INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY: APPLIED BUSINESS AND EDUCATION RESEARCH
2024, Vol. 5, No. 4, 1127 – 1133
http://dx.doi.org/10.11594/ijmaber.05.04.01

Research Article

Antibacterial Activity of Stingless Bee Propolis *(Heterotrigona itama)* Ethanol Extract on Dental Root Canal Bacteria Growth in Vitro

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Article history:
Submission January 2024
Revised March 2024
Accepted April 2024

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ABSTRACT

Introduction: Pulp and periapical tissue problems are 7th out of the top 10 diseases. Propolis from stingless bees is a natural substance that has been proven its efficacy in treating the diseases. Additionally, it is categorized safe for humans. This study aimed to determine the antibacterial activity of stingless bee propolis *(Heterotrigona itama)* ethanol extract on dental root canal bacterial growth in vitro. **Method:** This study was an experimental study with a posttest-only control group design and consisting of six treatment groups with concentrations of 3,000; 6,000; 12,000; and 24,000 ppm of stingless bee propolis, as well as a positive and negative control. Each treatment was repeated four times. **Result:** The result showed that there were no clear zones at concentrations of 24,000 ppm, but a clear zones appeared at concentrations of 3,000; 6,000; and 12,000 ppm. Stingless bee propolis ethanol extract is efficient against bacteria. At a concentration of 3,000 ppm, the inhibition zone's maximum circumference was visible.

Keywords*: Antibacterial, Propolis, Root canal bacteria, Stingless bee*

How to cite:
Takdir, A. Q., Purnamasari, C. B., Ompusunggu, N. Y., Paramita, S., & Yani, S. (2024). Antibacterial Activity of Stingless Bee Propolis *(Heterotrigona itama)* Ethanol Extract on Dental Root Canal Bacteria Growth in Vitro. *International Journal of Multidisciplinary: Applied Business and Education Research*. *5*(4), 1127 – 1133. doi: 10.11594/ijmaber.05.04.01

Introduction

Pulp disease often occurs due to the invasion of microorganisms that enter the exposed pulp through gaps in the dentin that experience caries or tooth fractures. Infected pulp can cause inflammation of the pulp or pulpitis (Bidjuni & Harapan, 2019; Yuwono, 2015). Inflammation of the pulp, known as pulpitis, can persist even after removal of the stimulus or resolve and return to its normal state. There are two types of pulpitis, namely reversible pulpitis and irreversible pulpitis (Yoga et al., 2018). Irreversible pulpitis is a persistent dental pulp inflammation. In this condition, the pulp cannot return to its normal state, and the treatment for this condition is root canal treatment (Gopikrishna, 2021; Garg & Garg, 2010).

Root canal treatment is a type of endodontic treatment that aims to eliminate microorganisms present in the root canals and restore the condition of the diseased tooth so that the surrounding biological tissue can accept it (Mubarak et al., 2016; Wahjuningrum & Subijanto, 2014). Root canal treatment consists of three stages known as the endodontic triad, including biomechanical root canal preparation (cleaning and shaping), sterilization, and hermetic obturation (Novitasari & Nugroho, 2017). The root canal sterilization stage aims to eliminate microorganisms in the root canals using irrigants and intracanal medicaments (Febrianifa et al., 2016). Chlorhexidine gluconate is one of the irrigants used in root canal treatment.

Chlorhexidine gluconate is an irrigating agent that acts as a broad-spectrum antibacterial agent. Chlorhexidine gluconate can work as a bacteriostatic and bactericidal, depending on the solution concentration (Anastasia et al., 2022). The concentration of chlorhexidine gluconate used as a root canal irrigation agent is 2% (Tanumihardja, 2010). The community widely uses natural ingredients with excellent and safe properties; one is propolis from bees (Lutpiatina, 2015). Propolis is a substance produced by bees containing bee saliva, beeswax, and a mixture of resins collected by bees from flowers, leaf buds, and exudates of various plants—secondary metabolites contained in propolis, namely flavonoids, tannins, alkaloids, and saponins (Pribadi, 2020; Zulfa et al., 2022).

The ethanol extract of stingless bee propolis effectively inhibits the growth of various bacteria, including *Streptococcus mutans*. These studies show that research on root canal bacteria has yet to be conducted. Therefore, researchers are interested in examining the antibacterial effectiveness of stingless bee propolis *(Heterotrigona itama)* ethanol extract on the growth of root canal bacteria.

Material and Methods

Research Design

This research was an experimental study that has been approved by the Health Research Ethics Commission, Faculty of Medicine, Mulawarman University, No. 04/KEPK-FK/I/2023, conducted from January to February 2023. In this study, there were two groups, including control and treatment group. Control group consisted of positive control (2% Chlorhexidine gluconate) and negative control (DMSO) while the treatment group consisted of four treatments with propolis extract (3,000; 6,000; 12,000; and 24,000 ppm). However, the post-test was only conducted to the control group. The research was repeated four times for each treatment group.

Materials and tools

The materials and tools used in this study were 96% ethanol, stingless bee propolis *(Heterotrigona itama)*, root canal bacterial cultures, 2% chlorhexidine gluconate, DMSO, 70% ethanol, paper points, Mueller Hinton Agar (MHA), Brain Heart Infusion Broth (BHIB), Whatman No. 42 filter paper, label paper, disc paper, sterile cotton swab, yellow tip, blue tip, Personal Protective Equipment (PPE), scissors, knife, glass bottle, Eppendorf tube, micropipette, spatula, glass jar, plastic wrapping, aluminum foil, petri dish, tweezers, beakers, Erlenmeyer flasks, ovens, incubators, autoclaves, rotary evaporators, vortex mixers, Buchner funnels, test tubes, calipers, spectrophotometers, ultrasonic cleaners, vials, digital scales, analytical balances and Biological Safety Cabinet Class II (BSC II).

Preparation of stingless bee propolis extract

The stingless bee propolis extract was obtained using maceration. The stingless bee

propolis was cut, weighed, and put into a glass bottle filled with 96% ethanol for three days and shake it every day for five minutes.

The maceration results were filtered using filter paper on the third day, and the filtrate was collected. The filtrate was concentrated with a rotary evaporator at 50°C for 60 minutes to get a thick extract. Furthermore, the propolis extract was diluted with DMSO to obtain 3,000; 6,000; 12,000; and 24,000 ppm of propolis extract.

Preparation of root canal bacterial samples

The root canal bacteria were taken from patients diagnosed (from drg. Dedy Sugiharto Clinic, Kecamatan Loa Janan Ilir, Kutai Kartanegara, East Kalimantan, Indonesia) with irreversible pulpitis in their teeth. The researcher inserted a sterile paper point into the root canal for 1 minute to collect the bacteria. Then, it was put into a test tube containing BHIB media and incubated at 37°C for 24 hours. After incubating the root canal bacteria for 24 hours, their turbidity level was measured using UV-Visible spectrophotometry until the turbidity level specified by the McFarland standard was 0.5. Subsequently, the suspension of root canal bacteria was inoculated onto MHA media.

Antibacterial test

The antibacterial test was carried out by placing a paper disc on the agar medium and dripping it with a sample of propolis extract, 2% Chlorhexidine gluconate, and DMSO using a micropipette. Next, the treated petri dish was incubated at 37°C in the incubator. After incubation, the inhibition zone was measured using a caliper.

Data analysis

The data obtained from the research results would be processed using Excel 2016 and SPSS for Windows Version 8.1 Pro software. The research data were tested for normality using the Shapiro-Wilk normality test, followed by a homogeneity test. The results indicated that the data distribution was normal and homogeneous ($p > 0.05$), allowing to perform the One-way ANOVA parametric statistical test.

Result and Discussion

Table 1 presents the results on the antibacterial effectiveness of the ethanol extract of stingless bee propolis *(Heterotrigona itama)* on the growth of root canal bacteria, 2% chlorhexidine gluconate, and DMSO (n=3).

Table 1. Average value of inhibition zone diameter (mm) of stingless bee propolis ethanol extract

Treatment group	Concentration	Mean (mm) ± SD
Stingless bee propolis *(Heterotrigona itama)*	3,000 ppm	2.16 ± 0.29
	6,000 ppm	1.63 ± 0.48
	12,000 ppm	1.00 ± 1.18
	24,000 ppm	0.00 ± 0.00
Chlorhexidine gluconate	2%	13.35 ± 0.68
DMSO		1.28 ± 0.38

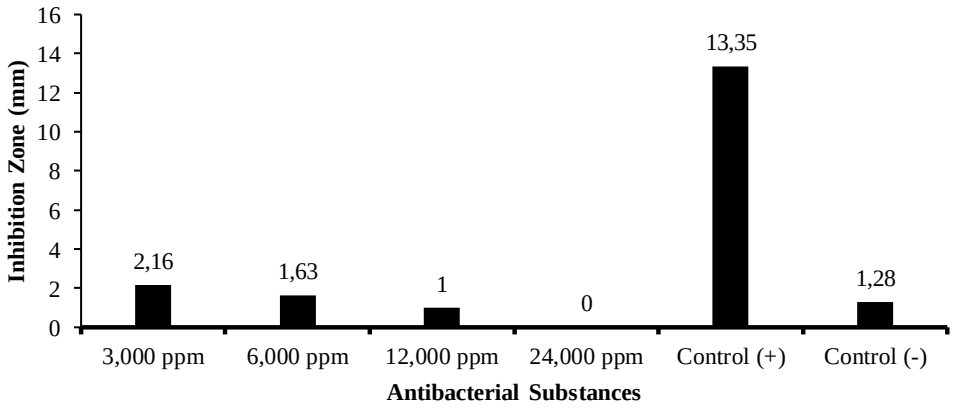

Figure 1. Average Value of Inhibition Zone Diameter (mm)

Table 1 shows that the ethanol extract of stingless bee propolis *(Heterotrigona itama)* has the largest diameter of the inhibition zone indicated at a concentration of 3,000 ppm with an average value of inhibition zone diameter of 2.16 mm. In contrast, 2% chlorhexidine gluconate as a positive control and DMSO as a control negative have an average value of the diameter of the inhibition zone, about 13.35 and 1.28 mm, respectively.

Figure 1 shows a decrease in the diameter of the inhibition zone of the stingless bee propolis *(Heterotrigona itama)* ethanol extract at concentrations of 3,000; 6,000; and 12,000 ppm. The One-way ANOVA test results obtained p= 0.00 (p <0.05). So, it shows a significant difference in each treatment group. A post hoc Tukey analysis test could be conducted to determine the differences between treatment groups (Table 2).

Table 2. Post hoc tukey analysis test results

Treatment group		Average difference	Sig.
3,000 ppm	6,000 ppm	0.53	0.80
	12,000 ppm	1.16	0.16
	Control (+)	-11.18*	0.00
	Control (-)	0.88	0.39
6,000 ppm	3,000 ppm	-0.53	0.80
	12,000 ppm	0.63	0.69
	Control (+)	-11.71*	0.00
	Control (-)	0.35	0.94
12,000 ppm	3,000 ppm	-1.16	0.16
	6,000 ppm	-0.63	0.69
	Control (+)	-12.34*	0.00
	Control (-)	-0.28	0.97
Control (+)	3,000 ppm	11.18*	0.00
	6,000 ppm	11.71*	0.00
	12,000 ppm	12.34*	0.00
	Control (-)	12.06*	0.00
Control (-)	3,000 ppm	-0.88	0.39
	6,000 ppm	-0.35	0.94
	12,000 ppm	0.28	0.97
	Control (+)	-12.06*	0.00

Note: * gave a significant different (p < 0.05)

The results of post hoc Tukey showed that there were significant and no significant differences between one treatment group and another. The results indicated that the ethanol extract of stingless bee propolis *(Heterotrigona itama)* could inhibit the growth of root canal bacteria at concentrations of 3,000; 6,000; and 12,000 ppm. The clear zone around the paper disc indicates that the inhibition of bacterial growth is due to the presence of an antibacterial compound in the extract (Ariyani et al., 2018; Silviana & Asri, 2022). Several factors, such as the concentration of the extract, the content of antibacterial compounds, the extract's diffusivity, and the type of inhibited bacteria, can influence antibacterial activity (Goetie et al., 2022).

The diameter of the inhibition zone formed from the ethanol extract of stingless bee propolis *(Heterotrigona itama)* gradually decreased at concentrations of 3,000; 6,000; and 12,000 ppm, demonstrating that the substance has the antibacterial potency in a low concentration (Saroinsong et al., 2014). This result is in line with the research that found that the diameter of the inhibition zone does not always increase in proportion to the increase in the concentration of antibacterial compounds. This process could occur due to differences in the diffusivity of antibacterial compounds in the agar

medium. In addition, different types and concentrations of antibacterial compounds can also provide different diameters of inhibition zones for a certain length of time (Septiani et al., 2017). The dilution factor can also affect the lack of diffusivity of antibacterial compounds in agar media. As the concentration of the extract increases, its solubility decreases, leading to a more concentrated extract. It makes it difficult for the extract to diffuse optimally into the agar media. It can happen because a higher concentration of the extract can cause saturation, causing the active ingredient compounds contained in the extract not to dissolve entirely into the agar medium (Zeniusa et al., 2019; Nomor et al., 2019).

Propolis has a varied chemical composition. Environmental factors, plant diversity and availability in a geographic area, and the location of propolis can influence the quality and quantity of propolis collected by honey bee species (Šuran et al., 2021; Selvan & Prabhu, 2010). Taking propolis at different locations will undoubtedly affect the production of propolis and the compounds contained therein (Arung et al., 2022). Honey bee species collect resin (45-55%) from surrounding plants, which is the main chemical composition of propolis. In addition, other compositions of propolis consist of beeswax and fatty acids (25-53%), 10% essential oil, 5% protein, and 5% organic and mineral compounds (Starr, 2021; Wardaniati & Gusmawarni, 2021; Lim et al., 2023). Propolis contains secondary metabolites in flavonoids, tannins, alkaloids, and saponins (Zulfa et al., 2022).

The type of bacteria used can also influence the difference in the diameter of the inhibition zone. In this study, the type of bacteria used was polymicrobial, originating from the root canal. Polymicrobial is a bacterial colonization consisting of 4-7 species, especially facultative anaerobic bacteria with an almost equal number of gram-negative and gram-positive bacteria (Indriana et al., 2017). The most common microorganisms found in root canals are anaerobic bacteria, facultative anaerobic bacteria, obligate aerobic bacteria, and obligate anaerobic bacteria (Garg & Garg, 2010; Mulyawati, 2011).

The root canal irrigation material used as a positive control in this study was 2% chlorhexidine gluconate. Chlorhexidine gluconate 2% is a broad-spectrum antimicrobial agent that can fight gram-positive and negative bacteria and yeast (Thakur et al., 2020). Chlorhexidine gluconate 2% in this study had an average diameter of the inhibition zone that was more significant than that of the other propolis extracts, which was 13.35 mm. Chlorhexidine gluconate in low concentrations can act as bacteriostatic by causing bacterial cell death so that bacterial cell leakage will occur, while chlorhexidine gluconate in high concentrations can act as bactericidal by coagulating the intercellular content of bacterial cells (Ristianti & Marsono, 2015).

Conclusion

Based on the research results, the ethanol extract of stingless bee propolis *(Heterotrigona itama)* effectively inhibits the growth of root canal bacteria. The concentration of 3,000 ppm showed the largest inhibition zone diameter. However, the inhibitory power of propolis extract falls into the weak category.

Acknowledgement

This study is supported by the Research Grant 2023, funded by the Faculty of Medicine, Mulawarman University.

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