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Preliminary Toxicity Assessment of Black Forest Honey and Garlic Extract for Postpartum Perineal Wound Healing

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Abstract

Postpartum perineal wound infection remains a significant cause of maternal morbidity. Interest in natural therapies has encouraged the investigation of black forest honey and garlic as potential wound-healing agents. This study evaluated the preliminary toxicity of black forest honey from Ujung Kulon, Banten, combined with garlic (*Allium sativum*) extract for potential postpartum perineal wound management. A laboratory experimental study employed the Brine Shrimp Lethality Test (BSLT) to assess toxicity and the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) assay to evaluate antioxidant activity. Artemia salina larvae were exposed to different extract concentrations, and Probit analysis was used to determine the median lethal concentration (LC50). The combination exhibited biological activity, with an LC50 of 230.4 ppm (95% CI: 19.5–388.9 ppm), indicating moderate toxicity according to established classifications. The Probit model demonstrated an adequate fit (Pearson Chi-square = 10.128; p = 0.429), supporting the toxicity estimate. Potential bioactive constituents include phenolic compounds, flavonoids, hydrogen peroxide, and allicin, which may contribute to antimicrobial and antioxidant properties. The combination demonstrated biological activity and moderate toxicity, suggesting potential for further investigation in postpartum perineal wound management. However, these findings do not establish clinical safety or efficacy. Further antimicrobial testing, in vivo studies, and clinical trials are necessary to determine its safety, therapeutic potential, and suitability for preventing postpartum perineal wound infections.

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1. INTRODUCTION

Maternal infection remains one of the leading causes of maternal morbidity and mortality worldwide, particularly in developing countries. Although substantial progress has been achieved in maternal health services, postpartum infections continue to contribute significantly to adverse maternal outcomes. One of the most common postpartum complications is perineal wound infection following spontaneous perineal laceration or episiotomy during vaginal delivery. The perineal area is highly susceptible to microbial contamination because of its proximity to the urinary and gastrointestinal tracts, thereby increasing the risk of bacterial colonization and delayed wound healing (Chen et al., 2021; Cao et al., 2025).

Conventional management of postpartum perineal wounds generally includes proper hygiene, nutritional support, mobilization, and antibiotic therapy. However, the increasing concern regarding antimicrobial resistance has encouraged researchers to investigate alternative and complementary therapies derived from natural products. Natural substances rich in bioactive compounds have attracted considerable scientific interest because they exhibit antimicrobial, anti-inflammatory, antioxidant, and tissue-regenerative properties that may support wound healing processes (Burayu & Kera, 2025; Ulfah & Marlina, 2026).

Black forest honey is one of the natural products known to possess various biologically active compounds, including flavonoids, phenolic acids, glycosides, and hydrogen peroxide. These compounds contribute to its antibacterial and antioxidant activities. Previous studies have demonstrated that black honey inhibits the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* aeruginosa, microorganisms frequently associated with wound infections (Živković et al., 2019; Al-Kafaween et al., 2023). In addition, honey has been reported to stimulate fibroblast proliferation, angiogenesis, and tissue regeneration while reducing excessive inflammatory responses during wound healing (Scepankova et al., 2021; Pleeging et al., 2022).

Garlic (*Allium sativum*) is another natural product widely recognized for its medicinal properties. The primary bioactive compound in garlic, allicin, exhibits broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria through disruption of bacterial cell membranes and inhibition of essential metabolic enzymes (Nakamoto et al., 2020; Jikah et al., 2024). Several studies have shown that garlic extract effectively inhibits the growth of Staphylococcus aureus, Escherichia coli, and other clinically important pathogens. Furthermore, garlic possesses antioxidant and anti-inflammatory properties that may facilitate tissue repair and protect cells from oxidative stress during the healing process (Abidullah et al., 2021).

Despite the growing body of evidence supporting the biological activities of black honey and garlic individually, studies evaluating the toxicity and antioxidant potential of their combination remain limited. Assessment of toxicity is a crucial preliminary step in the development of natural therapeutic agents because it provides information regarding biological safety and potential pharmacological activity. One of the most widely used screening methods is the Brine Shrimp Lethality Test (BSLT), which utilizes Artemia salina larvae as a bioindicator for determining the cytotoxic potential of natural compounds (Shahidi & Samarasinghe, 2025; Bolat et al., 2026).

The combination of Black Forest honey and garlic extract is hypothesized to provide complementary biological activities because both natural products contain distinct bioactive compounds with different mechanisms of action. Honey is rich in phenolic compounds, flavonoids, hydrogen peroxide, and organic acids that exhibit antibacterial, antioxidant, and anti-inflammatory properties while promoting tissue regeneration

(Pleeging et al., 2022; Al-Kafaween et al., 2023). In contrast, garlic contains allicin and other organosulfur compounds that exert potent antimicrobial activity by disrupting microbial cell membranes and inhibiting essential metabolic enzymes (Borlinghaus et al., 2021; Jikah et al., 2024). The combination of these natural products may therefore enhance biological activity through complementary mechanisms, potentially providing broader antimicrobial coverage, improved antioxidant protection, and greater support for wound healing compared with either substance used alone (Sun et al., 2024). Although the present study was not designed to evaluate synergistic interactions directly, investigating the toxicity profile of the combined extract represents an important preliminary step before further efficacy studies

Therefore, this study aimed to evaluate the toxicity of a combination of black forest honey from Ujung Kulon, Banten, and garlic (*Allium sativum*) extract using the Brine Shrimp Lethality Test (BSLT). The findings are expected to provide preliminary scientific evidence regarding the biological activity and safety profile of this natural combination as a candidate for further pharmacological investigation related to postpartum perineal wound management.

2. METHODS

This study employed an experimental laboratory design to evaluate the toxicity and antioxidant activity of a combination of black forest honey from Ujung Kulon, Banten, and garlic (*Allium sativum*) extract. The study was conducted as a post-test only laboratory experiment focusing on the biological activity of natural compounds intended for potential application in postpartum perineal wound management. Black forest honey was obtained from Ujung Kulon, Banten, Indonesia, while fresh garlic bulbs were collected from local agricultural suppliers. Both materials were processed and extracted prior to laboratory testing.

The toxicity assessment was performed using the Brine Shrimp Lethality Test (BSLT), a widely accepted preliminary screening method for evaluating the cytotoxic potential of natural products. Artemia salina Leach larvae aged 48 hours were used as biological indicators. The combination extract was prepared at concentrations of 10, 100, 500, and 1,000 ppm. Each concentration was tested in triplicate using ten larvae per replicate. A negative control group containing only the solvent was included in the experiment. The larvae were exposed to the test solutions for 24 hours, after which the number of dead and surviving larvae was recorded. Larvae were considered dead when no movement was observed during several seconds of observation. Mortality data obtained from each concentration level were used to determine the toxicity profile of the extract combination.

The antioxidant activity of the combination extract was evaluated using the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) free radical scavenging assay. A DPPH solution with a concentration of 40 ppm was prepared in methanol. Various concentrations of the extract were mixed with the DPPH solution and incubated in a dark environment for 30 minutes at room temperature. The absorbance of each sample was measured at a wavelength of 517 nm using a UV-Visible spectrophotometer. The percentage of radical scavenging activity was calculated by comparing the absorbance values of the control and treatment groups. The antioxidant capacity of the extract was subsequently expressed as the inhibitory concentration (IC50), representing the concentration required to inhibit 50% of DPPH free radicals.

Data collection was performed through direct laboratory observations. The independent variable was the concentration of the Black Forest honey and garlic extract combination (10, 100, 500, and 1000 ppm), whereas the dependent variable was the

mortality of Artemia salina larvae after 24 hours of exposure. Mortality was recorded as the number of dead larvae in each replicate and subsequently converted into percentage mortality for each concentration level.

The mortality data were analyzed using Probit regression analysis to estimate the median lethal concentration (LC50) together with its 95% confidence interval. Pearson's Chi-square goodness-of-fit test generated within the Probit regression model was used to evaluate whether the observed mortality responses adequately fitted the expected dose-response model. A non-significant Pearson's Chi-square value ($p > 0.05$) indicated that the Probit model appropriately represented the experimental data and that the estimated LC50 value was considered statistically reliable. All statistical analyses were performed using IBM SPSS Statistics software with a significance level of $p < 0.05$.

The independent variable was the concentration of the combination extract (10, 100, 500, and 1000 ppm), whereas the dependent variable was larval mortality after 24 hours of exposure. Mortality was expressed as the number and percentage of dead larvae in each concentration group. Prior to Probit analysis, mortality data were summarized for each concentration level and used to estimate the dose–response relationship. Pearson's Chi-square goodness-of-fit test generated within the Probit model was used to evaluate whether the observed mortality distribution adequately fitted the expected Probit regression model. A non-significant Pearson Chi-square value ($p > 0.05$) indicated that the model assumptions were satisfied and that the estimated LC50 was considered reliable. This study was approved by the Health Research Ethics Committee of the Banten Ministry of Health Polytechnic (Approval Number: DP.04.03/F.XXII/10779/2026). All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

3. RESULTS AND DISCUSSION

Figure 1 illustrates the overall experimental workflow used in this study, beginning with the preparation of Black Forest honey and garlic (*Allium sativum*) extract, followed by formulation of the extract combination, preparation of test concentrations, Brine Shrimp Lethality Test (BSLT), and statistical analysis for LC50 determination. This schematic diagram is presented to improve the clarity and reproducibility of the experimental procedures.

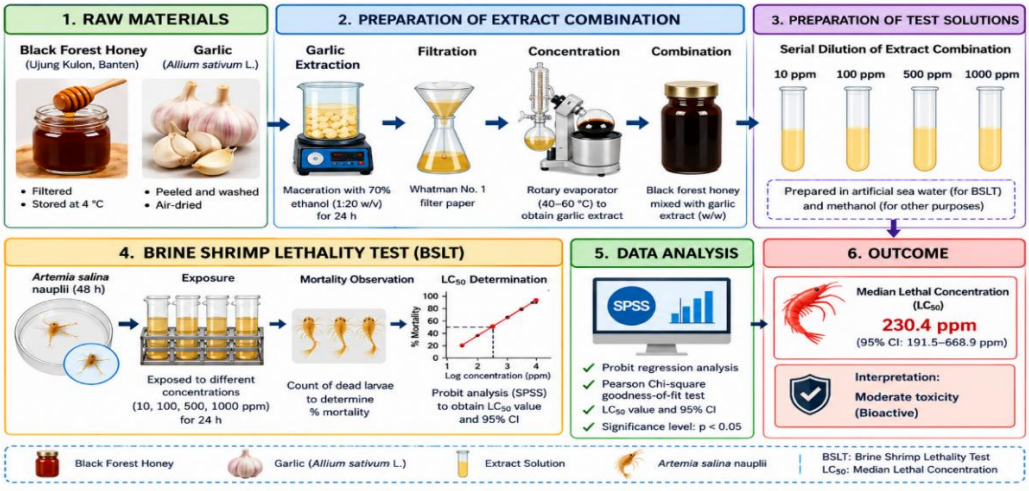


Figure 1. Experimental workflow of preparation and toxicity evaluation of Black Forest honey and garlic (*Allium sativum*) extract combination using the Brine Shrimp Lethality Test (BSLT).

The toxicity of the combination of black forest honey from Ujung Kulon, Banten, and garlic (*Allium sativum*) extract was evaluated using the Brine Shrimp Lethality Test (BSLT). This method was employed as a preliminary screening assay to assess the biological activity of the extract combination before further investigation as a potential natural agent for postpartum perineal wound management (Zakaria et al., 2023). The toxicity analysis was conducted using Artemia salina larvae exposed to four extract concentrations, namely 10, 100, 500, and 1000 ppm. Probit regression analysis was subsequently performed to determine the median lethal concentration (LC50) and evaluate the dose-response relationship between extract concentration and larval mortality (Noorhayati, Supomo, & Fatimah, 2024).

Table 1. Probit analysis results of the combination extract.

Parameter	Value
LC50	230.4 ppm
95% Confidence Interval	19.5–388.9 ppm
Pearson Chi-Square	10.128
Degree of Freedom	10
p-value	0.429

The probit analysis revealed an LC50 value of 230.4 ppm with a 95% confidence interval ranging from 19.5 to 388.9 ppm. This result indicates that the combination extract possesses considerable biological activity against Artemia salina larvae. According to Gutierrez Jr. (2021) extracts with LC50 values below 1000 ppm are categorized as toxic, while values above 1000 ppm are considered non-toxic. Therefore, the extract combination investigated in this study can be classified as toxic. Furthermore, based on the classification proposed by Karchesy et al., (2016) LC50 values ranging from 100 to 500 ppm are categorized as moderately toxic. Consequently, the toxicity level observed in this study falls within the moderately toxic category.

The validity of the Probit model was evaluated using Pearson's goodness-of-fit test. The analysis yielded a Pearson Chi-square value of 10.128 with a significance value of 0.429. Since the p-value was greater than 0.05, there was no statistically significant difference between the observed and predicted mortality responses. This finding indicates that the Probit model adequately represented the experimental data and that the estimated LC50 value was statistically reliable. Moreover, the absence of heterogeneity correction suggested acceptable consistency among experimental replications (Thakur et al., 2026).

Further evaluation of the residual analysis demonstrated that the Probit model provided accurate predictions at higher concentrations (500–1000 ppm), where residual values remained relatively small. In contrast, greater variability was observed at the lowest concentration (10 ppm). One replicate at this concentration produced a residual value of −2.679, indicating a discrepancy between observed and predicted mortality. Such variability at low concentrations is commonly encountered in BSLT studies and is generally attributed to biological variation among individual Artemia salina larvae. At concentrations substantially below the LC50 value, larval responses are often influenced by physiological differences rather than by the pharmacological effects of the tested extract. Despite this variation, the overall model remained valid as confirmed by the goodness-of-fit analysis. The toxicity observed in this study may be associated with the presence of bioactive compounds contained in both black honey and garlic. Black honey is known to contain flavonoids, phenolic compounds, glycosides, and hydrogen peroxide, all of which contribute to its biological activity. Previous studies have demonstrated that flavonoids and phenolic compounds exhibit antimicrobial, antioxidant, and anti-inflammatory properties

through inhibition of pro-inflammatory enzymes and induction of oxidative stress in microbial cells (Sun et al., 2024). Hydrogen peroxide produced enzymatically within honey also contributes to antimicrobial and cytotoxic activities (Sun et al., 2024). These mechanisms may explain part of the biological activity detected in the present toxicity assay.

The present study was designed as a preliminary biological screening using the Brine Shrimp Lethality Test (BSLT), which is widely employed for the initial identification of biologically active natural compounds. Therefore, the LC50 value obtained in this study should be interpreted as an indicator of biological activity rather than as a therapeutic or clinical safety parameter. Although the present study did not directly evaluate antimicrobial activity or wound-healing efficacy, previous studies have demonstrated that phenolic compounds, flavonoids, and allicin possess antibacterial, antioxidant, and anti-inflammatory properties that may contribute to tissue repair and prevention of wound infection. Consequently, the moderate toxicity observed in this study supports further investigations involving antimicrobial assays, antioxidant evaluation, in vivo wound-healing experiments, and clinical studies before practical application in postpartum perineal wound management

Garlic contributes additional biological activity through allicin, a sulfur-containing compound recognized for its broad-spectrum antimicrobial effects. Allicin exerts its activity by reacting with sulfhydryl groups of essential microbial enzymes, thereby disrupting cellular metabolism and inhibiting bacterial growth (Borlinghaus et al., 2021). Previous studies have demonstrated that allicin is active against several clinically important pathogens, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (Borlinghaus et al., 2014; Wallock-Richards et al., 2014). The synergistic interaction between phenolic compounds from black honey and allicin from garlic may contribute to the toxicity response observed in the BSLT assay.

The findings of this study are particularly relevant to the prevention of postpartum perineal wound infection. Perineal wounds are highly susceptible to bacterial contamination because of their anatomical location and continuous exposure to lochia during the postpartum period. The presence of pathogenic microorganisms may delay wound healing and increase postpartum morbidity (Okeahialam et al., 2023). The antimicrobial properties of allicin, flavonoids, and phenolic compounds may potentially inhibit bacterial colonization, while the antioxidant and anti-inflammatory effects of these compounds may support tissue regeneration and accelerate wound healing (Zulkefli et al., 2023; Chow et al., 2025; Liu et al., 2026).

Nevertheless, it is important to emphasize that the present study represents an initial screening stage using Artemia salina larvae as a bioindicator model. The LC50 value obtained in this study should not be directly interpreted as an effective or safe dose for human use. The BSLT assay primarily serves as a rapid and inexpensive method for identifying biologically active compounds with potential pharmacological applications (Ismail et al., 2025; Rasyid et al., 2020). Therefore, further investigations are required, including antimicrobial testing against specific postpartum pathogens, acute toxicity studies in animal models, formulation development, and controlled clinical trials before the extract combination can be recommended for practical application in postpartum care. Overall, the combination of Black Forest honey and garlic extract demonstrated moderate toxicity with an LC50 value of 230.4 ppm, indicating the presence of biologically active compounds according to established BSLT toxicity classifications. These findings provide preliminary evidence supporting further pharmacological investigations. Future studies should focus on evaluating antimicrobial activity against pathogens associated with postpartum perineal wound infection, antioxidant capacity using complementary assays,

formulation stability, animal toxicity testing, and wound-healing efficacy before translation into clinical applications.

The biological activity observed in this study is consistent with previous reports demonstrating that honey-derived phenolic compounds and garlic-derived organosulfur compounds exhibit broad-spectrum biological activities. Honey contains phenolic acids, flavonoids, hydrogen peroxide, and other bioactive constituents that contribute to antibacterial, antioxidant, and anti-inflammatory effects, thereby promoting wound healing through reduction of oxidative stress, stimulation of fibroblast proliferation, angiogenesis, and tissue regeneration (Becerril-Sánchez et al., 2021; Cianciosi et al., 2018). Likewise, allicin, the major organosulfur compound in garlic (*Allium sativum*), has been reported to inhibit a wide range of clinically important pathogens by disrupting microbial cell metabolism while simultaneously modulating inflammatory responses (Bhatwalkar et al., 2021; Sahidur et al., 2023; Shang et al., 2019). Although direct comparison of LC50 values among studies should be interpreted cautiously because of differences in extraction methods, biological models, and experimental conditions, the moderate toxicity observed in the present study indicates the presence of biologically active constituents that warrant further pharmacological investigations, including antimicrobial, antioxidant, and wound-healing evaluations. These findings are in agreement with recent evidence highlighting the therapeutic potential of honey-based natural products as promising candidates for wound management and tissue repair (Nhamussua et al., 2026).

The main limitation of this study is the use of the Brine Shrimp Lethality Test (BSLT) as the sole method for toxicity assessment. Although this assay provides preliminary evidence of biological activity, it cannot establish the safety or therapeutic efficacy of the honey and garlic extract combination for postpartum perineal wound management. Further studies using relevant mammalian cell models and in vivo wound-healing models are required to validate its safety and potential clinical application.

4. CONCLUSION

The combination of black forest honey from Ujung Kulon, Banten, and garlic (*Allium sativum*) extract demonstrated biological activity, with an LC50 value of 230.4 ppm, indicating moderate toxicity based on the Brine Shrimp Lethality Test (BSLT). This activity may be associated with bioactive compounds, including phenolics, flavonoids, hydrogen peroxide, and allicin. These findings provide preliminary evidence supporting further investigation of this combination as a potential candidate for postpartum perineal wound management. However, the BSLT results do not establish its safety or therapeutic efficacy in humans. Further antimicrobial, antioxidant, in vivo toxicity, and clinical studies are therefore required to determine its safety and effectiveness for postpartum perineal wound care.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL CONSIDERATIONS

This study was approved by the Health Research Ethics Committee of the Banten Ministry of Health Polytechnic (Approval Number: DP.04.03/F.XXII/10779/2026). All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Siti Rusyanti contributed to conceptualization, methodology, investigation, data analysis, and writing the original draft. Nani Yuningsih contributed to data collection, laboratory investigation, and data validation. Dwie Yunita Baska contributed to supervision, interpretation of results, and writing, review, and editing. All authors read and approved the final manuscript.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors declare that artificial intelligence tools, if used, were limited to supporting language refinement and improving the clarity and readability of the manuscript. No AI tools were used to generate scientific findings, analyze or interpret data, formulate research conclusions, or make research-related decisions. The research design, methodology, data collection, analysis, and interpretation were conducted by the authors. All authors reviewed and approved the final manuscript and accept full responsibility for its accuracy, originality, and scientific integrity.

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