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Original Article

PHYTOCHEMICAL ANALYSIS AND EFFECTIVENESS OF *STINGLESS* BEES PROPOLIS *(GENIOTRIGONA THORACICA)* ON MATRIX METALLOPROTEINASE-8 LEVELS IN PERIODONTAL THERAPY

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ABSTRACT

Objective: Propolis is a beehive-derived natural substance containing plant secondary metabolites and can potentially be used for periodontal therapy. This study aims to analyze the phytochemicals of Indonesian stingless bee propolis and its efficacy of propolis extract gel as an adjunctive periodontal therapy.

Methods: The qualitative and quantitative phytochemical analysis measured the total phenolic and flavonoid content. The gallic acid equivalent was used to measure phenolics, and the Quercetin equivalent was used to measure the flavonoids of the extract. The Kirby-Bauer method was used to analyze the antimicrobial activity. The study used periodontitis-induced Wistar rats and were treated by applying propolis gel with concentrations of 50%, 60%, and 70%. Matrix Metalloproteinase-8 (MMP-8) level was measured with the Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The qualitative and quantitative phytochemical analysis of the propolis extract detected the presence of phenol, flavonoid, alkaloid, triterpenoid, saponins, glycosides, and tannins. The Kirby-Bauer method showed that propolis gel with a concentration of 50%, 60%, and 70% had a significant difference in inhibition between treatment groups against *Porphyromonas gingivalis* ($p<0.05$). The 70% propolis gel appeared effective in inducing the expression of MMP-8 ($p<0.05$).

Conclusion: Propolis extract gel with 70% concentration could potentially be used as a supportive treatment for periodontal therapy.

Keywords: Stingless bees, Geniotrigona thoracica

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INTRODUCTION

Periodontitis is a chronic inflammatory condition caused by biofilms, characterized by the progressive destruction of the tooth's supporting tissues, which leads to tooth loss [1, 2]. Periodontitis is the most common cause of tooth loss in the adult population [1]. The Global Burden of Disease Study reports that periodontitis is the 6th most common disease in the world. The overall prevalence of periodontitis is 11.2% or 743 million people worldwide [3]. Periodontal inflammation is mediated by several cytokines that play a major role in bone resorption. Periodontitis treatment primarily targets periodontal pathogenic bacteria, which generally results in therapeutic benefits. However, antimicrobial treatment alone was not as effective as expected [1].

Combination therapies are needed in periodontal treatments, such as mechanical therapies accompanied by synthetic drugs or natural products [4, 5]. Propolis is a promising beehive-derived product with anti-inflammatory and anti-microbial properties for periodontitis treatment. Propolis is collected by bees from plant resins and plant exudates. Propolis comprises wax, resins, balsams, essential oils, and plant primary and secondary metabolites such as amino acids, vitamins, phenolics, terpenoids, tannins, and alkaloids [6, 7]. The main bioactive compounds in propolis are thought to be the plant secondary plant metabolites such as phenolics and terpenoids [8, 9].

There are many potential biomarkers to measure the healing rate of periodontal disease, namely macrophage inflammatory protein-1α (MIP-1α), neutrophil elastase, interleukin-1β (IL-1β), myeloperoxidase and neutrophil collagenase, also known as Matrix Metalloproteinase-8 (MMP-8) and gelatinase B, also known as matrix metalloproteinase-9 (MMP-9) [1, 10, 11]. Matrix Metalloproteinases (MMPs) play an essential role in various inflammatory processes related to tissue damage, such as the degradation of almost all basement membrane and peri-cellular components. MMP-8 is known as the main MMP in periodontitis [11]. Recently, MMP-8 was proposed as a diagnostic biomarker for an updated periodontitis classification system and demonstrated a lower risk of false positives compared to the traditionally used clinical measure of probing for bleeding.10 Salivary matrix metalloproteinase (MMP)-8 is a promising biomarker for diagnosing periodontitis; however, several recent studies showed conflicting results [12]. Involvement of MMPs in the destruction of periodontal tissues is strongly evident, and MMP-8 is the most important associated with collagen breakdown during periodontal destruction and is present in GCF and saliva of patients with chronic periodontitis [13]. Salivary MMP-8 was much lower than GCF in pretreatment and posttreatment of periodontitis patients [14]. Pathogen such as *Porphyromonas gingivalis*, which are the main components of pathogenic biofilm found in the gingival crevicular fluid, induces a cascade which leads to increased levels of MMPs. The persistent increase in MMP-8 in the gingival crevicular fluid indicates a high risk of poor response to periodontal therapy [15, 16].

Research, development, and commercialization of propolis is dominated by propolis for the European Honey Bee (*Apis mellifera*). Nevertheless, stingless bee propolis has also been proven as a potential and promising alternative source of propolis bioactive compounds. The biological activity of stingless bee propolis is primarily antimicrobial, antioxidant, anti-inflammatory, and anti-cancer. Other biological activities, such as wound healing, have also

been identified [17, 18]. This study aims to detect the matrix metalloproteinase (MMP)-8 levels to assess its diagnostic value in periodontitis. This research is expected to be the basis for developing complementary or adjunctive therapeutics for treating periodontitis based on Indonesian stingless bee propolis, especially that from the North Sumatra region.

MATERIALS AND METHODS

The study was approved by the Animal Research Ethics Committees/AREC of Universitas Sumatera Utara (protocol number 0850/KEPH-FMIPA/2022). The *in vitro* study is experimental laboratory research with posttest only control group design. *Porphyromonas gingivalis* ATCC 33277 was cultured using Brain Heart Infusion (BHI) broth at 37 °C for 24 h under anaerobic condition in anaerobic jar. The propolis extract was diluted into three different concentrations (50%, 60%, and 70%), chlorhexidine 0.2% as positive control and Dimethyl Sulfoxide (DMSO) as negative control. Chlorhexidine 0.2% was used as positive control because its usage as the golden standard to inhibit *P. gingivalis* in periodontitis therapy. 2 Sample size was determined using Federer formula with a minimum number of 5 samples for each group. Bacteria colonies were taken and planted on 5% Brucella Sheep Blood Agar with a four-quadrant streak scraping technique. The petri dishes were incubated at 25 °C for 24-48 h in anaerobic condition. The Minimal Inhibitory Concentration (MIC) was obtained by calculating the number of colonies with Colony Forming Units per millimeter (CFU/ml) using a colony counter tool.

The Kirby-Bauer method measured the antimicrobial activity of propolis gel to *Porphyromonas gingivalis* (*in vitro*). The qualitative and quantitative phytochemical analysis of the propolis extract was done to detect the secondary metabolites of the propolis extract. The study protocol was created according to Animal Research Reporting *In vivo* Experiments (ARRIVE) guidelines. The research was done in the animal housing facility at the Faculty of Mathematics and Sciences at Universitas Sumatera Utara. Thirty male Wistar rats (weighing 200-250 g), aged 8-12 w with lower central incisors and good health, were used. The animals were housed in groups of 3 under a 12 h light-dark cycle at room temperature with free access to water and food.

Animals were periodontitis-induced by ligation and bacterial infection (fig. 1). Briefly, *Porphyromonas gingivalis* ATCC 33277 was cultured at the Microbiology Laboratory at Universitas Sumatera Utara Hospital. Ligation with Silk 3/0 was done and placed simultaneously around incisor teeth in the subgingival position. The animals were then infected with *Porphyromonas gingivalis* ATCC 33277 at the gingival sulcus. All experimental procedures were performed by an experienced clinician. Anesthesia was performed with ketamine before the induction, and the treatment was applied to the animals. After seven days, the periodontitis-induced Wistar rats were examined clinically and swabbed to check for *P. gingivalis* to confirm whether the periodontitis induction was successful.

In this study, the propolis used was sourced from the hives of *Geniotrigona thoracica*/kelulut bees from Kebun Efi, Kabanjahe. The hydroglyceric extract of stingless bee propolis was produced using a proprietary method that involves heating followed by filtration steps where the final product contains 100 % (w/v) propolis extract. The propolis extract was then diluted and mixed accordingly to produce a gel that had 30% (w/w)–80% (w/w) hydroglyceric propolis extract. The gel products were made in the Pharmacy Lab of Universitas Sumatera Utara. The gel contained propolis extract, aquadest, triethanolamine, and carbopol. Metronidazole gel was used as the positive control because its usage as adjunctive therapy for periodontitis treatment with its antibacterial and anti-inflammation properties. 19 Metronidazole gel was obtained from Ti-es, Indonesia.

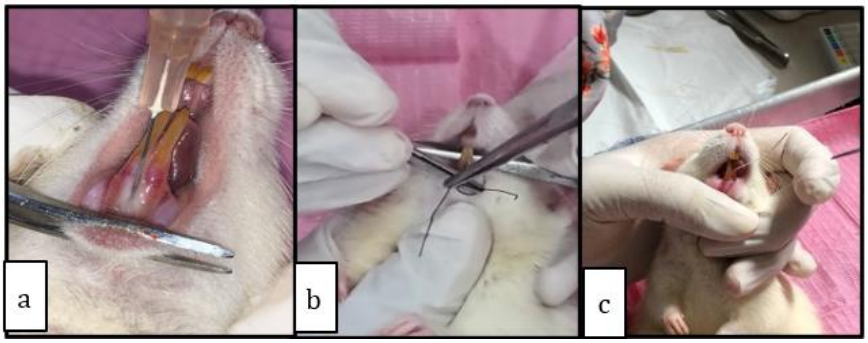

Fig. 1. a) Periodontitis induction with *P. gingivalis*. b) ligation with silk. c) obtaining gingival crevicular fluid with the paper point

There were three treatment groups: the periodontitis-induced rats treated with 50% (I), 60% (II), and 70% (III) propolis extract gel. The positive control was the periodontitis-induced rats treated with metronidazole gel 25%. The negative control was the periodontitis-induced rats treated with placebo gel. Mechanical therapy was done by using a manual scaler. Then, depending on the group, the gel was applied once after the mechanical therapy was done into the pocket of the gingiva by using a disposable tip. Five rats from each group were anesthetized with ketamine on the baseline before applying the gel, and gingival sulcular fluid was taken with a paper point into the pocket (fig. 1c). The paper points were put into the 1,5 ml Eppendorf tube with 0,5 ml phosphate-buffer saline (PBS). The same procedure was performed on the Wistar rats on the 7th d.

The clinical parameter evaluated was the MMP-8 levels from the gingival crevicular fluid using Enzyme-Linked Immunosorbent Assay (ELISA) (Merck, Germany) at Integrated Laboratory, Faculty of Medicine, Universitas Sumatera Utara. An experienced clinician examined the clinical parameters. The kit made use of a biotinylated rat MMP-8 detection antibody. The sample from gingival crevicular fluid in the Eppendorf tubes was centrifuged to dilute the supernatant in PBS for 10 min at 24 °C and 3500 rpm. The total MMP-8 level was determined in nanograms (ng), and the concentration in each sample was calculated by dividing the enzyme amount by the sample volume (ng/ml). The absorbance of the substrate color reaction was read on an ELISA reader using 450 nm wavelengths. Phytochemical analysis of propolis extract was done in quantitative and qualitative phytochemical tests at the Phytochemical Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. The gallic acid equivalent was used to measure phenolics, and the Quercetin equivalent was used to measure the flavonoids of the extract.

The data obtained from the study were quantitative in the form of laboratory parameters before and after treatment on days 0 (baseline) and 7. The Kruskal-Wallis test processed the data to see the difference between the groups on the baseline and seventh day.

RESULTS

Table 1 shows the results of the qualitative phytochemical analysis of the propolis extract. It contained alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids. Table 2 shows the total phenolics and flavonoids of the propolis extract: 4.51 mg/g and 13.92 mg/g, respectively. The results of the inhibition zone measurements for all treatment groups on the growth of the bacterium *P. gingivalis* ATCC 33277 are tabulated in table 3.

Table 1: Qualitative propolis extracts phytochemical test results

Secondary metabolites	Reactor	Results
Alkaloids	Dragendroff	+
	Bouchardat	+
	Meyer	+
Flavonoids	Mg Powder+Amyl Alcohol+HCl $_p$	+
Glycosides	Molish+H_2 SO $_4$	+
Saponins	Hot/shaken water	+
Tannins	$FeCl_3$	+
Triterpenoids/Steroids	Lieberman-Bourchat	+

The (+) sign indicates that the tested extract contains these metabolites.

Table 2: Quantitative propolis extract phytochemical test results

Types	Content (mg/g)
Total Flavonoids Levels	4.51 (0.45%)
Total Phenolics Levels	13.92 (1.39%)

Table 3: The resistance category and the differences of antibacterial effectiveness between treatment groups

Groups	Mean diameter of inhibition in mm (Zone±SD)	Resistance category	p-value
50%	11.58±0.14	Strong	0.002*
60%	12.50±0.00	Strong	
70%	13.25±0.25	Strong	
Chlorhexidine 0.2%	11.84±0.01	Strong	
DMSO	0±0	Weak	

≤5 mm (weak); 6-10 mm (moderate); 11-20 mm (strong); ≥21 mm (very strong), Kruskal-Wallis Test. *Significant (p<0.05)

Propolis extracts with a concentration of 70% have the largest average inhibition zone of 13.28 mm. They are included in the category of strong inhibition and comparable to 0.2 % chlorhexidine, which has an average inhibition zone of 11.84 mm.

The data analysis then proceeded using the Kruskal-Wallis and PostHoc Mann-Whitney tests. The Kruskal-Walli's test (table 4) was used to see if there was a significant difference between all treatment groups. There was a significant difference among the treatment groups. Furthermore, the Mann-Whitney test was carried out to test whether there was a difference in the diameter of the inhibition zone between each two of the test groups. The results of the Mann-Whitney test can be seen in table 5.

Table 4: The differences in the average diameter of the inhibition zone between treatment groups

Groups	P-value				
	50%	60%	70%	Chlorhexidine 0.2%	DMSO
Propolis 50%	-	0.000*	0.000*	0.023*	0.000*
Propolis 60%	0.000*	-	0.000*	0.000*	0.000*
Propolis 70%	0.000*	0.000*	-	0.000*	0.000*
Chlorhexidine 0.2%	0.023*	0.000*	0.000*	-	0.000*
DMSO	0.000*	0.000*	0.000*	0.000*	-

*Mann whitney test; significant (p<0.05)

The post Hoc test in table 5 shows that propolis extracts with concentrations of 50%, 60%, 70%, and 0.2% chlorhexidine showed a significant difference in inhibition between treatment groups against *P. gingivalis* (p<0.05). DMSO showed a significant difference in inhibition between treatment groups against *P. gingivalis* bacteria with a p<0.05. MMP-8 analysis was performed using the enzyme-linked immunosorbent assay (ELISA). The results of measuring the average concentration of MMP-8 in the rat gingival crevicular fluid in all treatment groups can be seen in table 5.

Table 5: The differences in GCF MMP-8 concentration between groups on day 7

Groups	Median (ng/ml)	Min-Max (ng/ml)	P-value
Propolis 50% H-7	1657.123	479.254-3849.752	
Propolis 60% H-7	4113.736	2498.324-5946.603	
Propolis 70% H-7	1954.632	781.922-2300.846	0.001*
Metronidazole H-7	2070.939	837.784-2981.886	
Placebo H7	1954.632	2201.205-3060.629	

*Kruskal-wallis; Significant (p<0.05), n=6

There was a significant difference in the mean MMP-8 concentration between treatment groups (p<0.05). The 70% propolis gel test group had the lowest concentration of the other.

DISCUSSION

Periodontitis is an inflammatory disease caused by the infection of

the periodontium. Various predisposing factors are involved in the development and progression of periodontal diseases. The hallmark features of periodontitis are activation of osteoclast genesis and consequent destruction of alveolar bone, which is irreversible and can lead to loss of tooth-supporting tissue [20]. The mechanisms underlying periodontal disease, particularly the delicate balance between the immune system and biofilms, have been the subject of research [21]. Research on natural ingredients in dentistry has continued to increase in recent years to find natural products with comparable therapeutic activity, lower toxicity, better biocompatibility, and improved affordability to the public [22, 23]. Propolis is a non-toxic resinous substance produced by bees that has antimicrobial, antifungal, anti-inflammatory, antioxidant, and antitumor properties and has attracted the attention of researchers both in the fields of medicine and dentistry [24, 25]. The chemical composition of propolis depends on geographical location, botanical origin, and bee species [6, 26]. However, its composition is quite complex. The main components of propolis are flavonoids and phenolics such as caffeic acid phenethyl ester [27, 28].

The propolis bioactive compounds are useful for treating aphthous ulcers, candidiasis, gingivitis, and periodontitis due to their therapeutic properties [29, 30]. In addition, the propolis from stingless bees (>600 species) also has similar biological activities and therapeutic benefits, namely antioxidant and anti-inflammatory properties and antimicrobial activity [18]. Table 1 illustrates the outcomes of a comprehensive qualitative phytochemical analysis performed on the propolis extract. The meticulous examination, employing various chemical tests, discerned the presence of specific secondary metabolites. It highlights the detection of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids. The notation of a positive sign in each instance indicates the unequivocal presence of these metabolites in the propolis extract. Table 2 outlines the quantitative analysis results for the propolis extract and details the amounts of two critical components in the propolis extract: 4.51 mg/g of flavonoids and 13.92 mg/g of phenolics. These findings are further detailed, indicating that flavonoids comprise 0.45% of the extract, while phenolics constitute a larger portion at 1.39%. Flavonoids are a very important class of polyphenols, as they are plant compounds with antimicrobial, antioxidant, and anti-inflammatory properties [31]. Their anti-inflammatory property stimulates phagocytic activity and cellular immunity. Propolis contains zinc and iron metal cations, which are essential during collagen synthesis, flavonoids and phenolic acid esters, that are effective in reducing the inflammatory response by inhibiting the arachidonic acid lipoxygenase pathway. In addition to its significant effect on the immune system, they promote cellular phagocytic activities [32]. The Caffeic Acid Phenethyl Ester (CAPE) also has a cytoprotective function and protects against the oxidative effects of inflammatory DNA pathologies [33]. One of the discussed possible mechanisms of the antimicrobial activity of propolis is the cinnamic acid and flavonoid components, which change the ion permeability of the inner bacterial membrane, causing membrane potential dissipation and inhibition of bacterial motility [34].

In the scope of this investigation, mice were deliberately exposed to *P. gingivalis*, an anaerobic gram-negative oral bacterium, which is considered a critical pathogen involved in the onset and development of periodontitis [35, 36]. The presence of *P. gingivalis* in the periodontal pocket has been reported to influence the development of periodontitis. The presence of *P. gingivalis* positively correlates with the periodontal pocket depth [37]. The fimbriae of *P. gingivalis* are thin and fibrous. These characteristics facilitate the adhesion of *P. gingivalis* to host cells and other bacteria and significantly promote biofilm development [38]. Table 3 presents the antibacterial effectiveness of different treatment groups, revealing that propolis extracts at a concentration of 70% exhibit the most robust antimicrobial activity. This is evidenced by the largest average inhibition zone observed, measuring 13.28 mm, and is categorized as a strong inhibitory effect.

Remarkably, this level of effectiveness is comparable to that of 0.2% chlorhexidine, as indicated by an average inhibition zone of 11.84 mm. These findings underscore the notable potency of the 70% propolis extract in inhibiting bacterial growth, emphasizing its potential significance in antimicrobial applications. The result, as presented in table 4, reveals statistically significant differences in inhibition zone diameters across all pairs of treatment groups ($p<0.05$). All three concentrations of propolis extract (50%, 60%, and 70%) exhibit statistically significant differences in inhibition zone diameters when compared to each other and DMSO. The 70% propolis extract stands out with the most notable difference compared to 0.2% chlorhexidine. This aligns with Yoshimasu *et al.* (2018) research, which proposed that propolis bioactive compounds can provoke *P. gingivalis* membrane depolarization, increasing its permeability. This observed effect suggests a potential antimicrobial mechanism of action for propolis against *P. gingivalis*.39,40 Nakao *et al.* (2020) stated that treatment with propolis significantly reduced pocket depth and improved clinical adhesion levels, along with a tendency to decrease *P. gingivalis*.41 Research by Veloz *et al.* (2019) stated that the polyphenols contained in propolis exhibit antimicrobial activity against Streptococcus mutans [42].

Research by Nazir *et al.* (2018) found that propolis extracted from stingless bees *Geniotrigona thoracica* contains terpenoid metabolite compounds, which have antibacterial, anti-inflammatory, and antioxidant activities [43, 44]. The anti-inflammatory effects of propolis are proven effective in improving the condition of periodontal disease based on Gingival Index (GI) and bleeding on probing (BOP) scores [45]. Although there is not much information regarding the mechanism of propolis' antimicrobial action, several researchers have stated that several propolis compositions play a role in this activity and that flavonoids are components commonly known to play a role in antimicrobial activity [6,46]. Research by Koo *et al.* (2002) stated that propolis plays a role in the enzymatic activity of *Streptococcus mutans* and *Streptococcus sanguis*, through the action of flavonoids, especially apigenin, as an inhibitor of the glucosyltransferase enzyme, which is an important factor in the formation of dental plaque [46]. Research by Coutinho (2012) states that subgingival irrigation with propolis extract as a periodontal support treatment is more effective than scaling and root planing alone, as assessed by clinical and microbiological parameters [47]. Research by Skaba *et al.* (2013) stated that there was a beneficial effect of propolis toothpaste gel on regular patients and periodontitis patients [48]. Research by Gonzalez *et al.* (2023) showed that propolis mouthwash was effective in reducing bacterial proliferation, especially in bacteria that were effective with chlorhexidine, and with the added benefit that propolis can avoid the typical staining of the mouth/tongue compared to using chlorhexidine [49].

Type I collagen represents the most significant component of the periodontal extracellular matrix; therefore, special attention has been paid to collagenase. Type I collagen contributes to a large amount of periodontal extracellular matrix, particularly collagenases and gelatinases such as MMP-8, MMP-13, MMP-2, and MMP-9 in periodontitis. Matrix metalloproteinases (MMPs) are essential proteases involved in periodontitis. They are associated with periodontal status [50, 51]. Matrix metalloproteinases (MMPs) are protein enzymes that have an important role in extracellular matrix degradation and reconstruction. MMPs are regulated by some inhibitors and are released and activated when necessary to degrade the extracellular matrix in some periodontal conditions, either healthy or pathogenic. Periodontopathogens can contribute to an imbalanced condition between MMPs and their inhibitors, resulting in host destruction [52]. Moreover, 90% to 95% of the collagenolytic activity in the gingival sulcus fluid comes from MMP-8. Therefore, matrix metalloproteinase-8 is considered one of the most promising biomarkers for analyzing the incidence and severity of periodontitis in gingival sulcus fluid [53].

The analysis of MMP-8 was conducted through enzyme-linked immunosorbent assay (ELISA), and the results detailing the average concentrations in the rat gingival crevicular fluid for all treatment groups are presented in table 5. The statistical analysis test reveals statistical significance ($p<0.05$), emphasizing differences in GCF MMP-8 concentrations among the treatment groups on day 7. Al-Majid *et al.* (2018) demonstrated that the increase in the level of active MMP-8 can differentiate periodontitis from gingivitis and predicted periodontal attachment loss. Furthermore, ongoing destruction of the

active periodontal tissues can be identified non-invasively in oral fluids by the pathologically elevated MMP-8 levels [12, 54, 55]. Previous longitudinal studies have also shown that MMP-8 levels can predict periodontitis progression and attachment loss. It also helps to monitor periodontitis treatment during the maintenance phase [14, 56]. Increased levels of salivary MMP-8 are correlated with periodontitis significantly [57–59]. Gupta *et al.* (2015) evaluated salivary MMP-8 levels in patients with chronic periodontitis compared to healthy individuals using the ELISA method. It was concluded that MMP-8 levels in the saliva of periodontitis patients were higher than those of healthy individuals [60]. De Morais *et al.* (2018) stated that MMP-8 concentrations were higher in periodontitis patients compared to the control group, and patients with advanced stages had higher MMP-8 concentrations. These findings indicate the potential use of MMP-8 as support in diagnosing periodontal disease [57]. The systematic review by Morais *et al.* (2018) stated that high MMP-8 levels correlated with developing gingivitis to periodontitis [57]. An essential biological function of MMP-8 is to facilitate the migration of leukocytes, especially granulocyte neutrophils, from the blood circulation to the periodontal sulcus by cleavage of collagen and other extracellular matrix components [61]. Other cell types, such as fibroblasts, also express MMP-8 when inflammation begins [62].

Miller *et al.* (2021) demonstrated that MMP-8 has the strongest positive correlation as salivary biomarker for discriminating periodontitis in the presence of diabetes, along with probing depth (PD)>5 mm ($p<0.001$) [63]. The findings indicate that high salivary concentrations of MMP-8 are associated with a patient having periodontitis [64, 65]. Soft tissue changes, including the destruction of the collagen matrix, can be assessed via MMP-8 and the production of pro-inflammatory mediators. MMPs are involved in tissue-destructive inflammatory processes, resulting in the breakdown of the membrane matrix, and MMP-8 is known to play a role in periodontitis.11 MMP-8 as a biomarker has been investigated, and Arakawa *et al.* showed that increased MMP-8 was detected in sites with ongoing bone loss [66].

Considering that MMP-8 is expressed in periodontitis in patients, this study observed MMP-8 levels in our mouse model of periodontitis and highlights the utility and translation of mouse studies back to humans. MMP-8 is an extremely valuable diagnostic tool in treating periodontitis, and future studies and healthcare policies should focus on implementing more accessible methods of chairside testing to reduce the prevalence of this disease [15]. MMP-8 can be detected in the sulcular fluid, which is associated with increased activity of annual vertical bone loss. In these cases, MMP-8 can be considered a possible marker for progressive bone loss in periodontitis. This finding is in line with other cross-sectional studies showing that GCF levels of MMP-8 is correlated with disease activity in patients with chronic periodontitis [58, 67]. Persistent increase of MMP-8 in GCF samples is considered a high risk of poor response to periodontal therapy [16]. The MMP-8 expression is stimulated by IL-1β and inhibited by insulin-like growth factor-1 [11, 68]. MMP-8 is one of the most abundant MMPs in periodontal tissues, and their level reflects the severity of the disease and its progression and response to treatment. They are secreted due to the infiltration of polymorphonuclear leukocytes and macrophages, plasma, and residual cells such as fibroblasts, endothelial cells, keratinocytes, and bone cells [69]. Proteolytic cascades can lead to extensive destruction of the periodontal tissue due to the activation of MMPs and could be an interesting target for diagnosis and therapy [15].

Low MMP-8 levels were associated with periodontal health, while the upregulation of MMP-8 levels denoted an increased risk for inflammation [70]. MMP-8 levels were related to the degree of healing process and osseointegration, indicating their functional role in the periodontal tissues [15]. Propolis groups and chlorhexidine, consistent with Konopka *et al.* (2012) and Zarch *et al.* (2021), showing that clinical parameters and the concentrations of humoral factors have no significant correlations one month after treatment. Longer follow-up of patients could have resulted in differences between groups in levels of MMP-8 reduction [52, 71]. Clinical trial research continuously needs to evaluate propolis formulations or population-based interventions to discover the potential of natural-based materials in gel preparations to support periodontitis treatment, which can affect the healing condition of periodontitis patients.

CONCLUSION

Propolis is a material that can be used as a supporting therapy for periodontitis, which can help eliminate inflammation and help the healing process. Propolis extract gel 70% is effectively used as a supportive treatment for periodontal therapy.

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AUTHORS CONTRIBUTIONS

PW, FZ, OAH, IE, N, JM, JMS, DZA, RL: Researcher, Critically Revised the Manuscript. PW: Writing, Analysis, Interpretation. PW, FZ, OAH, IE, N: Conception and Data Design. PW, FZ, JM, JMS, DZA: Analysis, Writing, and Performed the Experiments.

CONFLICT OF INTERESTS

Declare that there is no conflict of interest regarding the publications of this paper.

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