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In Vitro Antibacterial Effectiveness of Stingless Bee Propolis against Infected Breast Cancer Ulcer

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Abstract: Breast cancer is the most common cancer in women and often presents with malignant ulcers prone to infection. These wounds are difficult to manage, especially during chemotherapy, due to polymicrobial colonization and rising antibiotic resistance. Stingless bee propolis, rich in bioactive flavonoids, has shown promising antibacterial properties. This study aimed to evaluate in vitro antibacterial effectiveness of stingless bee propolis against bacteria from breast cancer ulcers. This was an in vitro study conducted at the Poltekkes Kemenkes Manado Medical Laboratory (July 2024–January 2025). Methanol-extracted stingless bee propolis was tested against MRSA and *P. aeruginosa* using disc diffusion, MIC, and MBC methods. Antibacterial activity was evaluated through inhibition zones and bacterial growth in serial dilutions. The outcomes were analyzed based on inhibition zone diameters and bacterial growth in serial dilutions. The results showed that propolis extract demonstrated antibacterial activity against MRSA and *P. aeruginosa*, with inhibition zones observed in both disc diffusion and dilution methods. Strong inhibition was noted at concentrations ≥80%, while minimal or no effect occurred below 30%. MIC and MBC were estimated at around 20%. Statistical analysis confirmed a significant dose-response relationship (ANOVA, p 0.0003), with stronger correlation in MRSA (R=0.84; p<0.001) than *P. aeruginosa* (R=–0.09; p 0.046). The minimum effective concentration was estimated at 32% for MRSA and 37% for *P. aeruginosa*. In conclusion, stingless bee propolis demonstrated concentration-dependent antibacterial activity against both Gram-positive (MRSA) and Gram-negative (*P. aeruginosa*) bacteria in vitro, with increased activity observed at higher concentrations. Minimum effective concentrations were 32% for MRSA and 37% for *P. aeruginosa*, supporting its potential use in treating infected breast cancer ulcers. These findings highlight propolis as a potential natural alternative for infection control and wound healing.
Keywords: breast cancer; ulcer; stingless bee; propolis; antibacterial effect

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in women, accounting for 22% of all new female cancer cases and representing the leading cause of cancer-related deaths in women globally, contributing to 14% of such mortalities.[1] In 2022, an estimated 2.3 million women were newly diagnosed with breast cancer, resulting in approximately 670,000 deaths worldwide. Notably, there is a striking disparity in disease burden based on levels of human development. In high Human Development Index (HDI) countries, one in twelve women is diagnosed with breast cancer during their lifetime, and one in seventy-one dies from it.[2]

In Indonesia, breast cancer ranks as the second most prevalent cancer and continues to show an increasing trend. According to GLOBOCAN 2020, Indonesia recorded 65,858 new cases and 22,430 deaths due to breast cancer, comprising 30.8% of all cancers in women. Globally, there were 2.26 million cases (11.7% of all cancers) and 684,996 deaths (6.9%), with women disproportionately affected, accounting for 24.5% of new cancer cases and 15.5% of cancer-related deaths.[3] Malignant wounds, such as those associated with breast cancer, result from tumor cell infiltration into surrounding skin tissues. These wounds are often malodorous, exudative, painful, and prone to infection. Diagnosing local infection in such wounds is challenging, especially in patients undergoing chemotherapy, which, while potentially beneficial in tumor reduction, can also impair wound healing and increase susceptibility to infection. The microbial flora in these chronic wounds typically consists of a complex mixture of aerobic and anaerobic organisms originating from skin, adjacent cavities, or the external environment. Common pathogens isolated from breast cancer ulcers include *Staphylococcus aureus* (particularly MRSA), *Pseudomonas aeruginosa*, *Corynebacterium striatum*, and *Proteus mirabilis*, along with anaerobes such as Bacteroides and *Escherichia coli*.[4]

Stingless bees, found in tropical and subtropical regions, produce a variety of biologically active substances including honey, pollen, wax, and propolis. Propolis, a resinous substance with complex chemical composition, has demonstrated anti-inflammatory, antibacterial, and antioxidant properties. These biological activities are largely attributed to its rich content of flavonoids, phenolic acids, and terpenoids. Flavonoids such as pinocembrin, galangin, and pinobanksin have been shown to increase bacterial membrane permeability, reduce membrane potential, and impair RNA polymerase function, thereby limiting bacterial resistance and motility.[5] In Indonesia, antibiotic misuse remains widespread, with a reported 86.1% of individuals using antibiotics without prescription (Riskesdas 2013). This contributes significantly to the rise of antibiotic resistance, underlining the urgent need for alternative antimicrobial therapies derived from natural products. Propolis is a promising candidate due to its broad-spectrum antimicrobial potential and accessibility.[6]

This study aims to evaluate the in vitro antibacterial effectiveness of stingless bee propolis against bacterial pathogens commonly associated with breast cancer ulcers, with a focus on determining its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

METHODS

This was an in vitro experimental study conducted at the Medical Laboratory of Poltekkes Kemenkes Manado, Indonesia, from July 2024 to January 2025. The study aimed to evaluate the antibacterial activity of stingless bee (*Trigona* sp.) propolis against *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, two common pathogens associated with breast cancer ulcers infections. Raw propolis collected from stingless bees in Gowa, South Sulawesi, was extracted using 100% methanol through maceration and concentrated using rotary evaporation. The final extract was standardized based on dry weight.

Clinical isolates of MRSA and *P. aeruginosa* were cultured on Mueller-Hinton Agar (MHA). Antibacterial sensitivity was assessed using the disc diffusion method with varying concentrations of propolis extract (10–100%) and meropenem (10 μg) as the positive control. Inhibition zones

were measured after 24-hour incubation at 37°C.

Serial dilutions of propolis extract were prepared to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). MIC was defined as the lowest concentration inhibiting visible bacterial growth, while MBC was determined by subculturing the contents onto MHA plates to observe bactericidal activity.

The independent variable was propolis concentration; the dependent variables were inhibition zone diameter, MIC, and MBC values. Results were recorded as mean inhibition zone diameters and presented in tabular format. Antibacterial strength was categorized as strong (>8 mm), moderate (4–8 mm), or weak (<4 mm) based on inhibition zones. All experimental procedures were approved by the institutional ethics committee.

RESULTS

The disc diffusion assay demonstrated that stingless bee (*Trigona* sp.) propolis has inhibitory effects against both MRSA and *Pseudomonas aeruginosa*. Table 1 showed that at 100% concentration, the extract produced inhibition zones of 9.0 0.15 mm for MRSA and 8.0±0.09 mm for *P. aeruginosa*. In contrast, the control antibiotic, meropenem (10 µg), generated significantly larger inhibition zones (MRSA: 19±0.05 mm; *P. aeruginosa*: 16±0.05 mm). The inhibition diameter decreased as the propolis concentration decreased. For MRSA, moderate inhibition (7 mm) was observed at 90%, and weak inhibition (1 mm) at 30%. For *P. aeruginosa*, inhibition was still observed down to 60% concentration, with no effect at concentrations below 20%.

In the dilution assay, propolis extract showed a concentration-dependent bacteriostatic and bactericidal effect. MIC and MBC values for both bacteria were estimated to be around 20%, with no visible growth at this concentration.

Table 1. Propolis concentration and average diameter of inhibition zone along with bacterial growth inhibition response

Indicator bacteria	Concentration of propolis methanolic extract (%)	Average inhibition zone diameter (mm)	Growth inhibition response	Bactericidal effect
MRSA	100	9+0.15	Strong	+
	90	7+0.25	Moderate	+
	80	4+0.18	Moderate	+
Meropenem	10ug	19+0.05	Very strong	+
P. aeruginosa	100	8+0.0.9	Strong	+
	90	7+0.05	Moderate	+
	80	6+0.10	Moderate	+
Meropenem	10ug	16+0.05	Very strong	+
MRSA	70	4+0.43	Moderate	+
	60	3+0.21	Weak	+
	50	2+0.11	Weak	+
P. aeruginosa	70	5+0.27	Moderate	+
	60	4+0.19	Moderate	+
	50	3+0.22	Weak	+
	40	2+0.08	Weak	+
MRSA	40	2+0.05	Weak	+
	30	1+0.10	Weak	+
	20	0	No inhibition	-
	10	0	No inhibition	-
P. *aeruginosa*	30	2+0.12	Weak	+
	20	0	No inhibition	-
	10	0	No inhibition	-

(+) = No bacterial growth, (-) = There was bacterial growth

Turbidity assessments and subculturing confirmed bactericidal activity at higher concentrations, especially ≥70%. Based on inhibition zone categories, propolis at 100% exhibited strong antibacterial activity; at 90–70% it showed moderate effects, and below 40% it was weak or inactive (Figure 1).

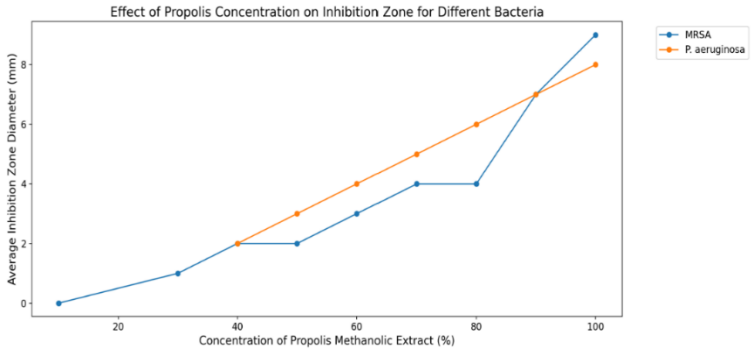

Figure 1. Effect of propolis concentration on inhibition zone for MRSA and *P. aeruginosa*

Statistical analysis One-way ANOVA showed that extract concentration significantly influenced inhibition zone diameter for both bacterial species (p=0.0003). Although *P. aeruginosa* exhibited a slightly greater mean inhibition zone (5.00 mm) than MRSA (3.56 mm), this difference was not statistically significant (p=0.288; T-test).

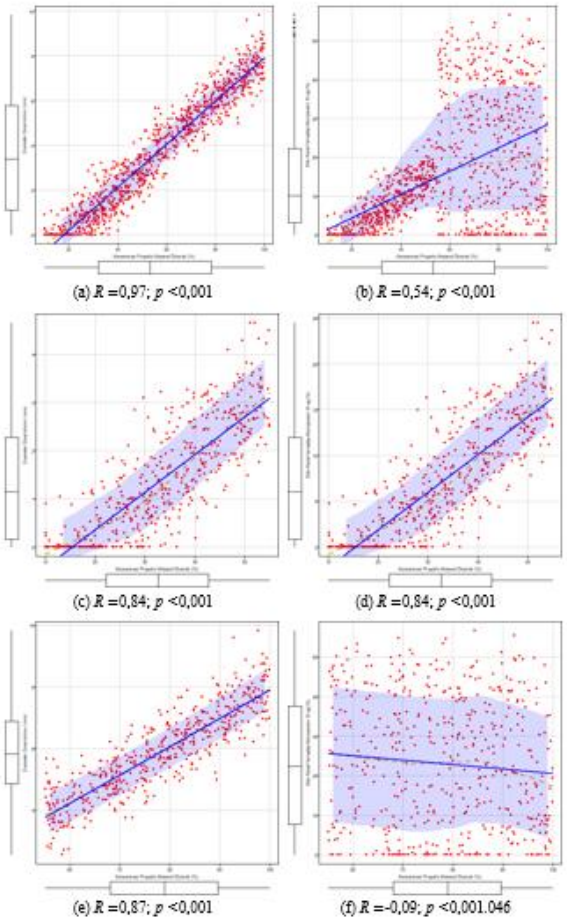

Figure 2. Corelation between propolis concentration, inhibition zone diameter, and relative antibacterial effectiveness based on comparison with meropenem inhibition zone diameter. R is Pearson's correlation coefficient. (N = 1000 replicates)

To further explore the relationship between propolis concentration and antibacterial activity, inhibition data were expanded through simulated replication (n=1000). A strong positive correlation was observed for MRSA (R=0.84; p<0.001), whereas *P. aeruginosa* showed a weak and inverse correlation (R=–0.09; p=0.046). The minimal effective concentration, defined by both absolute inhibition zone ≥1 mm and relative inhibition ≥5% compared to meropenem, was determined to be 32% for MRSA and 37% for *P. aeruginosa* (Figure 2).

DISCUSSION

This study demonstrates that the methanolic extract of stingless bee (*Trigona* sp.) propolis exhibits promising antibacterial activity against MRSA and *Pseudomonas aeruginosa*, the two predominant pathogens involved in infected breast cancer ulcers. The observed effects were concentration-dependent, with strong inhibition at ≥70% and diminished activity below 30%, confirming the dose-related efficacy of the extract.

The antibacterial effect is largely attributed to the presence of bioactive compounds in propolis, especially flavonoids (e.g., pinocembrin, galangin, quercetin, apigenin) and phenolic acids. These compounds disrupt bacterial cell walls, inhibit topoisomerases and ATPase activity, impair nucleic acid synthesis, and enhance membrane permeability, collectively weakening bacterial defenses.[1-3] The greater sensitivity of MRSA at higher concentrations and moderate susceptibility of *P. aeruginosa* align with earlier observations that propolis tends to be more effective against Gram-positive than Gram-negative bacteria due to outer membrane-related resistance mechanisms.[4]

Beyond its antibacterial effect, propolis also exerts important immunomodulatory and wound-healing actions. It modulates proinflammatory mediators such as TNF-α by inhibiting the NF-κB pathway, and enhances anti-inflammatory cytokines like IL-10, facilitating a controlled inflammatory response. Additionally, it promotes angiogenesis through upregulation of VEGF, granulation tissue formation, and collagen synthesis—critical processes in wound healing.[5,6] These properties are particularly valuable in managing chronic malignant wounds, where infection control and tissue repair must occur simultaneously.

The results of this study are consistent with in vitro evaluations from prior literature, in which flavonoids such as apigenin, kaempferol, and quercetin demonstrated activity against both Gram-positive and Gram-negative organisms, including resistant strains such as MRSA and *Enterobacter cloacae*. Notably, some flavonoids also exhibit synergistic effects when combined with conventional antibiotics, potentially enhancing therapeutic outcomes.[7-9]

The minimum inhibitory (MIC) and bactericidal concentrations (MBC) identified (~20%) provide a benchmark for further formulation development. While effective at these concentrations, the cytotoxicity and tolerability at the tissue level must be evaluated before clinical application. Given its broad-spectrum activity, anti-inflammatory potential, and natural origin, stingless bee propolis represents a compelling alternative for adjunctive therapy in ulcerative breast cancer infections, particularly where antibiotic resistance complicates treatment.[10]

However, this study has limitations. First, complete phytochemical profiling was not conducted, preventing quantification of specific bioactive compounds. Second, only qualitative flavonoid identification was performed, and the physiological activity of individual compounds may differ significantly. Third, toxicity and safety profiles of high-concentration propolis were not assessed in vivo or in cell-based assays. Therefore, these aspects should be addressed in future studies.

CONCLUSION

Stingless bee (*Trigona* sp.) propolis exhibits in vitro antibacterial activity against both Methicillin-resistant Staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa*, two key pathogens commonly associated with infected breast cancer ulcers. The antibacterial effect was shown to be concentration-dependent, with significant inhibition observed at concentrations above 30%, and optimal activity at 70% or higher. Minimum inhibitory concentrations were estimated at

32% for MRSA and 37% for *Pseudomonas*. *aeruginosa*. These results suggest that stingless bee propolis has potential as a natural antibacterial agent for managing malignant wound infections.

Future studies are recommended to investigate the safety, cytotoxicity, and clinical applicability of propolis in vivo, particularly when used at higher concentrations in the treatment of ulcerated breast carcinomas.

Conflict of Interest

The authors have nothing to declare.

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