

Phytochemical Composition, and Antioxidant Activity of Stingless Bee (*Heterotrigona Itama*) Propolis for Wound Healing Potentials.

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ABSTRACT

Background: Propolis from stingless bees *Heterotrigona itama*(*H.itama*) is a promising natural product with diverse biomedical applications. However, its bioactivity and antioxidant for wound healing remain poorly understood. This study aimed to evaluate phytochemical profile, and antioxidant activity of *H. itama* propolis collected from land forest in Kalimantan, Indonesia.

Methods: Qualitative phytochemical screening was performed to identify secondary metabolites, and antioxidant capacity was assessed using the DPPH radical scavenging assay with ascorbic acid as a reference standard. The result of the phytochemical content and antioxidant activity will be compared with the literature study on wound healing.

Results: Phytochemical tests showed the presence of flavonoids, saponins, tannins, phenolics, triterpenoids, terpenoids, and DPPH assay revealed antioxidant activity with an IC₅₀ value of 159.91 ppm compared to 2.71 ppm for ascorbic acid.

Conclusion: *H. itama* propolis biologically active, demonstrating antioxidant potential linked to its phenolic and flavonoid content. These findings support that phytochemical content and antioxidant activity are potentials for wound healing.

INTRODUCTION

Propolis, a resinous substance that bees get from plant exudates and mix with wax and salivary enzymes, is well-known for its many biological properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Salleh et al., 2021; Al-hatamleh et al., 2020). Stingless bee propolis, particularly that produced by *Heterotrigona itama*(*H.itama*), has attracted growing scientific attention due to its high content of bioactive compounds such as flavonoids, phenolics, terpenoids, and triterpenoids, which contribute to its therapeutic potential (Pratami et al., 2024; Balasopoulou et al., 2017). The chemical composition of propolis varies considerably depending on the plant sources available to the bees and the local environmental conditions, resulting in unique regional profiles (Rocha et al., 2023; Zuhendri et al., 2022)

This study aims to phytochemical content and antioxidant activity in stingless bee propolis forest land in Kalimantan; identify its phytochemical constituents through qualitative screening; and evaluate its antioxidant activity. The findings provide new evidence for chemical profile, and biomedical potential of propolis for wound healing derived from environmentally challenged ecosystems, contributing to sustainable natural product utilization and environmental bioprospecting in forest landscapes.

Propolis is rich in natural antioxidants that play an essential role in neutralizing reactive oxygen species (ROS), thereby reducing oxidative stress and preventing chronic degenerative diseases (Alsarayrah et al., 2025; Rozman et al., 2022). The antioxidant potential of propolis is largely attributed to its phenolic and flavonoid content, which may also reflect the resilience of local flora in disturbed or rehabilitated ecosystems (Salleh et al., 2021; Sanches et al., 2017).

Recent research on propolis and wound healing is rapidly developing. A recent meta-analysis (Machado Velho et al., 2023) summarizing numerous studies reported that propolis consistently increases the percentage of skin wound healing compared to conventional therapy (Machado Velho et al., 2023). A study by El-Sakhawy et al. (2024) also showed that propolis (as wound films and mesh scaffolds) can function as wound dressings due to its antimicrobial and anti-inflammatory properties (El-Sakhawy et al., 2024). *H. itama* propolis are rich in phenolic and terpenoid compounds that trigger high antioxidant activity (Jusril et al., 2025).

Several previous studies have examined the efficacy of propolis (both from *H. itama* and other species) in wound healing and fibroblast cell activity. Jusril et al. (2025) concluded that Malaysian *H. itama* propolis has antioxidant, antibacterial, and anti-inflammatory effects, and can accelerate wound healing. Research by Jongjitaree et al. (2022) found that propolis extract significantly stimulated the migration and proliferation of gingival fibroblast cells. These results are in line with Jacob et al. (2015), who reported that Malaysian propolis stimulates the rate of migration and proliferation of skin fibroblast cells at certain optimum concentrations (Jacob et al., 2015). Mechanistically, propolis components such as flavonoids and phenolic acids are known to suppress the activity of free radicals and proinflammatory cytokines, thereby supporting the epithelialization process in wounds (El-Sakhawy et al., 2024). Oryan et al., 2018a also revealed that propolis inhibits the formation of pathogenic bacterial biofilms and accelerates wound healing in animal models. The results of *H. itama* cultivation in East Kalimantan showed the ability of propolis to inhibit bacterial growth (Sarah Azzara Dikarulin, 2022). The results of this study provide a strong indication that propolis is a therapeutic agent in wound care by stimulating the tissue repair process, especially through its effects on fibroblast proliferation and migration.

METHODS

Propolis Collection and Extraction

Stingless bee (*H. itama*) propolis was collected at a stingless bee farm in Bukit Soeharto Forest, Kutai Kartanegara District, East Kalimantan, Indonesia (117°00'44.2"-1°10'33.6"). The bees were identified at the Forest Cultivation Laboratory, Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia. Propolis was extracted using 96% ethanol to create an ethanolic extract. After being broken up into tiny pieces, propolis was screened. making ethanol as a solvent. Ethanol and propolis were combined, agitated, and allowed to fully dissolve. The liquid was then filtered to eliminate any remaining particles. Distillation is then used to separate the mixture's ethanol and water. After being separated, the propolis extract was kept in an airtight container (Bankova et al., 2021; Basyirah Md Zin et al., 2018).




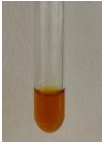

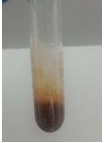








Phytochemical Screening



Qualitative phytochemical assays were performed to identify the major secondary metabolites present in the *H. itama* propolis extract. The tests included the foam test for saponins (El Aziz, M.M.A., Ashour, A.S. and Melad, 2019), Wagner's and Mayer's reagents for alkaloids (Oscar et al., 2020), the

Shinoda test using magnesium and hydrochloric acid for flavonoids (Sankhalkar & Vernekar, 2016), the ferric chloride test for phenolic compounds (Shaikh & Patil, 2020), and the hot water extraction followed by ferric chloride reaction for tannins (Rao et al., 2023). Steroids and terpenoids were detected using the Liebermann–Burchard reaction (S. K. Malik, 2023), while triterpenoids were identified by the Salkowski's test (Rajkumar et al., 2022). The appearance of characteristic color changes or precipitates was recorded as a positive indication for each class of compound.

Qualitative phytochemical analysis of propolis extract showed the presence of flavonoids, phenolics, tannins, saponins, terpenoids, and triterpenoids, while alkaloids and steroids were absent (Table 1).

Table 1: Qualitative Phytochemical Screening of Propolis Extract (*H.itama*) from land forest in Bukit Soeharto

Phytochemical class	Test performed/ Positive Indication	Before	After	Result
Saponins	Foam test; Foam is formed			+
Alkaloids	Wagner's and Mayer's test; Reddish brown precipitate			-
Flavonoids	Shinoda test; forms a red, orange, or pink color			+
Phenolics	Ferric chloride test; The solution is dark green / bluish black / purplis			+
Tannins	Ferric chloride test; Blackish blue/blackish green solution			+
Terpenoids	Salkowski's test; Forms A golden yellow layer			+
Triterpenoids	Liebermann–Burchard test; forms a reddish brown color			+

Steroid	Liebermann–Burchard test; Steroid: Forms a purplish green/brown color			–
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Antioxidant Activity

The antioxidant potential of propolis extracts was evaluated using the DPPH radical scavenging method. Briefly, propolis extracts were prepared in methanol at serial concentrations ranging from 0.78 to 500 ppm, depending on the sample. Each sample was mixed with 0.1 mM DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage inhibition was calculated, and IC₅₀ values (concentration required to inhibit 50% of DPPH radicals) were determined by nonlinear regression (four-parameter logistic model). Ascorbic acid served as the positive control (Gulcin & Alwasel, 2023), (Tang et al., 2020).

The extract from land forest Bukit Soeharto demonstrated dose-dependent DPPH radical scavenging activity with an IC₅₀ of 159.91 ppm, which was substantially weaker than the positive control ascorbic acid (IC₅₀ = 2.71 ppm). At the highest tested concentration (500 ppm), the propolis extract produced 69.16 ± 0.23% inhibition of DPPH radicals. The dose response relationship between concentration and radical scavenging activity for the propolis extract.

DISCUSSION

This study provides the first integrated assessment phytochemical profile, and antioxidant potential of *H. itama* propolis forest land in Kalimantan, Indonesia. The results demonstrate that the analyzed propolis samples maintain a diverse phytochemical composition and notable antioxidant activity.

Phytochemical Profile and Bioactive Constituents

Qualitative screening revealed the presence of saponins, flavonoids, phenolics, tannins, terpenoids, and triterpenoids, while alkaloids and steroids were absent. This pattern is consistent with previous reports on *H. itama* propolis from Malaysia and Indonesia, which highlight polyphenols and terpenoids as the dominant bioactive groups (Salleh et al., 2021). Flavonoids and phenolic compounds are well known for their antioxidant potential through free-radical scavenging and metal-chelating mechanisms, whereas terpenoids and triterpenoids contribute to antimicrobial and anti-inflammatory activities (Alsarayrah et al., 2025). The observed chemical profile indicates that bees can access diverse botanical exudates rich in secondary metabolites, reflecting the resilience and regenerative capacity of local vegetation (Withaningsih et al., 2023).

Antioxidant Activity and Biological Significance

The DPPH radical-scavenging assay demonstrated that propolis exhibited concentration-dependent antioxidant activity, classified as strong according to commonly used benchmarks (< 50 ppm) (Conte et al., 2022). The propolis IC₅₀ obtained here is similar to values reported for *H. itama* propolis from non-mining regions of Malaysia and higher than those from temperate bee species (Salleh et al., 2021; Mohan et al., 2024). This strong activity can be attributed to the presence of phenolics and flavonoids, which act as hydrogen-donating antioxidants, stabilizing reactive oxygen species and preventing oxidative damage (Hassanpour & Doroudi, 2023). The antioxidant potential observed also provides indirect evidence of the ecological health of post-mining landscapes (Salazar et al., 2024). Rehabilitated vegetation supplying resins to stingless bees may retain rich phytochemical diversity, supporting the production of bioactive propolis with pharmacological value (Pant, et al., 2024). Similar

correlations between botanical recovery and propolis quality have been noted in studies from Southeast Asia (Pratami et al., 2024).

Wound Healing Implications

Forestry Faculty of Forestry, Mulawarman University showed that *H.itama* propolis extract has inhibitory concentration (IC) or inhibitory concentration with IC₅₀ value of 112.53 µg/ml and IC₅₀ 165.382 µg/ml respectively so that it can inhibit the growth of staphylococcus epidermidis and Cutibacterium acnes bacteria. Other research on the content of *H.itama* cultivated in rice fields, secondary forests and urban areas that have been studied in East Kalimantan has antibacterial ability (Rusman & Arung, 2023; Sarah Azzara Dikarulin et al., 2022).

The antioxidant and antibacterial properties of propolis play a role in the wound healing process. This process is a complex biological response to tissue damage, involving dynamic interactions between cells, chemical mediators, and the extracellular matrix (Gonzalez et al., 2016). Disruption in any of the healing phases can lead to chronic wounds, which significantly impact quality of life and healthcare costs (Balasopoulou et al., 2017).

Challenges in wound management, particularly the wound healing process, include bacterial infection, prolonged inflammation, and limitations in tissue regeneration (Gonzalez et al., 2016; Powers et al., 2016; Choudhary et al., 2014). Therefore, the development of effective, safe, and natural resource-based alternative therapies is highly relevant.

A recent meta-analysis (Machado Velho et al., 2023) summarizing numerous studies reported that propolis consistently increases the rate of skin wound healing compared to conventional therapy (Machado Velho et al., 2023). A study by El-Sakhawy et al. (2024) also showed that propolis (as wound films and mesh scaffolds) can function as wound dressings due to its antimicrobial and anti-inflammatory properties. Phytochemically, Jusril et al. (2025) confirmed that *H. itama* propolis is rich in phenolic and terpenoid compounds that trigger high antioxidant activity.

Several previous studies have examined the efficacy of propolis (from both *H. itama* and other species) in wound healing and fibroblast cell activity. Jusril et al. (2025) concluded that *H. itama* Malaysian propolis has antioxidant, antibacterial, and anti-inflammatory effects, and can accelerate wound healing. Research by Jongjitaree et al. (2022) found that propolis extract significantly stimulated the migration and proliferation of gingival fibroblast cells. These results are in line with Jacob et al. (2015), who reported that Malaysian propolis stimulates the rate of migration and proliferation of skin fibroblast cells at certain optimum concentrations. Mechanistically, propolis components such as flavonoids and phenolic acids are known to suppress the activity of free radicals and pro-inflammatory cytokines, thereby supporting the epithelialization process in wounds (El-Sakhawy et al., 2024). Oryan et al. (2018) also revealed that propolis inhibits the formation of pathogenic bacterial biofilms and accelerates wound healing in animal models (Oryan et al., 2018b). The results of *H. itama* cultivation in East Kalimantan showed the ability of propolis to inhibit bacterial growth (Sarah Azzara Dikarulin, 2022). The results of this study provide a strong indication that propolis is a therapeutic agent in wound care by stimulating the tissue repair process, especially through its effects on fibroblast proliferation and migration.

The wound healing process goes through the stages of hemostasis, inflammation, proliferation, and remodeling. During the proliferation stage, fibroblast migration occurs. Fibroblast migration is an important and crucial process because it stimulates the formation of myofibroblasts. Myofibroblasts play a role in stimulating collagen matrix formation, angiogenesis, wound contraction, and wound closure (Singh et al., 2017; Plikus et al., 2021).

Environmental and Economic Implications

The combination of plow phytochemical content and antioxidant potential in *H. itama* propolis suggests that forest lands can sustainable apiculture (Harianja et al., 2023). Beekeeping in such areas may not only generate income for local communities but also contribute to ecological by promoting pollination and biodiversity restoration (Buchori et al., 2022).

Limitations and Future Directions

While the present study establishes baseline safety and bioactivity data, it is limited to in vitro antioxidant analysis and qualitative phytochemical tests. Further work employing chromatographic and spectrometric profiling would provide deeper insight into specific bioactive compounds. Additionally, assessment of other biological activities, such as antimicrobial or anti-inflammatory effects, along with in vivo toxicity evaluations would strengthen the biomedical relevance of this propolis. Long-term environmental monitoring in post-mining apiaries could also clarify potential variations in heavy metal uptake related to land rehabilitation practices.

CONCLUSION

H. itama propolis biologically active including flavonoids, saponins, tannins, phenolics, triterpenoids, terpenoids and demonstrating antioxidant potential linked to its phenolic and flavonoid content. These findings support that phytochemical content and antioxidant activity are potentials for wound healing.

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CONFLICTS OF INTEREST

No conflict of interest was found during the research

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