In Vitro Antibacterial Activity of a Combination of Stingless Bee Propolis and *Premna corymbosa* Leaves Extract Against *Streptococcus mutans*

Aktivitas Antibakteri *In Vitro* Kombinasi Propolis Lebah Kelulut Dan Ekstrak Daun *Premna corymbosa* Terhadap *Streptococcus mutans*

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Abstract. Dental caries remains one of the most common oral health problems and is closely associated with the activity of *Streptococcus mutans*. Natural products such as stingless bee propolis and *Premna corymbosa* leaves contain bioactive compounds has potential as antibacterial agents. This study evaluated the antibacterial activity of a combination of stingless bee propolis and *P. corymbosa* leaves extract against *S. mutans* using an in vitro microdilution method. Five formulations were prepared with different ratios of *P. corymbosa* leaves extract and propolis (100:0, 75:25, 50:50, 25:75, and 0:100). Clindamycin and ethanol were used as positive and negative controls, respectively. Phytochemical screening was performed using qualitative tests to identify major secondary metabolites. Based on these tests, the extracts were contained flavonoids, saponins, terpenoids, alkaloids, and tannins. Antibacterial activity was determined from optical density values and analyzed using one way ANOVA at a significance level of $p<0.05$. The results showed that antibacterial activity increased with higher proportions of propolis in the formulation. The strongest inhibition was observed in the propolis only formulation (57.51%), followed by the 25:75 combination (39.99%) and the 50:50 combination (26.11%). Statistical analysis indicated significant differences among treatment groups. The combination of *P. corymbosa* leaves extract and stingless bee propolis exhibits measurable antibacterial activity against *S. mutans*. Although the inhibition values were lower than those of clindamycin, the extract combination shows potential as a natural antibacterial agent for oral health applications.

Keywords: *Premna corymbosa*; propolis; *Streptococcus mutans*; antibacterial; microdilution

Abstrak. Karies gigi merupakan salah satu masalah kesehatan gigi dan mulut yang masih sering dijumpai terkait dengan aktivitas bakteri *Streptococcus mutans*. Pemanfaatan bahan alam seperti propolis lebah kelulut dan daun *Premna corymbosa* menjadi alternatif yang potensial karena keduanya mengandung berbagai senyawa bioaktif yang berperan sebagai antibakteri. Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri kombinasi propolis lebah kelulut dan ekstrak daun *P. corymbosa* terhadap pertumbuhan *S. mutans* secara in vitro menggunakan metode mikrodilusi. Formulasi kombinasi dibuat dalam lima variasi perbandingan ekstrak daun *P. corymbosa* dan propolis yaitu 100:0, 75:25, 50:50, 25:75, dan 0:100. Klindamisin digunakan sebagai kontrol positif dan etanol sebagai kontrol negatif. Skrining metabolit sekunder dilakukan secara kualitatif. Hasil uji fitokimia menunjukkan adanya senyawa flavonoid, saponin, terpenoid, alkaloid, dan tanin. Aktivitas antibakteri dianalisis menggunakan uji *one way* ANOVA. Hasil penelitian menunjukkan bahwa peningkatan konsentrasi propolis dalam kombinasi memberikan peningkatan aktivitas antibakteri. Daya hambat tertinggi diperoleh pada formulasi propolis murni sebesar 57,51%, diikuti kombinasi 25:75 sebesar 39,99% dan kombinasi 50:50 sebesar 26,11%. Hasil analisis statistik menunjukkan bahwa terdapat perbedaan yang signifikan antar perlakuan ($p < 0,05$). Berdasarkan hasil penelitian dapat disimpulkan bahwa kombinasi ekstrak daun *P. corymbosa* dan propolis lebah kelulut memiliki aktivitas antibakteri terhadap *S. mutans*. Kombinasi bahan alam ini berpotensi untuk dikembangkan sebagai alternatif agen antibakteri alami dalam bidang kesehatan gigi dan mulut

Kata kunci: *Premna corymbosa*; propolis; *Streptococcus mutans*; antibakteri; mikrodilusi

INTRODUCTION

Oral health plays an important role in maintaining overall health and quality of life. The dental caries remains one of the most frequently encountered conditions and affect a large portion of the population. The development of dental caries is strongly associated with cariogenic bacteria, such as *Streptococcus mutans*. This bacteria is able to produce organic acids from carbohydrate metabolism and survive in acidic environments (Amiqoh, 2022). These characteristics allow the bacteria to adhere to tooth surfaces and form biofilms that contribute to tooth demineralization (Liu et al., 2020). The pathogenicity of *S. mutans* has ability to produce acid and survive in acidic conditions. This bacterium can also synthesize extracellular glucan polymers. That facilitate adhesion to tooth surfaces and support biofilm formation. Dental plaque is a structured microbial biofilm and adheres to tooth surfaces. The development of caries was conducted when oral hygiene is poor (Aqawi et al., 2021). The dental caries was the most common oral health problems worldwide. It is estimated to affect about 60-90% of school aged children and most of adults in industrialized countries (Priamsari & Nuraida, 2022).

Propolis produced by stingless bees has attracted considerable attention because of its wide range of biological activities. It is known to possess antibacterial and antioxidant properties. These effects are largely associated with phenolic compounds and flavonoids present in propolis. Previous studies have reported that propolis is effective against various pathogenic microorganisms, including oral bacteria (Kustiawan et al., 2023). This activity is thought to be related to the presence of several bioactive compounds in propolis such as flavonoids and phenolic acids as well as esters, sugars, hydrocarbons, and minerals. These compounds contribute to its protective effects against bacterial, fungal, and viral infections (Khairunnisa et al., 2020).

In addition to propolis, medicinal plants such as *Premna corymbosa* have long been used by local communities in Kalimantan as traditional remedies. The young leaves are commonly prepared to help relieve inflammatory conditions such as gout. Previous phytochemical and pharmacological studies have indicated that *P. corymbosa* leaves possess antioxidant as well as antibacterial activities (Husniah & Gunata, 2020). Further phytochemical investigations have shown that the leaves contain various secondary metabolites, including steroids, alkaloids, polyphenols, flavonoids, terpenoids, and saponins, depending on the solvent fraction used (Melwita et al., 2014). These flavonoids are considered important contributors to antibacterial activity because they can disrupt bacterial cell membranes and lead to leakage of intracellular components. Several in vitro studies have also demonstrated that *P. corymbosa* leaves extract is able to inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus*, with inhibition increasing at higher extract concentrations (Anggraeni Putri et al., 2023; Suryana et al., 2017; Widiyastuti & Martina, 2017). Although *P. corymbosa* has shown antibacterial potential, its activity against oral pathogens is still not well understood. Studies focusing on *Streptococcus mutans* are particularly limited. The antibacterial effect of a combination of kelulut bee propolis and Premna corymbosa leaf extract against *S. mutans* has also not been reported. This combination is expected to produce better antibacterial activity because the bioactive compounds may act through different mechanisms. Therefore, this study evaluated the antibacterial activity of stingless bee propolis combined with *P. corymbosa* leaves extract against *S. mutans* using a microdilution method. The study also aimed to determine the most effective formulation and to support the development of natural antibacterial agents for oral health.

METHOD

MATERIALS

The materials used in this study consisted of stingless bee (*Heterotrigona itama*) propolis and *Premna corymbosa* leaves extract. The plant material was taxonomically identified at the Laboratory of Tropical Forest Ecology and Biodiversity, Faculty of Forestry, Universitas Mulawarman, under identification number 47/UN.17.4.08/LL/2025. The biological and assay materials included *Streptococcus mutans* bacterial suspension, Nutrient Broth (NB) medium (Oxoid, UK), clindamycin (Sigma-Aldrich, USA) as a positive control, crystal violet solution (Merck, Germany), and ethanol 96% (Merck, Germany) as a solvent and negative control. The qualitative phytochemical screening reagents included Mayer's, Wagner's, and Dragendorff's reagents for alkaloid determination, $FeCl_3$ 1% for tannins and phenolics, concentrated HCl and magnesium powder (Shinoda test) for flavonoids, and Liebermann-Burchard reagent for terpenoids and steroids, all purchased from Merck (Germany).

EQUIPMENT

The equipment used in this study included a water bath (Memmert, Germany), blender (Maspion), rotary evaporator (Buchi, Switzerland), oven (Memmert, Germany), analytical balance (Metter Toledo, Switzerland), glassware set (Pyrex, USA), spatula, porcelain dish, separating funnel (Pyrex, USA), Bunsen burner, filter paper (Whatman No.1, UK), autoclave (Hirayama, Japan), Laminar Airflow Cabinet (ESCO, SIngapore), Petri dishes (Anumbra), test tubes (Pyrex, USA), ose needle, micropipettes (Eppendorf, Germany), measuring cups (Pyrex, USA), hot plate (Corning, USA), flat bottom 96 well polystyrene microtiter plates (Nunc, Thermo Fisher Scientific, USA), microplate reader (Bio-Rad, USA).

RESEARCH DESIGN

This study was conducted as experimental research to evaluate the antibacterial activity of *P. corymbosa* leaves extract and stingless bee propolis against *S. mutans* (Pryambodho et al., 2022). The research included several stages starting from the extraction of plant materials and propolis. Followed by phytochemical screening and preparation of extract combinations before antibacterial testing was carried out. A completely randomized design was applied to compare the antibacterial effects of different formulations consisting of *P. corymbosa* leaves extract, propolis extract, and their combinations together with positive and negative controls. Antibacterial activity was assessed using a microdilution approach to observe the inhibition of bacterial growth, and the results obtained were analyzed statistically to determine differences among treatments and to identify the most effective formulation.

RESEARCH PROCEDURS

Preparation of Propolis Extract

Raw propolis were cleaned and cut into small pieces. A total of 50 g of propolis was macerated with 500 mL of 70% ethanol. The mixture was shaken until homogeneous. The solution was filtered and concentrated using a rotary evaporator to obtain a propolis extract.

Preparation of *Premna corymbosa* Leaves Extract

Dried leaves of *Premna corymbosa* were ground into powder and weighed to obtain 279 g of sample. The powdered material was placed in a maceration container and soaked in 700 mL of ethanol. The mixture was left to stand for 24 hours with occasional stirring and periodic shaking for about 20 minutes. The maceration was then repeated using half of the initial solvent volume. All filtrates were collected and concentrated using a rotary vacuum evaporator. The extract was further evaporated in a water bath until a thick extract was obtained (Arifin and Subandar, 2023).

Preparation of Extract Combinations

The extract combinations were prepared by mixing *P. corymbosa* leaves extract and stingless bee propolis in five different proportions. The combination as F1 (100:0), F2 (75:25), F3 (50:50), F4 (25:75), and F5 (0:100). These formulations were used to evaluate the antibacterial activity.

Analysis Methods

Phytochemical Screening of Extract

Qualitative phytochemical screening of the extract was conducted to identify the major groups of secondary metabolites following the unified standard procedures described by Hersila et al. (2023). Alkaloid detection was performed using Mayer, Bouchardat, and Dragendorff reagents. A positive result was indicated by the formation of white precipitates with Mayer reagent, brown precipitates with Bouchardat reagent, and reddish-brown precipitates with Dragendorff reagent. Flavonoids were identified using the magnesium hydrochloric acid test. The appearance of a yellow coloration in the amyl alcohol layer after the addition of magnesium powder and concentrated hydrochloric acid indicated a positive reaction. Tannin detection was carried out by adding ferric chloride solution (1% and 5%) to the extract. The formation of a black coloration indicated the presence of tannins. Saponins were identified using the foam test. The extract was treated with hot water and concentrated hydrochloric acid, and the formation of stable foam indicated a positive result. Steroid and terpenoid tests were conducted using acetic anhydride and concentrated sulfuric acid. The appearance of a greenish-blue color indicated the presence of steroids, while the absence of red coloration in the n-hexane and concentrated sulfuric acid test indicated that terpenoids were not present.

Antibacterial Activity

Nutrient broth medium was prepared by dissolving 0.8 g of NB powder in 100 mL of distilled water and heating until completely dissolved (Hidayati et al., 2021). A portion of the medium was transferred into test tubes for the preparation of *Streptococcus mutans* suspension and all media were sterilized in an autoclave at 121 °C for 15 minutes (Sapalina et al., 2022; Wasfi et al., 2018). Antibacterial activity was evaluated using a microdilution method in 96 well plates containing nutrient broth, bacterial suspension, and test samples followed by incubation at 37 °C for 24 hours (Anissa et al., 2022). Each well in columns A to G received 15 µL of bacterial suspension while column H served as the blank control containing only nutrient broth. The treatments consisted of *P. corymbosa* leaves extract, combinations of *P. corymbosa* extract and propolis, and propolis extract alone together with clindamycin as the positive control and ethanol as the negative control. After incubation the wells were emptied and rinsed before staining with crystal violet solution for 15 minutes to visualize bacterial growth (Siregar, 2018). The bound stain was dissolved with 96% ethanol and optical density values were measured using a microplate reader (Handoyo, 2020). The antibacterial activity of the *Premna corymbosa* leaves extract and *Heterotrigona itama* propolis formulations against *Streptococcus mutans* was quantified by measuring the optical density (OD) at 600 nm using a microplate reader. The percentage of bacterial growth inhibition for each treatment formulation (F1–F5) and the positive control was calculated relative to the negative control using the following equation:

$$\%\ Inhibition = \frac{A0 - A1}{A0} \times 100$$

Where A0 represents the mean optical density of the negative control (untreated bacterial suspension), and A1 represents the mean optical density of the bacterial suspension treated with the formulation or positive control. All measurements were performed in triplicate, and data were expressed as average percentages. The data obtained were averaged and analyzed statistically using one way ANOVA with a significance level of $p < 0.05$ (Ferdiana et al., 2022).

Statistical Analysis

All experimental assays for antibacterial activity were performed in triplicate, and data were expressed as the mean values. To evaluate the statistical significance of the treatments, the percentage of bacterial growth inhibition was analyzed using a One-way Analysis of Variance (ANOVA) after verifying data normality and homogeneity of variance. Statistical significance was established at a threshold of $p < 0.05$. Upon detecting a significant global effect, Tukey's Honestly Significant Difference (HSD) post-hoc test was subsequently applied to identify specific significant pairwise differences between individual formulation variants (F1–F5) and the controls.

RESULTS AND DISCUSSION

1. Yield of Extracts

Dried *Premna corymbosa* leaves were ground into powder and sieved using an 80-mesh sieve. A total of 279 g of Extraction of *Premna corymbosa* leaves using 70% ethanol yielded 34.26 g extract from 279 g of dried powdered material, corresponding to a yield of 12.2%. This relatively high yield indicates that hydroethanolic solvent is effective for extracting polar and semi-polar bioactive compounds from *P. corymbosa* leaves, which are commonly associated with antibacterial activity. In contrast, extraction of 1,021 g stingless bee (*Heterotrigona itama*) propolis resulted in a much lower yield of 0.6%. This finding is consistent with previous reports and may be attributed to the resinous and wax-rich composition of propolis, as well as the limited solubility of certain components in hydroethanolic solvents (Pratami et al., 2024). Despite its low yield, propolis is known to contain highly potent bioactive compounds, which may compensate for the limited extract recovery.

Phytochemical screening of *Premna corymbosa* leaves extract

Phytochemical screening of *P. corymbosa* leaves extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids. While terpenoids were not detected (Table 1).

Table 1. Secondary metabolite of *P. corymbosa* leaves extract

Phytochemical	Reagent	Indicator	Result
Alkaloids	Mayer + 2N HCl	White precipitate formed	(+)
	Bouchardat + 2N HCl	Brown precipitate formed	(+)
	Dragendorff + 2N HCl	Reddish-brown sediment formed	(+)
Flavonoids	Mg powder + 2N HCl + amyl alcohol	Yellow color formed in the amyl alcohol layer	(+)
Tannins	$FeCl_3$ 1% and 5%	Black coloration observed	(+)
Saponins	Hot water + concentrated HCl	Stable foam formed	(+)
Steroids	Anhydrous acetic acid + concentrated H_2SO_4	Green to bluish coloration observed	(+)
Terpenoids	n-Hexane + concentrated H_2SO_4	No red coloration observed	(–)

The presence of these secondary metabolites is relevant to the antibacterial potential of the extract. The antibacterial activity may be related to alkaloids and tannins, which have been reported to affect bacterial cell wall integrity (Kępa et al., 2025). Saponins may enhance antibacterial activity by increasing cell membrane permeability, thereby facilitating the penetration of other active compounds (Fink & Filip, 2023). Consequently, highly non-polar monoterpenes and sesquiterpenes remain poorly soluble in the hydroethanolic menstruum and are largely left behind in the plant matrix during maceration, falling below the detection threshold of qualitative screening (Liu & Chen, 2025). The diversity of secondary metabolites detected in *P. corymbosa* leaves extract supports its role as a complementary antibacterial agent when combined with other natural products.

2. Phytochemical Screening of Propolis Extract

Phytochemical analysis of *H. itama* propolis extract demonstrated the presence of flavonoids, saponins, and terpenoids, while alkaloids, tannins, and steroids were not detected (Table 2).

Table 2. Secondary metabolite of *H. itama* propolis extract

Phytochemical	Reagent	Indicator	Result
Alkaloids	Mayer + 2N HCl	No white precipitate formed	(–)
	Bouchardat + 2N HCl	No brown precipitate formed	(–)
	Dragendorff + 2N HCl	No reddish-brown sediment formed	(–)
Flavonoids	Mg powder + 2N HCl + amyl alcohol	Yellow color formed	(+)
Tannins	$FeCl_3$ 1% and 5%	No black coloration observed	(–)
Saponins	Hot water + concentrated HCl	Foam formed	(+)
Steroids	Anhydrous acetic acid + concentrated H_2SO_4	No bluish-green coloration observed	(–)
Terpenoids	n-Hexane + concentrated H_2SO_4	Red coloration observed	(+)

Flavonoids and terpenoids are widely reported as the main contributors to the antibacterial activity of propolis. These compounds exhibit antibacterial effects through membrane disruption, inhibition of bacterial enzyme activity, and suppression of virulence factors (Pitriani, 2022). The phytochemical profile of *H. itama* propolis observed in this study predominantly rich in flavonoids and terpenoids while lacking alkaloids and tannins aligns closely with previous investigations on Malaysian and Indonesian stingless bee propolis (Zain et al., 2025; Ibrahim et al., 2016).

3. Antibacterial Activity Against *Streptococcus mutans*

The antibacterial activity of *P. corymbosa* leaves extract, propolis, and their combinations against *Streptococcus mutans* is presented in Table 3 and Figure 1.

Table 3. Optical density (OD) values and bacterial growth inhibition percentages of *P. corymbosa* leaves extract and *H. itama* propolis formulations against *S. mutans*

Test Treatment	R1 (OD)	R2 (OD)	R3 (OD)	Average	Percentage
Negative control	0.31	0.29	0.29	0.30	0.00%
Positive control	0.10	0.09	0.09	0.09	68.79%
F1	0.28	0.28	0.30	0.29	3.38%
F2	0.27	0.28	0.29	0.28	5.10%
F3	0.22	0.22	0.22	0.22	26.11%
F4	0.17	0.17	0.19	0.18	39.99%
F5	0.13	0.12	0.13	0.13	57.51%

Note: R1, R2, R3 = Replications 1, 2, and 3; OD = Optical Density measured at λ= 600 nm.
Values are expressed as mean (n = 3). Percentage of inhibition was calculated relative to the negative control.

The negative control showed the highest average absorbance value, confirming the absence of antibacterial effects, while the positive control (clindamycin) demonstrated strong inhibition, validating the reliability of the microdilution assay.

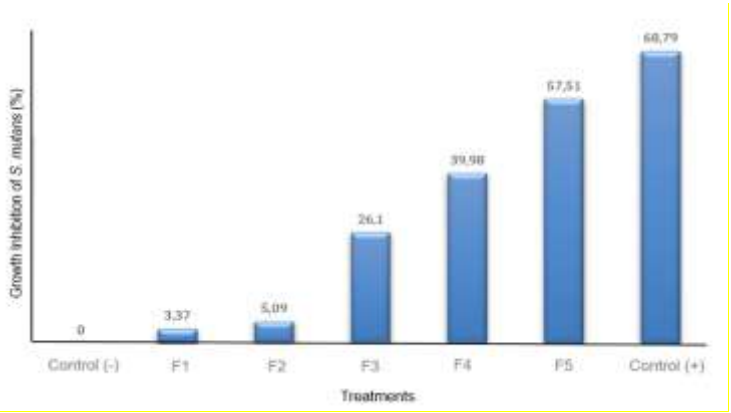

Figure 1. Percentage of *S. mutans* growth inhibition following treatment with *P. corymbosa* leaves and *H.itama* propolis formulations F1 (100:0), F2 (75:25), F3 (50:50), F4 (25:75), and F5 (0:100), compared with the positive control clindamycin. Values are expressed as mean (n = 3).

Formulations F1 and F2 showed weak antibacterial activit against *S. mutans*. This indicated that *P. corymbosa* leaves extract alone or in low proportion was insufficient to effectively inhibit *S. mutans* growth. A moderate inhibitory effect was observed in F3. That equal proportions of *P. corymbosa* leaves extract and propolis begin to response antibacterial activity. Stronger inhibition was observed in F4, while F5 (100% propolis) showed the highest antibacterial activity among all formulations.

This increasing trend demonstrates a clear dose dependent effect. The higher propolis content resulted in greater antibacterial activity. Propolis is known to inhibit *S. mutans* virulence by suppressing glycosyltransferase (GTF) enzymes involved in biofilm formation. It may explain its dominant contribution to bacterial growth inhibition. The enhanced activity observed in combined formulations (F3 and F4) suggests a synergistic interaction between the secondary metabolites present in *P. corymbosa* leaves extract and propolis. The contain of saponins and flavonoids may act together to disrupt bacterial cell membranes and metabolic processes (Dey et al., 2020).

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4. Statistical Analysis

One-way ANOVA analysis (Table 4) showed a highly significant difference among treatment groups ($p < 0.001$). The high F value indicates that the variation between treatments was substantially greater than the variation within groups, confirming that the observed antibacterial effects were caused by the different extract formulations.

Table 4. One-way ANOVA results of antibacterial activity of *Premna corymbosa* leaves extract and propolis combinations against *Streptococcus mutans*

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.085	6	0.181	386.421	0.000
Within Groups	0.007	14	0.000	-	-
Total	1.092	20	-	-	-

The statistical analysis supported the dose dependent antibacterial activity observed in the microdilution assay. The results showed that the combination of *P. corymbosa* leaves extract and stingless bee propolis significantly inhibited the growth of *S. mutans*. Statistical analysis using a one-way ANOVA demonstrated a highly significant difference in the bacterial growth inhibition percentages among the various treatment formulations and controls ($F(6, 14) = 386.42$, $p < 0.001$). This extremely low p value confirms that the alteration in the ratios of *P. corymbosa* leaves extract and *H. itama* propolis directly influenced the antibacterial efficacy against *S. mutans*. To determine the specific pairwise differences between individual treatments, a follow-up Tukey's Honestly Significant Difference (HSD) post-hoc test was performed ($p < 0.05$). The post-hoc analysis revealed that while low propolis formulations (F1 and F2) did not differ significantly from the negative control ($p > 0.05$), a stepwise increase in propolis concentration (from F3 to F5) resulted in significantly distinct and superior inhibition levels ($p < 0.05$), as detailed by the superscript notations in Table 3.

Interestingly, although *P. corymbosa* leaves extract possessed a wider diversity of secondary metabolite classes, *H. itama* propolis demonstrated a significantly higher antibacterial efficacy against *S. mutans*. This phenomenon is primarily governed by the quantitative density and specific chemical structures of the constituents rather than the sheer number of phytochemical classes. While plant leaves contain diffuse metabolites mixed with primary structural tissues, raw propolis is a highly concentrated, evolutionarily refined resin rich in specialized lipophilic flavonoids and terpenoids (Silva et al., 2025; Wiwekowati et al., 2025). Furthermore, the superior potency of propolis is driven by a profound synergistic mechanism; the abundant saponins act as natural surfactants that permeabilize the bacterial plasma membrane, thereby establishing a low resistance gateway for the highly concentrated flavonoids to rapidly flood the intracellular space and denature crucial bacterial proteins (Barboza et al., 2023). Consequently, this targeted biochemical synergy allows propolis to exert a more lethal antimicrobial effect than the qualitatively diverse but more diluted leaves extract.

Flavonoids, which were detected in both extracts can disrupt bacterial cell membranes and inhibit intracellular enzyme activity. This process increased membrane permeability and lead to leakage of cellular contents. Tannins and alkaloids found in *P. corymbosa* leaves extract may also contribute to antibacterial activity. This mechanism may inhibit the growth of gram-positive bacteria such as *S. mutans* (Priamsari & Nuraida, 2022). Saponins were also detected in both extracts. These compounds can cause membrane destabilization and cell lysis. This potential compound was enhanced overall antibacterial efficacy.

Although the maximum inhibition achieved by the natural extract combination (57.51% in F5) was lower than that of the positive control clindamycin (68.79%), the observed activity remains biologically and clinically relevant. Clindamycin has been widely reported to exhibit strong inhibitory activity against *Streptococcus mutans* at relatively low minimum inhibitory concentrations, demonstrating its effectiveness as a reference antibacterial agent (Kaspar et al., 2019). Similar findings have also been reported in studies evaluating salivary *Streptococcus* isolates and pediatric oral formulations containing clindamycin (Clark et al., 2017; Akhavan Karbassi et al., 2024).

Despite this difference in inhibitory magnitude, the present study demonstrated that the combination of *Premna corymbosa* leaves extract and stingless bee propolis exerted a statistically significant, dose-dependent antibacterial effect ($p < 0.05$). This linear, concentration dependent trend aligns specifically with the findings of Mohammed Ghilan et al. (2023), who reported that incremental doses of polyphenol rich natural extracts systematically amplify the inhibition of cariogenic bacteria. The precise correlation observed in both studies can be molecularly attributed to the cumulative concentration of total flavonoids and phenolic compounds delivered to the bacterial microenvironment. At lower formulations (F1 and F2), the active compounds are below the minimum inhibitory threshold required to

compromise *S. mutans*. However, as the proportion of propolis increases from F3 to F5, the concentration of lipophilic flavonoids and terpenoids reaches a critical mass. This high density of active components accelerates the rate of bacterial cell wall cross linking and multisite enzyme inactivation, mirroring the dose response dynamics observed in previous cariogenic biofilm interventions (Yang et al., 2025). This specific cross-study consistency strongly confirms that the antibacterial efficacy against *S. mutans* is heavily reliant on achieving a critical concentration of synergistic polyphenols to successfully breach the robust multi-layered cell wall of Gram-positive oral pathogens.

The presence of terpenoids in the propolis extract may further explain the increased antibacterial activity observed in formulations with higher propolis proportions. Terpenoids have been reported to inhibit bacterial adhesion and biofilm formation. Key virulence factors of *S. mutans*, which contributed to the superior inhibitory effects observed in formulations F4 and F5. This compositional difference supports the dose dependent trend observed in the antibacterial assay.

The combination of diverse secondary metabolites in *P. corymbosa* leaves and propolis suggests a multitarget antibacterial mechanism. The different compounds in both natural product act synergistically to inhibit bacterial growth. This synergistic interaction in the combined formulations exhibited stronger antibacterial activity than *P. corymbosa* leaves extract alone. At a molecular level, this synergy is driven by a multitarget mechanism that simultaneously disrupts the structural integrity and metabolic pathways of *S. mutans*. The saponins present in both extracts act as amphiphilic surfactants, binding to the cholesterol-free lipid bilayer of the bacterial plasma membrane and altering its fluidity. This membrane destabilization creates transient pores effectively acting as a low resistance gateway (Modi et al., 2023).

Through these newly formed pathways, the highly concentrated flavonoids from propolis and the alkaloids from the leaf extract can rapidly flood the intracellular space without meeting cellular resistance. Once inside, the flavonoids inhibit bacterial DNA gyrase and topoisomerase, arresting nucleic acid synthesis, while the tannins crosslink with vital cytoplasmic proteins and induce immediate protein denaturation (Donadio et al., 2021; Sharma et al., 2025). This sequential, dual-action assault where one class of compounds breaches the physical defenses (membrane) to allow another class to destroy the internal machinery (DNA/proteins). These findings provide solid biochemical references for these antibacterial effects and strongly support the potential application of this extract combination as a natural, multi-target antibacterial agent for oral health management. Overall, these findings support evidence of the antimicrobial potential of natural products derived from plants and bee products. The inhibitory activity against *S. mutans* provides a scientific basis references for further studies. These finding was potential to development of herbal based dental preparations to enhance therapeutic efficacy.

CONCLUSION

The combination of *Premna corymbosa* leaves extract and stingless bee propolis demonstrated antibacterial activity against *Streptococcus mutans* with a clear dose related response. The formulation with the highest proportion of propolis showed the greatest antibacterial potential, indicating that propolis plays a major role in the observed activity. This effect is associated with the presence of bioactive compounds such as flavonoids, saponins, terpenoids, alkaloids, and tannins, which may inhibit bacterial growth through complementary mechanisms. Overall, the findings suggest that the combination of *Premna corymbosa* leaves and stingless bee propolis is a promising natural antibacterial candidate for oral health applications and provides scientific support for its use as a traditional remedy.

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