessed the titles and abstracts against the predefined eligibility criteria, and potentially relevant articles were moved to a "Candidate" folder. Subsequently, the full texts of these candidate studies were rigorously reviewed by four independent reviewers for compliance with the inclusion and exclusion criteria. To ensure methodological rigor, any disagreements that arose during the selection process at both the abstract and full-text stages were

resolved through comprehensive discussion and consensus among the reviewers[17]. This detailed and transparent selection procedure is fully documented in the PRISMA flow diagram (**Figure 1**), which outlines the precise number of studies identified, screened, excluded, and ultimately included in the final synthesis.

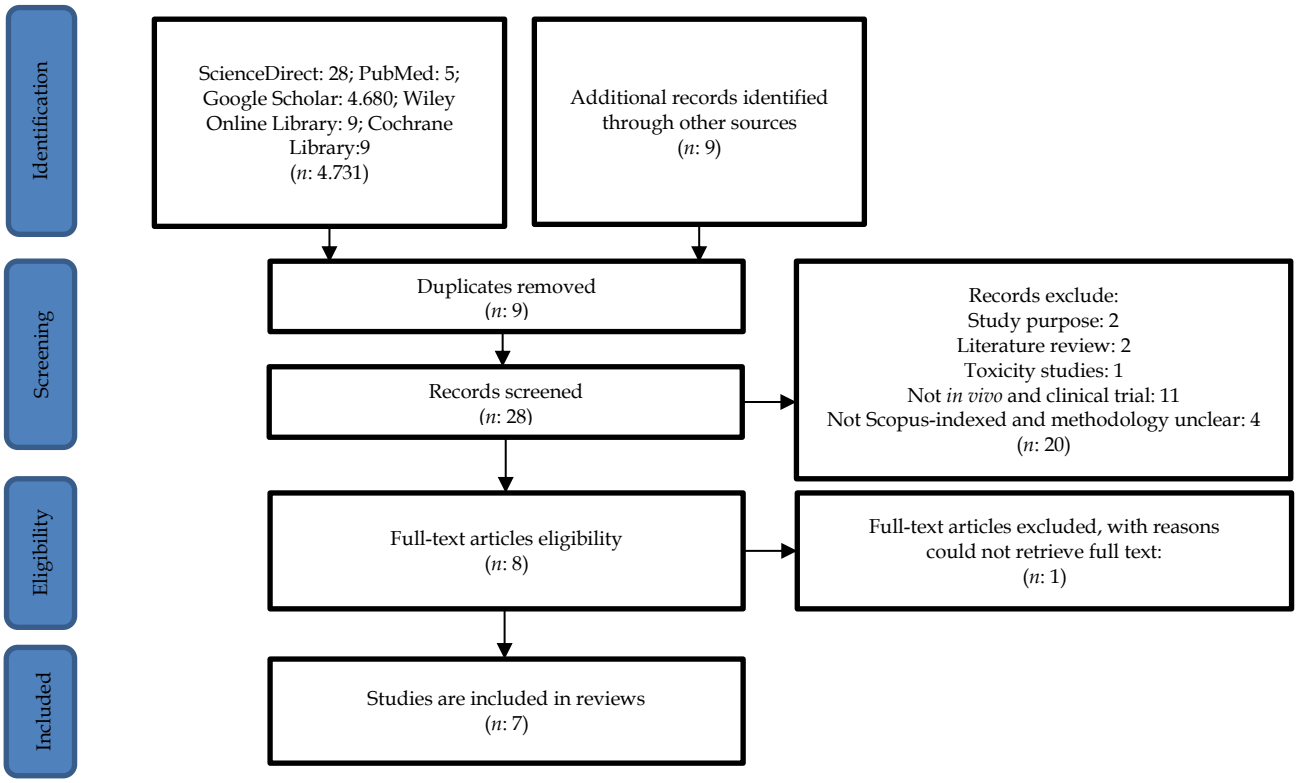

Figure 1. PRISMA guidelines in this study.

Data extraction

To ensure uniformity and minimize potential bias, data extraction was performed independently by all authors using a standardized data collection form developed in Microsoft Excel. The following essential information was meticulously extracted from each included study[18]:

1. Bibliographic information (first author, year of publication, and country of origin)
2. Study characteristics (including research design, experimental model, and sample type)
3. Intervention details (such as the specific extract or compound type, precise dosage, route of administration, and treatment duration)
4. Main pharmacological activities and measured outcomes
5. Key findings or conclusions.

In cases where multiple publications presented overlapping results derived from the same original dataset, only the most comprehensive or methodologically rigorous publication was retained for the final synthesis. Any disagreements that arose during the independent extraction process were resolved through constructive team discussion to guarantee high inter-reviewer consistency and data accuracy[19].

Data analysis

Data synthesis

Given the significant methodological and outcome heterogeneity observed across the included studies, a qualitative descriptive synthesis was performed instead of a quantitative meta-analysis[20]. The reported findings were systematically organized and synthesized thematically according to the primary pharmacological activities investigated: fibrinolytic, antibacterial, hepatoprotective, neuroprotective, and anticancer effects. Within each thematic category, data were narratively integrated by meticulously comparing the variations in experimental designs, biological models (both *in vivo* and *in vitro*),

and the proposed pharmacodynamic mechanisms[21]. This qualitative approach ensures a detailed and integrated understanding of the broad therapeutic relevance and mechanistic diversity demonstrated by *L. rubellus*.

Risk of bias and methodological quality assessment

The methodological quality and risk of bias for each included study were rigorously assessed by two independent reviewers, with verification provided by a senior reviewer. To ensure high standards of reporting and minimize bias in preclinical data, the ARRIVE 2.0 guidelines were applied to all *in vivo* studies, with a focus on transparency of reporting, appropriate randomization, adequate blinding, and adherence to ethical standards[22]. For clinical trials, the methodological quality was evaluated using the JADAD scale, which assesses the adequacy of randomization procedures explicitly, the implementation of blinding methods, and the reporting of attrition and withdrawals. Based on the scoring outcomes from these tools, each study was subsequently categorized as having a low, moderate, or high risk of bias[23]. Any initial disagreements between the primary reviewers were resolved through thorough discussion until a consensus was unanimously reached, thereby ensuring the reliability and methodological rigor of the synthesized evidence[24,25].

RESULTS AND DISCUSSION

The systematic search across multiple databases initially yielded a total of 4,731 studies. The distribution of identified records was as follows: ScienceDirect (n = 28), PubMed (n = 5), Google Scholar (n = 4,680), Wiley Online Library (n = 9), the Cochrane Library (n = 9), and additional records (n = 9). Following the initial identification, the screening phase involved removing nine duplicate records. Subsequent screening of the remaining 28 records resulted in the exclusion of 20 that were deemed irrelevant or inappropriate based on their titles and abstracts. A further eight records were excluded as ineligible. Ultimately, after full-text assessment (with one excluded at this stage), seven reports met all predefined inclusion criteria and were incorporated into the final systematic review (**Figure 1**). These final seven studies comprised both *in vivo* (animal) experiments and clinical trials, providing a diverse evidence base for subsequent analysis (**Table I**).

The systematic review of pharmacological activities primarily focused on the clinical and preclinical efficacy of *L. rubellus* extract, specifically the standardized fraction DLBS1033[26,27]. Clinical trials demonstrated that DLBS1033 exhibits promising fibrinolytic activity, with its bioactive protein maintaining a measurable half-life in a steady-state condition for approximately 8.6 hours[28]. A series of randomized controlled trials consistently confirmed the clinical benefits of DLBS1033 in patients suffering from acute ischemic stroke. These studies, conducted by Pinzon *et al*.[29] as well as Pinzon and Veronica[30], concluded that DLBS1033 offered superior efficacy in enhancing functional status and improving clinical prognosis compared to conventional therapy alone, all while maintaining a similar safety profile[29-31].

Beyond its established thrombolytic role, preclinical *in vivo* studies explored the extract's broader therapeutic potential. The extract showed significant hepatoprotective and antibacterial effects against *Salmonella typhimurium* infection in male Wistar rats, demonstrated by a reduction in AST and ALT enzyme levels and a decrease in bacterial colony counts[32]. Furthermore, *L. rubellus* was found to decrease oxidative stress markers, specifically malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, in rats infected with *S typhimurium*[33]. Intriguingly, preclinical research also indicated that a combination treatment involving *L. rubellus* may suppress colorectal cancer cell proliferation by regulating the expression of focal adhesion kinase (FAK) *in vivo*[34]. Collectively, these findings underscore the multifaceted therapeutic relevance of *L. rubellus* extract, particularly its established benefits in stroke management and its emerging potential in antibacterial, antioxidant, and anti-cancer applications.

The methodological quality of the clinical trials pertaining to *L. rubellus* was rigorously assessed using the JADAD score[35]. This evaluation was independently conducted by two researchers and the results are presented in **Table II**. The JADAD score is based on criteria including randomization, blinding, and accounting for all patients, with a score of 3 or higher typically signifying a good-quality trial. Among the four included manuscripts, Pinzon *et al*.[29] and Setyopranoto *et al*.[31] achieved the maximum score of 5, while the remaining studies scored 3 or 4. Notably, all manuscripts included in this systematic review scored 3 or greater, indicating that they possess adequate methodological quality for conveying reliable clinical trial information. It is important to emphasize that while a higher JADAD score reflects superior study design and execution, the score itself does not directly influence or validate the reported clinical outcomes of the study[36].

Table I. Studies selected for analysis.

Study	Year	Type	Pharmacological Activites	Aim	Methods	Results
Gayatri *et al*.[28]	2018	Clinical trial[37]	Fibrinolitic	Biological half life of bioactive protein	Clinical trial	The fibrinolytic effects of *L. rubellus* (DLBS1033) might be measured in steady state condition (8.6 hours)
Pinzon *et al*.[29]	2021	Clinical trial[37]	Acute ischemic stroke	Functional outcome in patients with acute ischemic stroke	Randomized control trial	In acute ischemic stroke patients, *L. rubellus* (DLBS1033) was more effective in improving functional status than conventional therapy alone, with a similar safety profile
Pinzon & Veronica[30]	2020	Clinical trial[37]	Acute ischemic stroke	Enhancement in functional status of acute ischemic stroke	Randomized control trial	*Lumbricus rubellus* (DLBS 1033) has shown superior efficacy in enhancing functional results compared to conventional treatment
Setyopranoto *et al*.[31]	2018	Clinical trial[37]	Acute ischemic stroke	Study on the hemostasis profile and clinical prognosis of individuals with acute ischemic stroke	Randomized control trial	*Lumbricus rubellus* DLBS1033 provided clinical benefits to those with ischemic stroke
Lestari *et al*.[32]	2019	*In vivo*[38]	Antibiotics	Reducing levels of ALT and AST enzymes and inhibiting the growth of *S. typhimurium* colonies *in vivo*.	Preclinical study	*Lumbricus rubellus* earthworm extract exhibits hepatoprotective and antibacterial effects by reducing AST and ALT levels and the quantity of *S.typhimurium* bacterial colonies in male Wistar rats
Samatra *et al*.[33]	2017	*In vivo*[38]	Antibiotics	The impact of *L. rubellus* on reducing MDA and 8-OHdG levels in male Wistar rats infected with *S. typhimurium*.	Preclinical study	*Lumbricus rubellus* may decrease the levels of MDA and 8-OHdG in male Wistar rats infected with *S. typhimurium*
Permana *et al*.[34]	2019	*In vivo*[38]	Colorectal cancer	Regulating the expression of focal adhesion kinase and IL-1β in colorectal cancer.	Preclinical study	The combination of 5-fluorouracil (5-FU) and CL may limit proliferation by decreasing FAK expression in colorectal cancer *in vivo*

Table II. JADAD Score.

Study	JADAD Score (Points)
Gayatri *et al*.[28]	3
Pinzon *et al*.[29]	5
Pinzon & Veronica[30]	4
Setyopranoto *et al*.[31]	5

Analysis of the included studies, summarized in **Table III**, revealed significant deficiencies in reporting quality, with 33.33% of the publications failing to adequately detail crucial methodological and ethical information. While some core methodological items demonstrated excellent compliance, several critical areas suffered from inconsistent or unclear reporting. Crucially, all included studies achieved 100% reporting for items related to sample size, inclusion/exclusion criteria for animals (3a), randomisation, protocol registration, and declaration of interests (21a, 21b). This indicates a high commitment to transparency regarding the planning and fundamental execution of the experiments[39,40].

However, significant deficiencies were noted in reporting key elements related to animal welfare and specific methodological detail. Over 66% of studies either failed to report or reported unclearly on items related to Housing and Husbandry (15a, b) and detailed descriptions of the Experimental Animals (8a, b) used, making replication challenging[41]. Furthermore, crucial details regarding the Blinding (n/a) of the outcome assessor or researchers were unclear in 66.67% of the reports. Details on specific Outcome Measures (6b, c) were also unclear in 66.67% of studies, indicating variability in defining and reporting endpoints[42-44]. Although the majority of studies reported on the Study Design (1) and Statistical Methods (7a-d) (66.67% each), the remaining percentage being unclear suggests ambiguity that warrants attention[45].

Key missing elements included justifications for the choice and use of animal species and models, the number and composition of experimental and control groups, details regarding allocation methods, comprehensive descriptions of statistical methods, specific units of analysis for each dataset, and clear articulation of outcomes and result interpretation[46,47]. Critically, these reports also often lacked discussions regarding the Implications for Replacement, Refinement, or Reduction (the 3Rs) in the use of animals. This pervasive lack of transparency suggests that the authors of the original manuscripts did not consistently adhere to established reporting standards, such as the ARRIVE Guidelines[48-50]. This methodological inconsistency hinders the reproducibility and critical appraisal of the findings, demonstrating a need for improved adherence to best practices in preclinical scientific reporting.

Table III. ARRIVE Guidelines Checklist.

Item	ARRIVE Guidelines Checklist	Reported (%)	Unclear (%)	Not Reported (%)
Study Design	1	66.67	33.33	0.00
Sample size	2	100.00	0.00	0.00
Inclusion and exclusion criteria	3	100.00	0.00	0.00
	3a	100.00	0.00	0.00
	3b	66.67	0.00	**33.33**
Randomisation	4	100.00	0.00	0.00
Blinding	5	100.00	0.00	0.00
	n/a	33.33	66.67	0.00
Outcome measures	6	66.67	33.33	0.00
	6a	66.67	0.00	**33.33**
	6b, 6c	33.33	66.67	0.00
	6b	66.67	33.33	0.00
Statistical methods	7a, b, c, d	66.67	33.33	0.00
Experimental animals	8a, b	33.33	66.67	0.00
Experimental procedures	9a, b, c	66.67	33.33	0.00
Results	10	66.67	33.33	0.00
	10a	100.00	0.00	0.00
	10b, c	66.67	33.33	0.00
Abstract	11a, b	66.67	0.00	**33.33**
Background	12	66.67	33.33	0.00
Objectives	13	66.67	33.33	0.00
	13a	33.33	33.33	**33.33**
	13b	66.67	0.00	33.33
	13c	100.00	0.00	0.00
Ethical statement	14	66.67	33.33	0.00
Housing and husbandry	15a, b	33.33	66.67	0.00
Animal care and monitoring	16	66.67	0.00	**33.33**
Interpretation/scientific implications	17a, b	66.67	33.33	0.00
Generalisability/translation	18	66.67	33.33	0.00
	18a	66.67	0.00	33.33
	18b	66.67	33.33	0.00
	18c	66.67	0.00	**33.33**
Protocol registration	19	100.00	0.00	0.00
Data access	20	66.67	33.33	0.00
Declaration of interests	21a	100.00	0.00	0.00
	21b	100.00	0.00	0.00

Acute ischemic stroke (AIS) remains a leading cause of global morbidity and mortality. While current reperfusion therapies, such as recombinant tissue plasminogen activator (rtPA) and mechanical thrombectomy, are effective, their utility is severely restricted by narrow therapeutic time windows and the substantial risk of hemorrhagic complications[51,52]. This critical need for safer adjunctive agents is addressed by lumbrokinase (DLBS1033), a derivative of *L. rubellus*. Lumbrokinase offers superior hemostatic stability by promoting targeted fibrin degradation while minimizing systemic plasminogen activation, thereby lowering the bleeding risk associated with conventional thrombolytics[53,54]. This mechanism is particularly beneficial for patients who have contraindications for rtPA[55]. Clinical trials confirm this advantage: Pinzon *et al*.[29] demonstrated that DLBS1033 significantly improved functional outcomes in AIS patients without increasing hemorrhagic events.

Furthermore, Setyopranoto *et al*.[31] reported enhanced hemostasis profiles compared to combined aspirin and clopidogrel therapy. Crucially, DLBS1033 has a significantly longer biological half-life of approximately 8.6 hours, compared to rtPA (less than 5 minutes), which potentially extends the crucial therapeutic window for reperfusion[30,56]. Beyond its fibrinolytic

effects, DLBS1033 exhibits neuroprotective properties by modulating oxidative stress and inflammation, which may further contribute to improved neuronal survival and recovery[29]. These findings position *L. rubellus*-derived lumbrokinase as a highly promising adjunctive therapy, combining efficient clot dissolution with an enhanced safety profile.

The global challenge of antimicrobial resistance necessitates the search for alternative natural therapeutics[57]. *Lumbricus rubellus* extracts have demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens, including *Staphylococcus aureus* and *S. typhimurium*[32,33]. This antimicrobial effect is primarily attributed to the extract's bioactive constituents, which include proteolytic enzymes, lysozymes, and antimicrobial peptides that disrupt bacterial cell walls and induce oxidative stress[58,59]. These compounds also exert immunomodulatory effects, enhancing host defense mechanisms while mitigating inflammation and oxidative injury associated with severe infection[33,60]. Preclinical evidence, as reported by Lestari *et al*.[32] demonstrated that *L. rubellus* extract effectively reduced bacterial load and normalized elevated liver enzyme levels (AST and ALT) in *S. typhimurium*-infected rats, confirming its dual antibacterial and hepatoprotective properties. Similarly, Samatra *et al*.[33] documented a reduction in oxidative stress markers, such as MDA and 8-OHdG.

This combined biological versatility extends to its fibrinolytic potential. The lumbrokinase enzyme acts through a dual mechanism, directly degrading fibrin clots and activating plasminogen into plasmin, thereby enhancing the body's intrinsic fibrinolytic activity[61]. Its prolonged half-life of 8.6 hours provides sustained thrombolytic action compared to conventional drugs[30]. The confirmed safety and efficacy, with improved patient outcomes and no increased risk of bleeding[29,31], along with its anti-inflammatory and endothelial-stabilizing properties, suggest its broader application in various thrombotic conditions beyond stroke, such as myocardial infarction[62].

In the management of highly prevalent malignancies, such as colorectal cancer (CRC), bioactive compounds from *L. rubellus* coelomic fluid exhibit synergistic potential with chemotherapy[63,64]. Permana *et al*.[34] demonstrated that coelomic fluid, in combination with 5-FU, significantly reduced the expression of FAK and Interleukin-1β (IL-1β). Focal adhesion kinase and IL-1β are key molecules involved in tumor proliferation, inflammation, and metastasis, suggesting that the combination therapy enhances chemosensitivity and mitigates inflammatory signaling[65-68]. Furthermore, the inherent antioxidant properties of *L. rubellus* reduce oxidative stress markers (MDA and 8-OHdG), minimizing DNA damage and promoting malignant cell apoptosis[70,71].

Collectively, *L. rubellus* exhibits a remarkable spectrum of pharmacological activities, including fibrinolytic, antibacterial, hepatoprotective, and anticancer properties, making it an exceptional candidate for the development of multi-target natural therapeutics. The organism's biological versatility, improved safety profile, and sustainable origin align perfectly with the growing demand in modern drug discovery. However, the existing evidence, while compelling, necessitates more rigorous investigation. Future research should focus on standardizing extraction and purification methods, characterizing the molecular constituents precisely, and conducting large-scale, high-quality multicenter clinical trials to substantiate its translational potential across multiple disease states and bridge traditional knowledge with modern pharmacological science.

CONCLUSION

Lumbricus rubellus is confirmed to possess a broad spectrum of scientifically substantiated pharmacological activities, demonstrating efficacy across several key therapeutic domains. The primary bioactive constituents, notably lumbrokinase and various coelomic fluid metabolites, contribute to diverse biological effects, including potent fibrinolytic, neuroprotective, antibacterial, hepatoprotective, and anticancer activities. These beneficial pharmacological actions are mechanistically linked to the compounds' ability to degrade fibrin, modulate inflammatory signaling, significantly attenuate oxidative stress, and regulate critical cellular processes such as proliferation and apoptosis. Through these integrated, multi-target mechanisms, *L. rubellus* presents a unique and compelling natural resource for managing complex diseases such as ischemic stroke, bacterial infections, and colorectal cancer. This systematic review consolidates the existing experimental and clinical evidence, underscoring the translational relevance of *L. rubellus*. Further research focused on meticulous molecular characterization, standardization of extraction protocols, and rigorous clinical validation is essential to advance the development of *L. rubellus*-derived therapeutic agents and facilitate their integration into modern, evidence-based medicine.

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AUTHORS' CONTRIBUTION

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Project administration: Iyan Hardiana
Resources: -
Software: -
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Visualization: Iyan Hardiana
Writing - original draft: Iyan Hardiana
Writing - review & editing: Iyan Hardiana, Elly Wahyudin, Muhammad Aswad, Rina Agustina

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declared no conflict of interest related to this research.

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