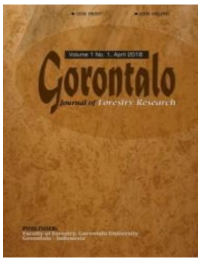

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THE POTENTIAL OF *Heterotrigona itama* BEE BREAD IN INHIBITING THE GROWTH OF PATHOGENIC BACTERIA

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ABSTRACT

Bacterial infections remain a major problem for Indonesians. Bacterial resistance caused by inappropriate antibiotic use is a major factor in the prevalence of bacterial infections in the community. Therefore, the use of natural ingredients is needed as an alternative to natural antibiotics. One natural ingredient with antibacterial potential is bee bread, produced by the stingless bee species *Heterotrigona itama.* The aim of this study was to determine the antibacterial activity contained in *Heterotrigona itama* bee bread extract in fighting pathogenic bacteria. The test used the well method. The growth medium used was Mueller Hinton Agar (MHA) with a 24-hour incubation time. The concentrations used were 500, 250, and 125 μg/well. The percentage and inhibition zone of *Heterotrigona itama* bee bread extract varied among bacterial species. Based on the diameter of the inhibition zone at a concentration of 500 μg/well, inhibition of gram-positive bacteria tended to be greater than that of gram-negative bacteria. Bee bread produced by *Heterotrigona itama* bees has inhibitory activity against five types of pathogenic bacteria. The results also provide opportunities for further research, including the formulation and production of applicable products.

Keywords: Antibacteria**;** Bee Bread; *Heterotrigona itama*; Pathogenic Bacteria; East Kalimantan

INTRODUCTION

Infection is a disease caused by the entry and proliferation of microscopic organisms such as fungi, bacteria, parasites, and viruses, which damage the host's body, resulting in disease symptoms (Mandell et al., 2010). Bacterial infections are a major source of infectious diseases in Indonesia. Several key factors contributing to the massive proliferation of these bacteria include geographic conditions, climate conditions, population density, and community habits. Indonesia faces significant challenges in addressing bacterial infections in the community. This is due to the widespread use of inappropriate antibacterial doses due to poor drug oversight (Rahmadi et al., 2024). One of the most commonly used classes of antibacterial agents is antibiotics. Antibiotics are chemical agents produced by microorganisms or synthesized for therapeutic purposes that inhibit bacterial growth or cause bacterial death (Halawa et al., 2024). Several natural products are known to contain natural antibacterial properties that can combat resistant pathogens (Rossiter et al., 2017).

The stingless bee, *Heterotrigona itama*, is better known as the kelulut in East Kalimantan and is popular among beekeepers due to its health and environmental benefits (Priawandiputra et al., 2020). Beebread is a natural product produced by this bee. Beebread is derived from flower pollen collected by worker bees and carried to the hive. The pollen then mixes with saliva and digestive enzymes, a small amount of honey, and nectar, which ferments in the hive (Didaras et al., 2021).

Bee bread contains bioactive compounds, including phenolic compounds. These phenolic compounds play a role in health. Phenolic compounds such as phenolic acids, flavonoids, and proanthocyanidins have antioxidant and anti-inflammatory properties that are beneficial for reducing the risk of chronic diseases (Zhang et al., 2016). Ethanol extract of *H. itama* bee bread from Malaysia has inhibitory activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhi* (Akhir et al., 2018).

Although several studies have reported the antibacterial activity of bee bread produced by stingless bees, scientific evidence focusing on *Heterotrigona itama* bee bread from different geographical regions remains limited. Most previous studies investigated samples from Malaysia and other regions, while data from eastern Indonesia, particularly East Kalimantan, are still scarce. This study presents novel evidence on the antibacterial potential of *H. itama* bee bread cultivated in Mugirejo Village, East Kalimantan, Indonesia, against a combination of oral, skin, and foodborne pathogenic bacteria. Furthermore, this research provides comparative inhibition data across multiple bacterial groups, highlighting differential sensitivity between Gram-positive and Gram-negative bacteria. Therefore, this study contributes new regional and biological insights into the antibacterial properties of *H. itama* bee bread and supports its potential development as a locally sourced natural antibacterial agent.

RESEARCH METHOD

Bee bread samples were obtained from Mugirejo Village, Samarinda, East Kalimantan, Indonesia. The obtained bee bread was separated from the resin pot. Maceration was carried out using 96%

Ethanol solvent for 3x24 hours. After that, the filtrate and residue were separated. The filtrate concentration process was carried out using a Rotary Vacuum Evaporator machine to obtain a dry extract. A total of 25 mg of *Heterotrigona itama* bee bread extract was dissolved in 1 mL of DMSO (dimethyl sulfoxide), so that a stock concentration of 25,000 ppm was obtained. The stock concentration was reduced so that there were 4 test concentrations, namely 25,000, 12,500, 6,250, and 3,125 ppm. The final concentrations in each well were 500, 250, 125, and 62.5 ppm.

Antibacterial assay was conducted using the well diffusion method, referring to the method of Poelongan et al. (2006) with modifications. The well method is known to be more effective in inhibiting bacteria than the disc method (Nurhayati et al., 2020). Agar diffusion in this method of assay is measured by observing the penetration of the diffusing substance into the specimen being studied (Turunen, 1975). The bacterial growth medium was Mueller Hinton Agar (MHA) dissolved in distilled water. The media dissolution process was carried out on a heater until homogeneous. The equipment was sterilized using an autoclave at 121˚C for 15 minutes. A total of 15 mL of agar media was poured into a petri dish and waited until solid.

The bacterial suspension used the McFarland standard by comparing the turbidity level between the test colony suspension and the 0.5 McFarland standard or approximately 1.5 x 108 CFU/mL. The standardized bacterial suspension was then inoculated into the test medium in a zigzag pattern and evenly distributed over the entire surface. The wells were made using a cork borer. 20µl of the test extract was inserted into each well. Incubation was carried out for 18-24 hours at 37°C. The inhibition zone was measured by the clear diameter in 3 measuring directions (**Figure 1**).

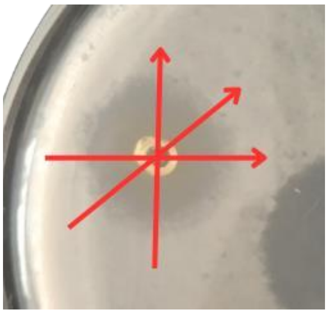

Figure 1. Measuring directions

RESULTS AND DISCUSSION

The antibacterial activity of bee bread *Heterotrigona itama* cultivated in the Mugirejo Village area produced different levels of inhibition for each bacterium. The results of the percentage test of bee bread ethanol extract against five pathogenic bacteria, namely *Streptococcus mutans*, *Streptococcus sobrinus*, *Cutibacterium acne*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Salmonella typhi* at 3 series of concentrations (500, 250, and 125 µg/well) when compared with the positive control chloramphenicol we used the concentration of 10g/20L in each well (Kusuma et al., 2014) (Table 1).

Chloramphenicol is used as a positive control, because this type of antibiotic is able to inhibit bacteria in a broad spectrum, where this substance is able to inhibit gram-positive and gram-negative bacteria (Pratiwi, 2008).

Table 1. **Inhibition activity of pathogenic bacteria by ethanol extract of bee bread *Heterotrigona itama***

Concentration (*µg/well*)	Inhibition Percentage (%)					
	S. mutans	*S. sobrinus*	*C. acne*	*S. epidermidis*	*E. coli*	*S. typhi*
500	43.14	48.59	46.86	50.00	33.83	36.32
250	37.26	35.29	35.27	42.16	18.91	27.63
125	17.16	18.63	13.04	20.59	7.46	11.19
+	100	100	100	100	100	100
Concentration (*µg/well*)	Inhibition Zone Diameter (mm)					
	S. mutans	*S. sobrinus*	*C. acne*	*S. epidermidis*	*E. coli*	*S. typhi*
500	9.8±0.69	10.3±0.88	10.8±0.88	11.3±3.21	7.6±0.51	8.1±0.69
250	8.4±1,00	8.0±1,00	8.1±0.57	9.6±3.37	4.2±3.67	6.1±0.19
125	3.9±3.32	4.2±0.47	3.0±3.46	4.7±4.22	1.7±2.89	2.5±2.87
+	22.7±0.58	22.7±5.77	23±0,00	22.7±0.58	22.3±0.83	22.3±1.76

Bee bread extract of *H. itama* can inhibit the growth of all bacteria at almost all concentrations. The highest percentage of inhibition was found at a concentration of 500 µg/well. The inhibition of *S. epidrmidis* bacteria (50%), followed by *S. sobrinus* bacteria (48.59%), *C. acne* (46.86%), *S. mutans* (43.14%), *S. typhi* (36.32%), and *E. coli* (33.83%). There is bacterial inhibitory activity by bee bread extract against all types of pathogenic bacteria from a concentration of 500-125 µg/well. *S. epidermidis* and *C. acne* bacteria are bacteria located on the surface of the skin, especially facial skin which can cause skin problems such as acne in an unbalanced state (Dréno et al., 2017). *S. mutans* and *S. sobrinus* are the most common bacterial species found in the oral cavity associated with dental caries (Nurelhuda, et al., 2010). *S. typhi* and *E. coli* are bacteria that usually contaminate food (Nindi, et al., 2021).

Based on the diameter of the bacterial inhibition zone, at a concentration of 500 µg/well, inhibition in *S. epidrmidis*, *S. sobrinus*, and *C. acne* bacteria is classified as a strong inhibition category. At a concentration of 250 µg/well, the antibacterial ability of the extract is classified as moderate, and at a concentration of 125 µg/well, the ability is classified as weak. While inhibition in *S. mutans*, *E. coli*, and *S. typhi* bacteria is included in the moderate category at a concentration of 500 µg/well. and at concentrations of 250 and 125 μg/well it has weak inhibition. This grouping is based on the theory by Davis & Stout (1971), which states that the diameter of the inhibition zone <5 mm has weak inhibition, the inhibition zone with a range of 5-10 mm has moderate bacterial inhibition, 10-20 mm has strong bacterial inhibition, and for the diameter of inhibition > 20 mm has very strong inhibition.

Chloramphenicol was used as a positive control in antibacterial assay because it is a type of antibiotic that can inhibit protein synthesis in bacteria (Oong & Tadi, 2023). In this test, chloramphenicol had very strong inhibitory power on all types of bacteria with an inhibition zone diameter of >20 mm.

The inhibition percentages for *E. coli* and *S. typhi* were relatively low compared to the inhibition percentages for the other four types of bacteria. Both of these bacteria are gram-negative bacteria. Gram-negative bacteria have an outer membrane rich in lipopolysaccharides, making it difficult for bioactive compounds in the extract to penetrate the outer membrane of these bacteria (Nazzaro et al., 2013). This is in contrast to gram-positive bacteria, which in this observation were represented by *S. mutans*, *S. epidermidis*, *S. sobrinus*, and *C. acne*, which had a higher inhibition percentage. This is due to their thin outer membrane layer, which makes these bacterial species more susceptible to extracts or essential oils (Olajuyigbe et al., 2018). This phenomenon is why the same extract produced different levels of bacterial inhibition and the strength of the extract for each type of bacteria. The flavonoid group of phenolic compounds in bee bread also plays a role in antibacterial activity (Sari et al., 2024).

The antibacterial activity observed in *H. itama* bee bread may be attributed to the presence of various bioactive compounds, including phenolic acids, flavonoids, and organic acids, which have been widely reported in bee products. Previous studies have shown that phenolic compounds exert antibacterial effects by disrupting bacterial cell membranes, inhibiting nucleic acid synthesis, and interfering with energy metabolism (Daglia, 2012; Cushnie & Lamb, 2011). Variations in antibacterial potency among bee bread samples are strongly influenced by botanical origin, geographic location, and fermentation processes occurring within the hive (Othman et al., 2019; Didaras et al., 2021). Therefore, the antibacterial activity of bee bread from East Kalimantan observed in this study may reflect unique environmental and floral characteristics of the region, which could influence its bioactive compound profile.

The findings of this study contribute to the growing body of knowledge on stingless bee products by demonstrating that *Heterotrigona itama* bee bread from East Kalimantan exhibits measurable antibacterial activity against clinically relevant pathogenic bacteria. The observed higher susceptibility of Gram-positive bacteria compared to Gram-negative bacteria reinforces existing theories regarding bacterial cell wall permeability and bioactive compound diffusion. Importantly, this study expands current literature by providing region-specific antibacterial data and highlighting the potential application of bee bread as a natural antibacterial candidate for oral and topical formulations. These results may serve as a scientific basis for future studies focusing on compound isolation, formulation development, and in vivo evaluation of bee bread-derived antibacterial agents.

The results clearly demonstrate that the ethanol extract of *Heterotrigona itama* bee bread exhibits differential antibacterial activity across tested bacterial species. At the highest concentration (500 µg/well), the inhibition zones against Gram-positive bacteria (*Staphylococcus epidermidis*, *Streptococcus sobrinus*, *Cutibacterium acne*, and *Streptococcus mutans*) were consistently larger than those observed for Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). This pattern suggests that the antibacterial efficacy of bee bread extract is strongly influenced by bacterial cell wall structure.

Gram-positive bacteria possess a thick peptidoglycan layer without an outer lipopolysaccharide membrane, allowing easier penetration of phenolic and flavonoid compounds present in bee bread. In contrast, Gram-negative bacteria contain an outer membrane rich in lipopolysaccharides that acts as a permeability barrier, limiting diffusion of hydrophobic bioactive compounds and reducing antibacterial effectiveness (Nazzaro et al., 2013; Halawa et al., 2024).

These findings are consistent with recent reports indicating that natural products derived from bee products, including bee bread and propolis, generally exhibit stronger antibacterial activity against Gram-positive bacteria than Gram-negative strains (Didaras et al., 2021; Sari et al., 2024).

A clear concentration-dependent inhibition pattern was observed across all tested bacteria. Increasing extract concentration from 125 to 500 µg/well resulted in a proportional increase in both inhibition zone diameter and percentage inhibition. This dose–response relationship confirms that the antibacterial activity of *H. itama* bee bread is not incidental but reflects genuine biological activity of its bioactive constituents.

Similar dose-dependent antibacterial trends have been reported for bee bread extracts from different geographic regions, emphasizing the importance of extract concentration in determining antibacterial potency (Othman et al., 2019; Didaras et al., 2021). The moderate to strong inhibition categories observed at higher concentrations indicate that bee bread extract could be further optimized through purification or formulation processes to enhance its antimicrobial effectiveness.

The antibacterial activity observed in this study is likely associated with the high content of phenolic compounds and flavonoids present in bee bread. Phenolic acids and flavonoids are known to exert antibacterial effects through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, and interference with bacterial energy metabolism (Cushnie & Lamb, 2011; Daglia, 2012).

Recent studies on *H. itama* bee bread from Southeast Asia reported the presence of quercetin, kaempferol derivatives, caffeic acid, and ferulic acid, all of which have been associated with antibacterial and antioxidant activities (Sari et al., 2024). The fermentation process occurring inside the hive may further enhance the bioavailability and antibacterial potency of these compounds by converting pollen-bound polyphenols into more active forms (Didaras et al., 2021).

One of the significant contributions of this study lies in its regional specificity. Bee bread samples analyzed in this research originated from Mugirejo Village, East Kalimantan, an area with distinct floral diversity and climatic conditions. Botanical origin is a well-known determinant of bee product composition, influencing phenolic profiles and biological activity (Othman et al., 2019).

The antibacterial potency observed in this study may therefore reflect the unique plant biodiversity of East Kalimantan. This supports the hypothesis that bee bread from different regions cannot be considered chemically or biologically identical, even when produced by the same bee species. Consequently, regional characterization of stingless bee products is essential for their standardization and future medicinal application.

Furthermore, although inhibition against *E. coli* and *S. typhi* was lower, measurable antibacterial activity was still observed, indicating possible applications in food safety or as a complementary antimicrobial agent. These findings align with current global interest in developing plant- and bee-derived antimicrobials as alternatives to conventional antibiotics amid rising antimicrobial resistance (Halawa et al., 2024).

Despite its promising results, this study is limited to in vitro antibacterial assays. Further research is needed to isolate and characterize individual bioactive compounds responsible for antibacterial activity, evaluate synergistic effects among compounds, and assess cytotoxicity and safety profiles. In vivo studies and formulation development will be critical steps toward translating these findings into practical antibacterial products.

CONCLUSION

Bee bread produced by *Heterotrigona itama* bees cultivated in Mugirejo Village, East Kalimantan, has inhibitory activity against five types of pathogenic bacteria (*S. epidermidis, S. sobrinus, S. mutans, S. typhi, C. acne, and E.coli*) at a concentration of 500 μg/well. The flavonoid contained in bee bread has a role on antibacterial activity. The significant differences in bee bread inhibition are influenced by several factors, such as the type of bacteria and the bioactive compound content, which play a significant role in this assay. This research is expected to serve as a basis for further studies on the potential and benefits of *Heterotrigona itama* bee bread. The results also provide opportunities for further research, including the formulation and production of applicable products.

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REFERENCES

Akhir, R. A. M., Bakar, M. F. A., & Sanusi, S. B. (2017, October). Antioxidant and antimicrobial activity of stingless bee bread and propolis extracts. In *AIP conference proceedings* (Vol. 1891, No. 1, p. 020090). AIP Publishing LLC.

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents, 38*(2), 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology, 23*(2), 174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Davis W.W. and T.R Stout. 1971. Disc Plate Methode of Microbiological Antibiotic Assay. Microbiol. 22: 659-665

Didaras, N., Kafantaris, I., Dimitriou, T., Mitsagga, C., Karatasou, K., Giavasis, I., ... & Mossialos, D. (2021). Biological properties of bee bread collected from apiaries located across greece. Antibiotics, 10(5), 555. https://doi.org/10.3390/antibiotics10050555

Dréno, B., Martin, R. J., Moyal, D., Henley, J. B., Khammari, A., & Seité, S. (2017). Skin microbiome and acne vulgaris: staphylococcus, a new actor in acne. Experimental Dermatology, 26(9), 798-803. https://doi.org/10.1111/exd.13296

Halawa, E., Fadel, M., Al-Rabia, M., Behairy, A., Nouh, N., Abdo, M., ... & Abdeen, A. (2024). Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Frontiers in Pharmacology, 14. https://doi.org/10.3389/fphar.2023.1305294

Kusuma, I.W., Murdiyanto, E.T. Arung, Syafrizal, Y. Kim, (2014). Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Science and Human Wellness. 3, (3–4). https://doi.org/10.1016/j.fshw.2014.12.004

Mandell GL, Bennet JE, Dolin R. Principles and Practice of Infectious Diseases. Elsevier Book Aid; 2010.

Nazzaro, F., Fratianni, F., Martino, L. D., Coppola, R., & Feo, V. D. (2013). Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6(12), 1451-1474. https://doi.org/10.3390/ph6121451

Nindi, I. A., Kenconojati, H., Prayogo, Putriantini, I.N., Inaiyah. (2021). Microbiological Examination of Escherichia coli and Salmonella on Fishery Products at Fish Quarantine Station, Quality Control and Safety of Fishery Products Yogyakarta. Journal of Aquaculture Science. October 2021 Vol 6 (2): 76-82

Nurelhuda, N., Al-Haroni, M., Trovik, T. A., & Bakken, V. (2010). Caries experience and quantification of streptococcus mutans and streptococcus sobrinus in saliva of sudanese schoolchildren. Caries Research, 44(4), 402-407. https://doi.org/10.1159/000316664

Nurhayati, L.S., Yahdiyani N., Hidayatulloh, A. (2020). Comparison Of The Antibacterial Activity Of Yogurt Starter With Disk Diffusion Agar And Well Difussion Agar Methods. Jurnal Teknologi Hasil Peternakan, 1(2):41-46

Olajuyigbe, O. O., Olajuyigbe, A. A., & Afolayan, A. J. (2018). Ultrastructure and x-ray microanalysis of the antibacterial effects of stem bark ethanol extract of acacia mearnsii de wild against some selected bacteria. Journal of Pure and Applied Microbiology, 12(4), 2217-2228. https://doi.org/10.22207/jpam.12.4.61

Oong G. C. & P. Tadi. (2023). Chloramphenicol. https://www.ncbi.nlm.nih.gov/books/NBK555966/

Othman, Z. A., Noordin, L., Ghazali, W. W., Omar, N., & Mohamed, M. (2019). Nutritional, phytochemical and antioxidant analysis of bee bread from different regions of Malaysia. *Indian J. Pharm. Sci*, *81*(5), 955-960.

Poelongan, M., Chairul, Komala, I., Salmah, S., & Susan, M. N. (2006). Aktivitas Antimikroba dan Fitokimia dari Beberapa Tanaman Obat. Seminar Nasional Teknologi Peternakan dan Veteriner.

Priawandiputra, W., Muhammad Giffary Azizi, Rismayanti, Kartika Martha Djakaria, Anggun Wicaksono, Rika Raffiudin, Tri Atmowidi, & Damayanti Buchori. (2020). Lebah Tanpa Sengat (Stingless Bees) Panduan Budidaya Lebah Tanpa Sengat (Stingless Bees) di Desa Perbatasan Hutan. In ZSL Indonesia (Cetakan 1). ZSL Indonesia.

Rahmadi, A., S. I. Susilowati, A. Pahriyani. (2024). Profil Sebaran Antibiotik Berdasarkan Klasifikasi AWaRe dan Potensi Risiko Resistensi di Indonesia. Indonesian Journal of Pharmaceutical Education, 2024; 4 (2): 325 – 335

Sari, E., D. A. S. Wardhani, V. Agustina, Agussalim, L. Hakim, R. Wulandari, N. A. Wibowo, L.S. Chua. (2024). Identification of bioactive compounds and encapsulation of bee bread from Heterotrigona itama using a spray dryer with its antioxidant activity. Biodiversitas. 25(7): 2857-2865

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science*, *8*, 33-42.

Turunen, M. J. (1975). A Finite Difference Method for the Determination of Diffusion Coefficient. phys. stat. sol. (a) 31, 489.

Pratiwi. 2008. Mikrobiologi Farmasi. Jakarta: Erlangga.