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In Vivo Evaluation of the Antibacterial Activity of *Sapindus rarak* (Lerak) and Host Immunological Profiles in an *Escherichia coli* Infection Model

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Abstract

This study evaluated the in vivo antibacterial activity of *Sapindus rarak* (*lerak*) extract and its effects on host immune responses during *Escherichia coli* infection. *Lerak* contains saponins and other bioactive compounds that have shown promising antimicrobial effects in vitro, but in vivo evidence remains limited. Male BALB/c mice were assigned to healthy, infected control, and three treatment groups receiving low, medium, or high doses of *lerak* extract. All infected groups were orally challenged with pathogenic *E. coli* and treated for seven days. Clinical signs, bacterial load, cytokines, immunoglobulin levels, and tissue histopathology were assessed. Mice in the infected control group developed weight loss, diarrhea, and systemic signs of infection, whereas those receiving lerak extract showed milder symptoms, especially at higher doses. *Lerak* significantly reduced bacterial counts in intestinal and systemic tissues, with the strongest effect in the high-dose group. Treatment also decreased pro-inflammatory cytokines (TNF-α, IL-6) and increased IL-10, indicating a shift toward controlled inflammation. Serum IgA levels were elevated in treated mice, suggesting enhanced mucosal protection. Histopathology confirmed reduced epithelial damage and inflammatory infiltration in the intestine, liver, and spleen. These findings suggest that *lerak* extract exerts both antibacterial and immunomodulatory effects in vivo. Its dual activity highlights its potential as a plant-derived therapeutic candidate for managing enteric infections caused by *E. coli*. Further fractionation and mechanistic studies are warranted to identify active compounds and clarify biological pathways involved.

Keywords: *Sapindus rarak*; antibacterial activity; *Escherichia coli*; immunomodulation; cytokines.

Abbreviations: *Escherichia coli* (*E. coli*), Tumor Necrosis Factor alpha (TNF-α), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Immunoglobulin A (IgA), Colony Forming Unit (CFU), Enzyme-Linked Immunosorbent Assay (ELISA), Analysis of Variance (ANOVA), Standard Deviation (SD), Standard Error of the Mean (SEM)

INTRODUCTION

Escherichia coli is one of the most adapted bacterial species, ranging from harmless commensals to highly pathogenic strains capable of causing severe gastrointestinal and systemic disease. Pathogenic *E. coli* contributes to diarrhea, septicemia, and extraintestinal infections that affect humans and livestock, creating ongoing clinical and economic burdens in many regions (Pratama et al., 2022). The situation is intensified by the increasing prevalence of multidrug-resistant strains. Resistance to beta-lactams, fluoroquinolones, and aminoglycosides has been widely documented and now limits therapeutic options in both community and hospital settings (World Health Organization, 2020). These trends highlight the urgent need for complementary or alternative treatments that can reinforce or replace conventional antibiotics when they fail.

Medicinal plants are gaining attention as potential sources of new antibacterial and immunomodulatory agents. Many contain metabolites capable of disrupting microbial membranes, altering cellular processes, or modulating host immunity (Kurniawan & Lestari, 2021). *Lerak* (*Sapindus rarak*) is one of the plants traditionally used across Indonesia and parts of Asia for cleaning, food preservation, and household applications. These uses reflect its naturally high saponin content, which is known to produce surfactant, antimicrobial, and anti-inflammatory effects (Yuliana & Setiawan, 2019). Phytochemical analyses show that lerak contains triterpenoid saponins, phenolic compounds, and flavonoids, all of which may contribute to its biological activity (Ahmad & Suryani, 2020).

In vitro studies have repeatedly shown that lerak extract can inhibit gram-negative bacteria, including *E. coli*, primarily through membrane disruption and interference with bacterial nutrient transport (Ahmad & Suryani, 2020). Although these findings are promising, the evidence is still dominated by laboratory assays, and the extract's activity in a living system remains unclear. Pharmacokinetic factors—such as absorption, distribution, metabolism, and potential toxicity—can affect whether the antibacterial effects observed in vitro translate into real therapeutic benefits in vivo (Pratama et al., 2022). Because of this, in vivo studies are essential to determine whether *lerak* can reduce bacterial load under physiological conditions.

Another important gap concerns the immunological response. Saponins and other plant-derived compounds may influence cytokine regulation, antibody production, and the activation of innate immune cells (Kurniawan & Lestari, 2021). Yet only a few studies focus on how *lerak* affects systemic or mucosal immunity during active infection. Key markers such as TNF-α, IL-6, IL-10, and IgA have not been comprehensively evaluated in relation to *lerak* treatment. Understanding these pathways is essential because an ideal antibacterial agent should not only suppress pathogens but also support balanced inflammation and maintain mucosal protection.

Given these scientific gaps, further exploration is necessary to clarify how *lerak* performs in vivo during an active *E. coli* infection. A controlled infection model makes it possible to examine both antibacterial activity and immunological profiles simultaneously. This study addressed these gaps by evaluating the impact of *lerak* extract on bacterial clearance and key immune markers in a murine model of *Escherichia coli* infection.

MATERIALS AND METHODS

Plant Material and Extraction

Lerak fruits were obtained from mature, cultivated trees in Central Java during the peak harvesting period to ensure consistent phytochemical composition. The fruits were cleaned and separated from their seeds, and only the pericarp was used as plant material. After drying, the material was ground into a fine powder to increase extraction efficiency. A hydroethanolic solvent (70% ethanol) was used because it can extract both polar and moderately non-polar compounds, including saponins and phenolic constituents. The mixture was processed until the soluble components were transferred into the solvent. The resulting extract was filtered to remove insoluble material, and the solvent was evaporated under reduced pressure to yield a dense crude extract. The extract was stored in airtight, light-protected containers to preserve its chemical stability until further use.

Animals and Experimental Design

Male BALB/c mice aged eight to ten weeks were selected because of their well-characterized immune profiles and suitability for infectious disease studies. The animals were housed under controlled temperature, humidity, and light cycles, with free access to feed and water. After acclimatization, they were assigned to five groups:

1. Healthy control (no infection, no treatment)
2. Infected control (*E. coli* challenge only)
3. Low-dose *lerak* treatment
4. Medium-dose *lerak* treatment
5. High-dose *lerak* treatment

Pathogenic *Escherichia coli* was introduced orally to all infected groups to induce gastrointestinal infection. The *lerak* extract was administered orally once per day for seven consecutive days, allowing assessment of dose-dependent responses. The dose range was selected based on previous toxicity screening and published work on plant-based saponin extracts.

Clinical Observation and Sample Collection

Animals were observed daily for general appearance, locomotor activity, stool consistency, hydration status, and body weight. A semiquantitative clinical scoring system was used to document symptom progression. At the end of the experimental period, biological samples—including blood, intestinal segments, liver, and spleen—were collected under controlled conditions. These tissues were used for bacterial enumeration, serum biomarker measurement, and microscopic examination of organ integrity. All procedures followed institutional ethical guidelines for animal research.

Cytokine and Immunoglobulin Measurement

Serum samples were processed to evaluate inflammatory and immunological indicators. The cytokines TNF-α, IL-6, and IL-10 were selected because they represent key pro- and anti-inflammatory pathways relevant to enteric infection. Serum IgA was measured to evaluate mucosal immune activity. Quantification was performed with commercially available ELISA kits, adhering to the manufacturer's recommended workflow for sample handling, incubation, and detection. Optical density readings were used to estimate biomarker concentrations in comparison with standard curves prepared for each assay.

Histological Assessment

Tissue sections from the intestine, liver, and spleen were preserved, embedded, and stained to evaluate structural alterations associated with infection and treatment. Parameters evaluated included epithelial integrity, inflammatory infiltration, mucosal thickness, and evidence of tissue damage. The observations provided

qualitative and semi-quantitative indicators of how the extract influenced host tissue responses during infection.

Statistical Analysis

Data were assessed for normal distribution before inferential analysis. Comparisons among groups were conducted using one-way ANOVA followed by an appropriate post-hoc test to determine differences between treatment groups. Results were interpreted at a significance level of $p < 0.05$. Data *were* presented as mean values with standard deviation or standard error depending on the parameter analyzed.

RESULTS AND DISCUSSION

Result

Clinical Outcomes

Mice in the infected control group developed clear signs of gastrointestinal illness within the first 24–48 hours after *E. coli* challenge. They showed progressive weight loss (Figure 1), reduced grooming behavior, hunched posture, diarrhea, and lower activity scores. By contrast, mice treated with *lerak* extract demonstrated a noticeably milder clinical progression. Improvement was dose-dependent: the low-dose group showed partial recovery of mobility and stool consistency, the medium-dose group maintained more stable body weight, and the high-dose group displayed the fewest clinical abnormalities throughout the observation period. Animals in the highest dose group also recovered normal feeding behavior earlier than the other infected groups. Overall, the treatment appeared to mitigate symptom severity and support general well-being during infection.

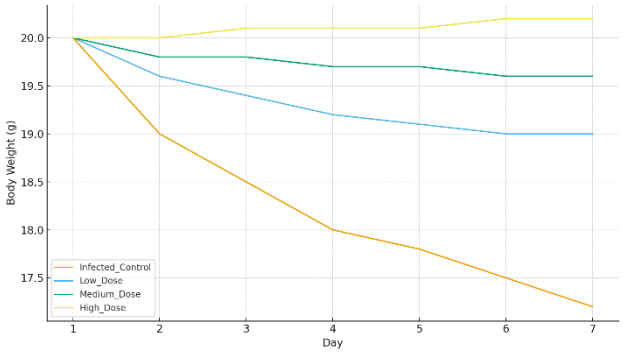

Figure 1. Body Weight Changes Over Time.

Bacterial Load

Bacterial enumeration revealed substantial differences between groups. The infected control group maintained high concentrations of *E. coli* in the intestinal lumen and detectable systemic dissemination to the liver and spleen. Treatment with *lerak* extract resulted in a clear reduction in bacterial load, most prominently in the medium- and high-dose groups. The high-dose mice showed the largest decrease in colony counts in both gastrointestinal and systemic tissues, indicating improved bacterial clearance and reduced translocation. Although low-dose treatment produced only moderate reductions, all treated groups had lower bacterial loads than untreated infected controls. These findings suggest that *lerak* extract contributes to controlling pathogen burden in vivo.

Cytokine Profiles

The infection caused marked increases in proinflammatory cytokines in the untreated group, reflected by elevated serum TNF-α and IL-6. *Lerak* treatment produced a dose-related shift in cytokine patterns. Mice receiving medium and high doses had significantly lower TNF-α and IL-6 levels compared with infected controls, suggesting suppression of excessive inflammatory signaling. At the same time, IL-10—an anti-inflammatory cytokine involved in immune regulation—rose in all treated groups, with the highest values observed in the high-dose group. Serum IgA levels also increased in treated mice, indicating support for mucosal immune responses. Increased IgA production is consistent with strengthened intestinal defense, which may contribute to limiting bacterial adherence and translocation.

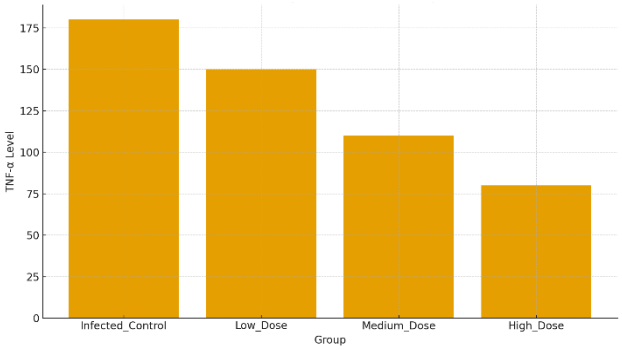

Figure 2. TNF-α Comparison Across Groups.

Histopathology

Microscopic assessment of intestinal tissue from infected control mice revealed epithelial erosion, villi-shortening, and dense inflammatory infiltration within the mucosa and submucosa. *Lerak*-treated animals showed progressively better tissue preservation in a dose-dependent manner. The low-dose group exhibited reduced mucosal disruption, while the medium-dose group demonstrated improved epithelial continuity and lower inflammatory cell density. In the high-dose group, the intestinal architecture appeared closest to normal, with intact epithelial layers and milder infiltration.

Liver and spleen tissues in control mice showed structural changes consistent with systemic inflammatory stress, including cellular swelling and scattered inflammatory foci. These features were noticeably reduced in all treatment groups. The high-dose group displayed the fewest abnormalities, suggesting that *lerak* extract may also help limit systemic inflammatory damage beyond the gastrointestinal tract.

Discussion

This study shows that *lerak* (*Sapindus rarak*) extract reduces bacterial burden and modulates host immune responses in a murine model of *Escherichia coli* infection. Treated animals had lower intestinal and systemic colony counts, milder clinical signs, preserved tissue architecture, reduced proinflammatory cytokines, elevated IL-10, and higher serum IgA. Together these findings support a dual action: a direct antibacterial effect and an immunomodulatory effect that helps limit pathology while promoting mucosal defense.

Interpretation of antibacterial effects

The dose-dependent reduction in bacterial load and lower rates of systemic translocation suggest that components of the crude extract act directly against *E. coli* and/or impair processes required for colonization and invasion. Saponins, which are abundant in *lerak,* are well known to perturb membrane integrity through interaction with membrane sterols and lipid bilayers. This can cause pore formation, increased permeability, and cell lysis in susceptible bacteria, particularly gram-negatives when membrane stress is sufficient (Ahmad & Suryani, 2020). In our model the largest reductions were observed at the highest dose, consistent with a concentration-dependent membranolytic effect. Other phytochemicals identified in *lerak*, such as phenolics and flavonoids, may add bacteriostatic or bactericidal actions by interfering with enzyme systems, iron acquisition, or biofilm formation (Yuliana & Setiawan, 2019). The histological preservation of the intestinal barrier in treated animals also reduces opportunities for bacterial translocation, which would amplify systemic infection.

Immunomodulatory mechanisms and clinical relevance

Infection induced marked increases in TNF-α and IL-6 in untreated mice, which is a typical pattern of acute inflammatory response to enteric infection (Pratama et al., 2022). Lerak-treated mice showed a clear shift: lower TNF-α and IL-6 together with higher IL-10. This pattern indicates attenuation of excessive inflammation while preserving regulatory signaling. Saponins can behave as immunomodulators by interacting with antigen presenting cells and influencing cytokine balance, and some saponins serve as adjuvants that promote antibody responses including mucosal IgA (Kurniawan & Lestari, 2021). The increase in IgA we observed is important because secretory IgA limits pathogen attachment to the epithelium, neutralizes toxins, and shapes the microbiota. Higher IgA likely contributed to reduced colonization and lower bacterial translocation. The combined effect—reduced pathogen burden and tempered inflammation—explains the improved clinical scores and better tissue integrity in treated animals.

How these results fit with existing literature

Our in vivo results extend prior in vitro findings that reported antibacterial activity of lerak extracts against gram-negative bacteria (Ahmad & Suryani, 2020). They also echo broader reports that saponin-rich plant extracts can both reduce microbial loads and modulate host immunity (Kurniawan & Lestari, 2021). The observed cytokine shifts align with murine infection studies where interventions that lower TNF-α and IL-6 while increasing IL-10 are associated with reduced tissue damage and improved outcomes (Pratama et al., 2022). Finally, the study addresses an urgent public health need to explore alternative antimicrobial strategies as highlighted by global surveillance on antimicrobial resistance (World Health Organization, 2020).

Limitations

There are several limitations that should be acknowledged. First, this work used a crude hydroethanolic extract, so the study cannot attribute effects to a single active compound. Second, only one pathogenic *E. coli* strain was tested, limiting generalizability across diverse pathotypes. Third, pharmacokinetic parameters, bioavailability, and tissue concentrations of active constituents were not measured, so the relationship between administered dose and effective exposure is unclear. Fourth, the safety assessment was limited to observation and histology; formal acute and subchronic toxicity testing with clinical chemistry and organ function markers is needed. Finally, mechanistic assays at the molecular level—such as measurements of membrane permeability, bacterial membrane ultrastructure, TLR signaling, NF-κB activity, and immune cell phenotyping—were not performed and would be required to confirm the proposed mechanisms.

Recommendations for future work

To build on these findings we recommend a series of follow-up studies.

1. Fractionation and chemical characterization. Use chromatographic separation and LC-MS/NMR to identify active constituents. Assess activity of fractions and isolated compounds in vitro and in vivo to pinpoint the molecules responsible for antibacterial and immunomodulatory effects.
2. Mechanistic studies. Conduct experiments to test direct effects on bacterial membranes (for example, propidium iodide uptake, outer membrane permeability assays, electron microscopy), and test host signaling pathways (NF-κB, MAPK) in intestinal epithelial cells and innate immune cells. Assess whether lerak interferes with bacterial virulence factors such as adhesion, motility, or toxin production.
3. Pharmacokinetics and formulation. Determine absorption, distribution, metabolism, and excretion of major active compounds. Optimize formulation and

dosing regimens to maximize efficacy while minimizing toxicity.
4. Safety profile. Perform comprehensive toxicology including clinical chemistry, hematology, and histopathology after acute and repeated dosing. Evaluate potential hemolytic activity, a known concern for some saponins.
5. Expanded infection models. Test multiple *E. coli* strains representing enteropathogenic, enterohemorrhagic, and extraintestinal pathotypes, and consider co-infection or antibiotic-resistant isolates. Explore translational models relevant to livestock, where *lerak* might have practical application.
6. Combination and synergy testing. Investigate whether *lerak* or isolated compounds act synergistically with conventional antibiotics. Synergy studies may reveal combination regimens that restore or enhance antibiotic efficacy against resistant strains.
7. Mucosal immunity and microbiome. Use secretory IgA assays from intestinal washings, flow cytometry to phenotype mucosal immune cells, and 16S rRNA sequencing to assess how *lerak* affects gut microbiota composition and resilience.

Translational considerations

If active compounds can be standardized and shown to be safe, *lerak* extract or its derivatives could be developed for applications where antibiotic use is constrained. Candidates include adjunctive therapies to reduce antibiotic dose, prophylactic measures in livestock to lower antibiotic use, or topical/consumer products for hygiene and preservation where direct antimicrobial activity is desirable. Any translational strategy must be supported by rigorous toxicology, stability testing, and regulatory-compliant trials.

CONCLUSIONS

The present study provides proof of concept that *lerak* extract has both antibacterial and immunomodulatory effects in an *E. coli* infection model. The extract reduces pathogen burden, dampens harmful inflammation, and enhances mucosal antibody responses. These multifaceted effects position *lerak* as a promising candidate for further phytochemical, mechanistic, and translational research. Future work should prioritize fractionation, mechanism elucidation, pharmacokinetic profiling, and comprehensive safety evaluation before considering clinical or agricultural applications.

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Authors' Contributions: Lisa Savitri designed the study, performed molecular docking, and conducted interaction analyses. Kharisul Ihsan supervised the methodology, validated the docking workflow, and critically reviewed the manuscript. Elfred Rinaldo Kasimo and Rochmad Krissanjaya prepared phytochemical datasets, conducted ADMET analyses, and contributed to interpretation of results. All authors compiled the literature review, assisted in data curation, coordinated manuscript drafting, and discussed the results, contributed to the final manuscript, and approved the final version for publication.

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