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Antibacterial Activity of Pegagan (*Centella asiatica*) Extract Against Bacteria Causing Postpartum Wound Infections

Indriani Febrishaummy Gunawan* & Tania Aprilianti
Faculty of Health and Sciences, Universitas Bhakti Asih Tangerang, Tangerang, Indonesia
*Corresponding Author e-mail: indrianifeb@gmail.com

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Abstract
Postpartum perineal wounds are highly susceptible to bacterial infection, particularly by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Although *Centella asiatica* is widely recognized for its wound-healing properties, quantitative data on its antibacterial potency against postpartum-related pathogens remain limited. This study aimed to evaluate the antibacterial activity of ethanol extract of *C. asiatica* against *S. aureus* and *P. aeruginosa* by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Fresh leaves of *C. asiatica* were extracted using 96% ethanol. Ethanol was selected as the extraction solvent due to its ability to dissolve both polar and semi-polar bioactive compounds, including flavonoids, tannins, and triterpenoids, which are associated with antibacterial activity. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, alkaloids, and triterpenoids. Antibacterial activity was assessed using the broth microdilution method. The extract exhibited concentration-dependent inhibition, with MIC values of 12.5% against *S. aureus* and 3.12% against *P. aeruginosa*, while bactericidal activity for both strains was observed at 25%. These findings provide quantitative evidence of the antibacterial potential of *C. asiatica*ethanol extract against key bacteria associated with postpartum wound infections, highlighting its potential as a natural antibacterial agent for postpartum wound management.

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INTRODUCTION

Postpartum perineal wounds are highly susceptible to microbial contamination due to tissue disruption, local humidity, and continuous exposure to endogenous flora during and after delivery. When bacterial colonization is not adequately controlled, these wounds may progress to infection, leading to delayed wound healing, increased pain, higher maternal morbidity, and potential postpartum complications. *Staphylococcus aureus* and Pseudomonas aeruginosa are among the most frequently reported bacterial pathogens in wound infections and are known for their ability to develop antibiotic resistance and form biofilms, which further complicate therapeutic management. Infection of postpartum wounds therefore remains an important concern in maternal health care, particularly in settings with limited access to optimal wound management (Prasetyawan et al., 2025; Saraswati et al., 2025)

The escalating prevalence of antimicrobial resistance has intensified the search for alternative or complementary antibacterial agents derived from natural products. Medicinal plants that contain diverse secondary metabolites are promising candidates because they may act via multiple targets and therefore reduce the likelihood of rapid resistance development. *Centella asiatica* (pegagan, gotu kola) is widely documented for its wound-healing properties and has been used traditionally for topical treatment of skin lesions; however, literature on its direct, quantitative antibacterial activity against clinically relevant postpartum wound pathogens remains limited. Due to its rich phytochemical composition and multitarget antimicrobial mechanisms, *C. asiatica* represents a potential natural source of antibacterial agents for postpartum wound management (Aulia Azmi & Dewi Dwiyanti, 2020; Qurrotuaini et al., 2022; Andriyanto et al., 2025).

Among various pathogenic microorganisms, *Staphylococcus aureus* and Pseudomonas aeruginosa are frequently isolated from infected wounds. S. aureus is a common skin commensal capable of invading damaged tissue, whereas P. aeruginosa thrives in moist environments and demonstrates strong resistance to many antimicrobial agents. The ability of P. aeruginosa to form biofilms further complicates infection control, as biofilm-associated bacteria are less responsive to host defenses and antibiotic treatment (Nazilinly et al., 2024).

Phytochemical studies of *C. asiatica* consistently report high levels of triterpene glycosides (notably asiaticoside and madecassoside), triterpene acids (asiatic acid, madecassic acid), flavonoids, tannins, saponins, and other phenolic compounds. These constituents contribute differently to biological activity: triterpene glycosides (asiaticoside, madecassoside) primarily modulate wound-healing processes through stimulation of collagen synthesis, fibroblast proliferation, and anti-inflammatory signaling; flavonoids and phenolics provide antioxidant activity and exhibit direct antibacterial effects often mediated by membrane interaction, enzyme inhibition, and interference with nucleic acid function; saponins and certain triterpenoids may disrupt membrane integrity and potentiate permeability of bacterial cells. Together, these chemical classes can act synergistically to reduce microbial burden and support tissue repair (Diniz et al., 2023; Zhou et al., 2023).

From a chemical perspective, asiaticoside is a triterpene glycoside whose aglycone (asiatic acid) and sugar moiety influence bioavailability and interaction with biological membranes; experimental evidence indicates asiaticoside and related triterpenoids reduce inflammatory mediators and promote matrix remodeling, effects that indirectly limit the niche available for pathogen proliferation in wounds.

Flavonoids (polyphenolic structures) exert antibacterial actions that depend on substitution patterns (hydroxylation, methoxylation, glycosylation) and can cause cytoplasmic membrane perturbation, inhibition of energy metabolism, and suppression of nucleic acid synthesis. Triterpenoids may insert into lipid bilayers, increasing membrane permeability and leading to leakage of cellular contents; they also possess anti-inflammatory properties that are advantageous in infected wounds. Elaborating these chemical roles clarifies the mechanistic basis by which *C. asiatica* extracts may exert both antimicrobial and wound-healing effects (Hein et al., 2025; Zhou et al., 2023)

Extraction solvent selection is critical because different solvents yield different chemical profiles: polar and semi-polar solvents (e.g., ethanol, methanol) typically extract flavonoids, tannins, saponins, and glycosylated triterpenes, whereas nonpolar solvents (e.g., hexane) favor lipophilic terpenes and fatty acids. Ethanol is commonly used in *C. asiatica* extraction because it provides a balance between polarity and solubility, allowing efficient recovery of both polar and semi-polar antibacterial constituents (flavonoids, tannins, triterpene glycosides) and often results in higher phenolic and flavonoid yields compared to water or nonpolar solvents. This chemical rationale underpins the selection of ethanol for extraction in studies that aim to evaluate antibacterial potential(Wong & Ramli, 2021).

Although *Centella asiatica* has been widely reported for wound healing, quantitative antibacterial evaluation using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays against postpartum wound pathogens remains limited. Postpartum perineal wounds are at high risk of infection by antibiotic-resistant bacteria, and the lack of quantitative MIC/MBC data restricts the translation of *C. asiatica* into evidence-based topical antibacterial formulations.

To address this gap, this research evaluates the antibacterial activity of an ethanol extract of *C. asiatica* against *Staphylococcus aureus* and Pseudomonas aeruginosa by determining MIC and MBC values and relating the observed activity to the presence of key phytochemical classes, including flavonoids, tannins, saponins, and triterpenoids (Chonsut et al., 2024; Diniz et al., 2023).

METHOD

An experimental laboratory approach was applied to examine the antibacterial effect of ethanol extract of *Centella asiatica* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Fresh leaves of *Centella asiatica* were collected, washed with distilled water, air-dried at room temperature, and ground into fine powder. Extraction was carried out using 96% ethanol in a Soxhlet apparatus for 6–8h. Ethanol was selected as the extraction solvent due to its ability to dissolve both polar and semi-polar secondary metabolites, including flavonoids, tannins, and triterpenoids, which are associated with antibacterial activity. The solvent was removed under reduced pressure using a rotary evaporator, and the crude extract was stored at 4 °C until further analysis (Diniz et al., 2023; Wong & Ramli, 2021).

Qualitative phytochemical analysis was conducted to identify major secondary metabolites present in the extract, including flavonoids, tannins, saponins, alkaloids, and triterpenoids. For antibacterial testing, bacterial suspensions were prepared from 24-hour cultures and adjusted to a turbidity equivalent to 0.5 McFarland standard. Serial dilutions of the extract were prepared to obtain concentrations ranging from 1.56% to 25% (Nazilinly et al., 2024)

Antibacterial activity was evaluated using the broth microdilution technique. The bacterial strains employed in this study were ATCC strains obtained from a certified microbiology laboratory. The MIC was determined as the lowest extract concentration that inhibited visible bacterial growth after incubation at 37 °C for 18–24 hours. To determine the MBC, samples from wells without visible growth were subcultured onto agar and incubated for an additional 24 hours. The lowest concentration resulting in no bacterial colony formation was defined as the MBC. All experiments were conducted in triplicate (Nazilinly et al., 2024).

RESULTS AND DISCUSSION

Phytochemical Profile of *Centella asiatica* Extract

Phytochemical screening confirmed that the ethanol extract of *Centella asiatica* contains multiple classes of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and triterpenoids. The presence of these compounds suggests that the antibacterial activity of the extract may result from the combined action of various bioactive constituents rather than a single compound.

Table 1. Phytochemical Screening of Ethanol Extract

Phytochemical Screening	Test Method	Centella asiatica extract
Tannins	$FeCl_3$ test	+
Flavonoids	Shinoda test	+
Saponins	Froth test	+
Alkaloids	Mayer, Dragendorff	+
Steroids	Liebermann-Buchard	+

(+) : presence detected, (-) : not detected

Flavonoids exhibit antibacterial activity mainly through disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, and interference with cellular energy metabolism. Tannins contribute to antimicrobial effects by forming complexes with bacterial cell wall proteins, thereby altering membrane permeability and inhibiting key enzymatic activities. Saponins increase membrane permeability by interacting with lipid bilayers, which can lead to leakage of intracellular components and eventual cell lysis. Alkaloids interfere with DNA replication and protein synthesis, whereas steroids and triterpenoids destabilize bacterial membranes and suppress essential metabolic processes. These multitarget mechanisms support the synergistic antibacterial action of complex phytochemical mixtures present in *Centella asiatica* extract (Hein et al., 2025; Horváth et al., 2016).

One of the major bioactive constituents of *Centella asiatica* is asiaticoside, a triterpenoid saponin that has been extensively associated with wound-healing activity through stimulation of fibroblast proliferation and enhancement of extracellular matrix synthesis, thereby promoting tissue regeneration and remodeling. Asiaticoside and related triterpenoids also exhibit anti-inflammatory properties that facilitate wound contraction and accelerate the healing process (Diniz et al., 2023).

The presence of these phytochemicals provides a strong biochemical basis for the antibacterial activity observed in this study. The combined effects of membrane disruption, inhibition of essential enzymatic pathways, and interference with nucleic acid synthesis may account for the bacteriostatic and bactericidal activities of the *Centella asiatica* extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Flavonoids and tannins are known to alter membrane permeability and inhibit key metabolic enzymes, whereas saponins and triterpenoids destabilize lipid bilayers, leading to leakage of intracellular components. Such multitarget mechanisms are characteristic of plant-derived secondary metabolites and may reduce the likelihood of rapid resistance development compared with single-compound antibiotics, thereby supporting their potential as alternative or complementary antibacterial agents (Hein et al., 2025; Horváth et al., 2016; Manilal et al., 2023; Zhou et al., 2023).

Antibacterial Activity

The antibacterial activity of *Centella asiatica* ethanol extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was evaluated using the broth microdilution method to determine the MIC and MBC. These two bacterial species were selected because they are commonly associated with wound infections, including postpartum perineal wounds, which are highly susceptible to microbial colonization due to tissue trauma, moisture, and reduced local immunity after childbirth. The results are presented in Tables 2 and 3. In these tables, the symbol (+) indicates visible bacterial growth, whereas (–) indicates no visible growth after incubation

Table 2. Minimum Inhibitory Concentration (MIC) against bacterial growth

Bacteria	M	M+B	Centella asiatica extract				
			25	12.5	6.25	3.12	1.56
Staphylococcus aureus	-	+	-	-	+	+	+
Pseudomonas aeruginosa	-	+	-	-	-	-	+

(-) : No visible growth; (+) visible growth

Table 3. Minimum Bactericidal Concentration (MBC) against bacterial growth

Bacteria	M	M+B	Centella asiatica extract				
			25	12.5	6.25	3.12	1.56
Staphylococcus aureus	-	+	-	+	+	+	+
Pseudomonas aeruginosa	-	+	-	+	+	+	+

(-) : No colony formation; (+) colony formation observed

The results demonstrated that the extract exhibited concentration-dependent inhibitory effects against both bacterial strains. The MIC values were observed at 12.5% for *S. aureus* and 3.12% for *P. aeruginosa*, whereas the MBC for both bacteria was recorded at a concentration of 25%. These findings indicate that the extract possesses both bacteriostatic and bactericidal properties. The lower MIC value observed for *P. aeruginosa* suggests that this bacterium was more susceptible to the extract compared to *S. aureus*.

In the context of postpartum wound infections, the differential susceptibility of the tested bacteria is clinically relevant. *Staphylococcus aureus*, a common skin commensal, readily colonizes disrupted perineal tissue, whereas *Pseudomonas aeruginosa* thrives in moist environments characteristic of postpartum wounds. The ability of *Centella asiatica* ethanol extract to inhibit both Gram-positive and Gram-negative bacteria indicates a broad-spectrum antibacterial effect, which is advantageous for controlling polymicrobial wound infections (Chonsut et al., 2024; Nazilinly et al., 2024).

Phytochemical screening demonstrated that the extract contains flavonoids, tannins, saponins, alkaloids, and triterpenoids. These classes of compounds contribute to antibacterial activity through complementary mechanisms, including disruption of membrane integrity, inhibition of key metabolic enzymes, interference with nucleic acid synthesis, and increased membrane permeability. Such multitarget actions provide a mechanistic explanation for the observed bacteriostatic and bactericidal effects and are consistent with previous reports on the antibacterial properties of *C. asiatica* and related medicinal plants (Hein et al., 2025; Zhou et al., 2023).

The bactericidal effect observed at a concentration of 25% reflects the relatively low potency of crude plant extracts compared with conventional antibiotics, which typically exhibit MIC and MBC values in the μg/mL range. From a chemical–pharmaceutical perspective, this high effective concentration indicates that the active antibacterial constituents are present at low proportions within the crude extract and may act synergistically rather than individually. This finding underscores the importance of fractionation or purification strategies to enhance antibacterial potency and reduce the required therapeutic dose (Diniz et al., 2023).

The increasing prevalence of antibiotic-resistant bacteria further supports the exploration of plant-based antibacterial agents, particularly in maternal health care, where treatment safety is critical. Medicinal plants such as *C. asiatica* offer advantages due to their chemical diversity, multitarget mechanisms of action, and potential for lower adverse effects compared with synthetic antibiotics. The present findings therefore reinforce existing evidence that plant-derived extracts can serve as complementary sources of antibacterial agents for wound management (Salsabila et al., 2024).

Despite these promising results, this research has limitations. The antibacterial activity was evaluated under in vitro conditions, which may not fully replicate the complex physiological environment of postpartum wounds. Factors such as wound exudate composition, local pH, host immune response, and microbiota interactions may influence antibacterial efficacy in vivo. Therefore, further studies involving formulation development, toxicity assessment, and clinical evaluation are necessary before clinical application can be recommended.

Overall, the results of this research provide strong evidence that *Centella asiatica* ethanol extract exhibits significant antibacterial activity against bacteria associated with postpartum wound infections. This supports its potential as a natural antibacterial agent for maternal wound care and highlights its relevance for further development in postpartum infection prevention strategies.

CONCLUSION

This research indicates that the ethanol extract of *Centella asiatica* exhibits measurable antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as demonstrated by MIC values of 12.5% and 3.12%, respectively, and an MBC of 25% for both bacteria. The antibacterial effect is supported by the presence of flavonoids, tannins, saponins, and triterpenoids, which act through multiple biological targets.

These findings provide quantitative evidence of the antibacterial potential of *C. asiatica* against bacteria associated with postpartum wound infections and suggest its possible application as a complementary natural agent in maternal wound care. Further in vivo and formulation-based studies are required to enhance its therapeutic relevance.

RECOMMENDATION

Based on the findings of this research, further investigations are recommended to explore the antibacterial potential of *Centella asiatica* extract in more comprehensive experimental settings. Future studies should include in vivo evaluations to confirm its effectiveness and safety under physiological conditions, as in vitro results may not fully represent the complex biological environment of wound sites.

Additionally, future research should focus on fractionation and characterization of the active constituents to enhance antibacterial potency and reduce the effective concentration required. The development of appropriate topical formulations, such as gels or ointments, is also suggested to improve stability, bioavailability, and practical applicability in postpartum wound management.

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