

Antioxidant and antimicrobial effects of polyherbal toothpaste against oral pathogens *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Candida albicans*

Euis Reni Yuslianti^{1*}, Agus Susanto², Afifah Bambang Sutjiatmo³, Wahyu Widowati⁴, Vini Ayuni⁵, Dwi Nur Triharsiwati⁵, Dhanar Septyawan Hadiprasetyo^{3,5}, Ignatius Marcelino Kurnia Putra⁶ and Jeffrey Jeffrey¹

¹Faculty of Dentistry, ³Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Cimahi 40531, Indonesia

²Faculty of Dentistry, Universitas Padjadjaran, Sumedang 45363, Indonesia

⁴Faculty of Medicine, Maranatha Christian University, Bandung 40164, Indonesia

⁵Biomolecular and Biomedicine Research Center, Aretha Medika Utama, Bandung 40163, Indonesia

⁶Faculty of Mathematics and Science Education, Universitas Pendidikan Indonesia, Bandung 40154, Indonesia

Received 28 April 2025; revised received 23 February 2026; accepted 26 February 2026

Maintaining oral health is essential for preventing dental plaque, and toothpaste is a primary tool for supporting oral hygiene. Herbal-based toothpaste is considered a safer alternative due to its natural bioactive compounds, which have antimicrobial and antioxidant properties. This study was conducted to assess the antioxidant and antimicrobial activities of a toothpaste formulation containing royal jelly, black cumin, ginger, and cinnamon extracts. Antioxidant activity was assessed using DPPH, ABTS, H₂O₂, and NO scavenging assays, while antimicrobial activity was evaluated by the disk diffusion method against *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Candida albicans*. The polyherbal toothpaste mix exhibited the highest scavenging activity in the DPPH (50.89%), ABTS (15.71%), NO (33.97%), and H₂O₂ (23.04%) assays, and showed significant antimicrobial activity against three representative oral pathogens at 100% concentration. The polyherbal toothpaste mix demonstrated significant antioxidant and antimicrobial properties, supporting its potential application as a natural dental care product.

Keywords: Antimicrobial, Antioxidant, Herbal, Oral Pathogens, Toothpaste

IPC code; Int. cl. (2021.01)– A61K 36/00, A61K 35/64, A61K 35/644, A61K 8/9783, A61K 8/98, A61P

Introduction

Dental plaque is an intricate biofilm that is formed on the surface of teeth as a result of bacterial colonisation and adhesion to the enamel. This biofilm consists of about 500 different bacterial species that colonise the teeth in an organised manner¹. Examples of microorganisms commonly found in dental plaque include *Streptococcus mutans*, *Porphyromonas gingivalis*, *Candida albicans*, and other pathogens. *S. mutans* is the predominant causative agent of dental caries. This bacterium produces glucosyltransferase enzymes that synthesise extracellular polysaccharides, forming a sticky glycocalyx layer that promotes bacterial adhesion and biofilm formation on the tooth surface². *P. gingivalis*, in contrast, is a key periodontal pathogen associated with chronic periodontitis. It induces pathological changes in periodontal tissues by activating

host immune and inflammatory responses, resulting in the breakdown of the teeth's supporting components³.

In addition to bacterial pathogens, fungal species such as *C. albicans* are also detected in dental plaque, particularly in children with severe early childhood caries. *C. albicans* is a yeast that commonly exists in the human oral cavity, contributing to the formation of dental plaque⁴. Unlike cariogenic and periodontopathic bacteria, *C. albicans* contributes to oral disease by adhering to oral surfaces, forming biofilms, and interacting synergistically with *S. mutans*. Co-infection with *S. mutans* enhances microbial colonisation and accelerates the progression of carious lesions⁵.

Several factors, including diet and immune response, can interfere with plaque stability, thereby playing a part in the development of oral conditions like dental caries. Consequently, the bacterial composition in diseased areas differs significantly from that in healthy sites⁶. The buildup of dental plaque can swiftly initiate gingivitis and, if left unmanaged over time, may progress to

*Correspondent author
Email: ery.unjani@yahoo.co.id

deterioration of periodontal attachment and the resorption of alveolar bone. In addition, chronic plaque can cause tooth demineralisation and decay through caries development. Effective plaque management encompasses the preservation of oral health, optimal treatment, and prevention of periodontal disease recurrence⁷. Oral hygiene has a critical role in controlling plaque formation; thus, efficient mechanical cleaning—such as toothbrushing—is necessary for maintaining oral health. This mechanical action can be enhanced by chemical agents, including toothpaste formulations containing antibacterial ingredients, which are vital for inhibiting plaque growth⁸. In addition to antibacterials, toothpaste should also contain antioxidant ingredients. The antioxidant agents can help prevent tooth and gum damage caused by bacteria. Bacteria in dental plaque can produce free radicals that can damage tooth and gum tissue and cause inflammation. Antioxidants can neutralise free radicals by either donating electrons to them or by preventing them from taking electrons from healthy cells⁹. Antioxidants are commonly found in various herbal plant constituents.

Herbal plants are now widely used as ingredients in a variety of products, including toothpaste. Nowadays, with the progression of technology and science, numerous toothpaste industries initiate innovation by adding substances that are advantageous for dental health. Of course, the ingredients in toothpaste should be safe and effective. According to SNI No. 12-3524-1994, the appearance of a good toothpaste should be soft, homogeneous, with no air bubbles, no lumps, no foreign objects, and no separated particles. In addition, the content it contains must not exceed the limits recommended by the health department. Common ingredients in toothpaste include fluoride and other functional additives that contribute to oral health. One of the predominant substances added to toothpaste is herbal ingredients. Various types of herbs can suppress the growth of microorganisms. In addition, since herbs come from plants, they are considered safe and organic. Herbal extracts derived from plants such as black cumin (*Nigella sativa* L.), ginger (*Zingiber officinale* L.), and cinnamon (*Cinnamomum burmannii* L.) are known for their ability as antimicrobial agents, thus making them potential ingredients for herbal toothpastes¹⁰⁻¹². Bioactive compounds in black cumin, including polyphenols, volatile oils, and various phytochemicals, have demonstrated antimicrobial properties by suppressing the growth and activity of microorganisms. Key constituents such as thymoquinone, thymol, and p-cymene contribute significantly to these effects. Ginger exhibits antimicrobial effects attributed to its

constituents, such as gingerol, paradol, shogaol, and zingerone¹³. In addition, cinnamon contains major bioactive compounds, such as cinnamaldehyde, eugenol, and cinnamic acid¹⁴.

Combining multiple herbal extracts has been shown to further enhance their synergistic effects, in which the active components of herbs work together to enhance their antimicrobial properties¹⁵. To date, there has been no specific investigation into the incorporation of a combined extract of black cumin, ginger, royal jelly, and cinnamon in toothpaste formulations. Other studies have explored different herbal plant combinations, such as orange peel extract (*Citrus sp.*) and mint leaf extract (*Mentha piperita* L.)¹⁶, as well as betel leaf extract (*Piper betle*) and lime peel extract (*Citrus limon burm f.*)¹⁷. In this study, a combination of black cumin, ginger, and cinnamon extracts was used as active ingredients in the toothpaste formulation named toothpaste mix extract (TPME). The addition of extract is expected to increase the effectiveness of toothpaste. Therefore, this study's objective is to evaluate the antioxidant by DPPH, ABTS, NO, and H₂O₂ scavenging activity and antimicrobial activity of TPME containing black cumin, ginger, and cinnamon extract towards *P. gingivalis*, *S. mutans*, and *C. albicans*.

Materials and Methods

Chemicals used

2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, D9132), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich, A1888), dimethyl sulfoxide (DMSO; Sigma-Aldrich, 1029521000), ferrous ammonium sulfate (Merck, 1.03792.1000), hydrogen peroxide (H₂O₂; Merck, 1.08597.1000), 1,10-phenanthroline (Merck, 1.07223.0010), sodium nitroprusside (SNP; Merck, 1065410100), sulfanilamide (Merck, 1117990100), phosphoric acid (H₃PO₄; Merck, 100573), and N-(1-naphthyl)ethylenediamine dihydrochloride (Merck, 1062370025) were used in this study. Mueller–Hinton agar (HiMedia, M173) was used for antimicrobial assays.

Toothpaste mix extract (TPME) preparation

The plant extraction process was carried out at PT Borobudur Industri Jamu, Semarang, and was standardised in accordance with the Indonesian Good Manufacturing Practice for Traditional Medicines (CPOTB/cGMP). The cinnamon (CoA No. 048PU01.7), black cumin (CoA No. 075PU02.1), and ginger (CoA No. 026PU01.6) extractions were carried out using 70%

ethanol solvent. All extracts were mixed with excipients (maltodextrin) to obtain a dry extract¹⁷. The standard toothpaste base consisted of fluoride, glycerin, CMC-Na, and methyl paraben. The extracts of cinnamon, secang wood, black cumin, ginger, and royal jelly were then mixed into the prepared standard formula paste base. Following formulation, the toothpaste was subjected to organoleptic testing by two assessors, who evaluated the product based on its visual appearance, odour, and taste. The toothpaste mixture was prepared in five different formulations, as shown in Table 1.

DPPH scavenging activity

DPPH scavenging activity was determined using a previously described method¹⁸. In brief, a 96-well plate was filled with 50 μ L of sample, followed by the addition of 200 μ L of 0.077 mmol DPPH. For the blank well, 250 μ L of 10% DMSO was added, while 250 μ L of 0.077 mM DPPH was added to the control well. The plate was incubated for 30 minutes at room temperature in the dark, and the absorbance was subsequently measured at 517 nm.

ABTS scavenging activity

ABTS reduction activity was measured using the previous method¹⁹. Two microliters of each sample concentration were added to individual wells of a 96-well plate, followed by the addition of 198 μ L of ABTS solution to each sample well. For the control, 200 μ L of ABTS solution was added without the sample. A blank was prepared by adding 200 μ L of 10% DMSO to a separate well. The plate was incubated for 6 minutes at 37°C. The absorbance was measured at 745 nm.

H₂O₂ scavenging activity

The scavenging activity of H₂O₂ was examined employing the previous method²⁰. 60 μ L of sample

extract was added to the 96-well plate. Following the addition of the sample, 12 μ L of 1 mM ferrous ammonium sulfate was administered to each well sample and well control. For the well control, 63 μ L of 10% DMSO was added; for the well sample, 90 μ L of 10% DMSO was added. Well samples were then treated with 3 μ L of 5 mM H₂O₂ and incubated at room temperature in the dark for 5 minutes. 75 μ L of 1,10-phenanthroline was added to the sample and control solutions, and the mixture was incubated again for 10 minutes in a dark room at room temperature. The absorbance value was obtained at 510 nm wavelength.

NO scavenging activity

The nitric oxide (NO) scavenging activity was assessed using the previously described method²¹. A volume of 10 μ L was dispensed into both the sample and blank wells. Subsequently, 40 μ L of SNP was added to the sample and control wells, whereas the blank well received 140 μ L of 10% DMSO, and the control well received 10 μ L of 10% DMSO. The microplate was then incubated under ambient light conditions for 2 hours. Following incubation, 100 μ L of Griess Reagent—comprising 1% sulfanilamide, 2% H₃PO₄, and N-(1-naphthyl)ethylenediamine dihydrochloride with a concentration of 0.1%—was added to the control and sample wells. Absorbance readings were obtained at 516 nm.

Antimicrobial assay

The antimicrobial assay was performed using the disk diffusion method²². The disk diffusion method was selected as a preliminary screening assay due to its simplicity, reproducibility, and suitability for evaluating the antimicrobial potential of semi-solid formulations such as toothpaste²³. A sterile cotton swab was inserted into the microbial suspension of *P. gingivalis* (ATCC 33277), *C. albicans*, and *S. mutans* (ATCC 25175), then inoculated on the surface of Mueller-Hinton Agar, and subsequently let stand for half an hour at room temperature. The 6 mm paper discs were soaked in the sample, positive, and negative control solutions, and then placed on the plate. The plates were incubated for 24 hours at 37°C under aerobic conditions for *S. mutans* and *C. albicans*. The *P. gingivalis* plates were incubated under anaerobic conditions for 24 hours at 37°C and 2% CO₂. The inhibition zone was measured with a vernier on each disc, and the results obtained were divided by three to determine the average value.

Table 1 — Composition of herbal toothpaste formulation

Ingredients	Formula (%)				
	F1	F2	F3	F4	F5
Royal jelly	0	0.2	0.4	0.6	0.8
Secang extract	0	0.2	0.4	0.6	0.8
Black cumin extract	0	0.2	0.4	0.6	0.8
Ginger extract	0	0.2	0.4	0.6	0.8
Cinnamon extract	0	0.2	0.4	0.6	0.8
Fluoride	0.24	0.24	0.24	0.24	0.24
Hydroxyapatite	45	50	50	50	50
Calcium carbonate	47	47	47	47	47
Sodium CMC	3.5	1.5	1.5	1.5	1.5
Xylitol	0.26	1.26	1.26	1.26	1.26

Statistical analysis

All statistical evaluations were conducted using SPSS version 20.0. Normality of distribution and homogeneity of variance were examined using the Shapiro–Wilk and Levene's tests. Parametric data were analysed using one-way ANOVA, followed by Tukey's HSD test for post hoc analysis. When the data did not meet parametric requirements, the Kruskal–Wallis test was used, and the Mann–Whitney U test was used for pairwise comparisons. Differences were considered statistically significant when $p < 0.05$.

Results

Organoleptic test

The TPME was characterised by its visual appearance, odour, and taste. The product has a light brown colour with a distinctive herbal aroma, a sweet taste with mint and ginger flavours, and producing moderate foam. All assessors who conducted organoleptic tests preferred the taste and aroma of multi-extract toothpaste. Therefore, the TPME can be used as an oral product, and further tests can be carried out.

DPPH scavenging activity

The DPPH assay is a well-known technique for assessing the ability of an antioxidant to neutralise free radicals that contribute to oxidative stress. Fig. 1a shows the ability of TPME to scavenge DPPH. An increase in DPPH radical scavenging activity was observed in a manner dependent on concentration as the concentration of TPME increased. The minimum activity was detected at 3.13 $\mu\text{g/mL}$ (23.25%), whereas the maximum was at 100 $\mu\text{g/mL}$ (50.89%), $p < 0.05$. According to Table 1, the IC_{50} value for TPME was $95.08 \pm 3.15 \mu\text{g/mL}$, indicating moderate antioxidant potential.

ABTS scavenging activity

The ABTS assay evaluates antioxidant capacity by measuring how effectively a sample reduces a blue-green radical solution. The ABTS scavenging activity of the multi-extract toothpaste is presented in Fig. 1b. The data indicate a positive correlation between the concentration of the sample and its reduction activity, suggesting that an increase in sample concentration results in higher reduction activity of TPME against

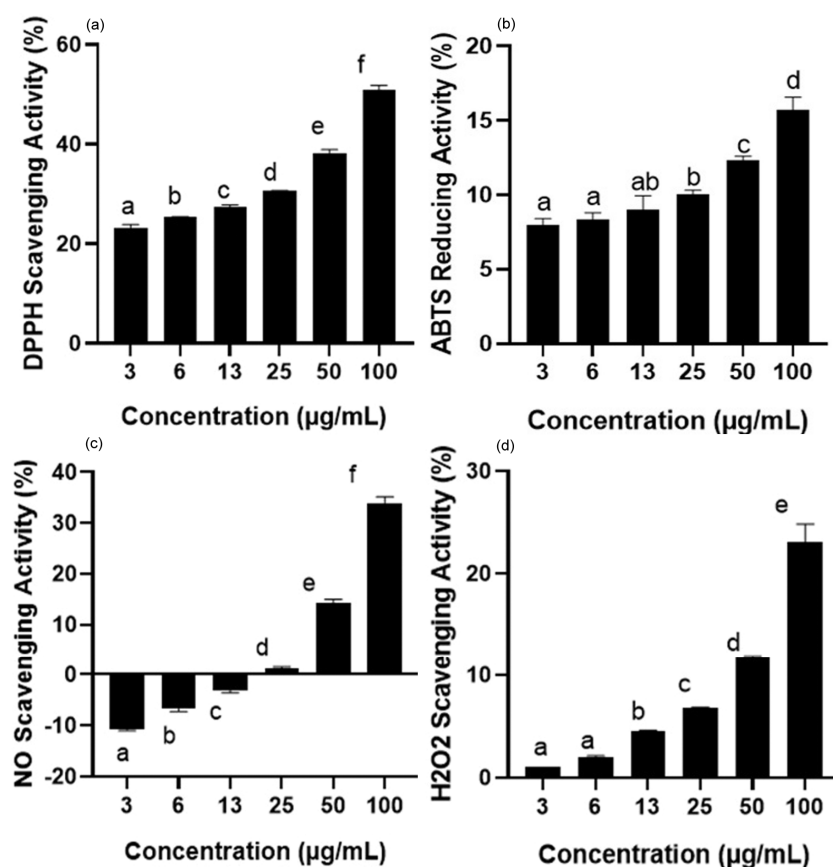


Fig. 1 — Effect of various concentrations of TPME on antioxidant scavenging activities: (a) DPPH, (b) ABTS, (c) NO, and (d) H₂O₂. *Data are presented as mean \pm standard deviation; tests were conducted in three replications. Different letters indicate significant differences between treatments by Post Hoc Tukey HSD test ($P < 0.05$).

ABTS radicals across all concentrations, as demonstrated in Fig. 1b. The peak ABTS radicals reduction activity was detected at a concentration of 100 $\mu\text{g/mL}$ ($p < 0.05$). Additionally, Table 1 presents the IC_{50} value of TPME, which is 548.90 ± 55.10 $\mu\text{g/mL}$.

NO scavenging activity

This test assesses a sample's capacity to neutralise nitric oxide radicals, reactive substances that, in excess, can cause tissue damage and inflammation. The NO scavenging activity of TPME is illustrated in Fig. 1c. The results show a direct relationship between the sample concentration and its scavenging activity, indicating that higher concentrations of the sample correspond to greater NO scavenging activity of TPME across all concentrations. TPME did not demonstrate any measurable NO scavenging activity at concentrations of 3.13, 6.25, and 12.5 $\mu\text{g/mL}$. The highest NO scavenging activity, with 33.97% inhibition, was observed at 100 $\mu\text{g/mL}$ ($p < 0.05$).

H_2O_2 scavenging activity

Reactive oxygen species, such as hydrogen peroxide, can generate more dangerous free radicals within cells. The ability of a material to neutralise or break down hydrogen peroxide before it causes harm is measured by the H_2O_2 scavenging assay. Fig. 1d shows the antioxidant activity of the toothpaste samples at varying concentrations. There was a statistically significant positive association found between sample concentration and H_2O_2 scavenging capacity ($p < 0.05$). An incremental increase in concentration corresponded with an enhanced ability to neutralise H_2O_2 . The maximum scavenging activity, observed at 100 $\mu\text{g/mL}$, was 23.04%.

The inhibitory concentration of TPME was assessed and is shown in Table 2. Antioxidant activity was determined using the IC_{50} value. This study found that TPME has higher antioxidant activity in the DPPH scavenging assay than in the ABTS, NO, and H_2O_2 scavenging assays. Based on the IC_{50} classification, TPME showed strong antioxidant activity in the DPPH assay (50–100 $\mu\text{g/mL}$), moderate activity in the NO assay (101–150 $\mu\text{g/mL}$), and weak activity in the ABTS and H_2O_2 assays.

Table 2 — IC_{50} value of TPME

Antioxidant Assay	IC_{50} ($\mu\text{g/mL}$)
DPPH	95.08 ± 3.15
ABTS	548.90 ± 55.10
NO	133.62 ± 3.63
H_2O_2	229.96 ± 9.28

Antimicrobial and antifungal activity of TPME against *S. mutans*, *C. albicans*, and *P. gingivalis*

Fig. 2 demonstrates that the TPME exhibited inhibitory activity against *C. albicans*, *S. mutans*, and *P. gingivalis*. For *C. albicans*, TPME exhibited the greatest inhibition at a 100% concentration, resulting in an inhibition zone of 4.23 mm, and maintained its

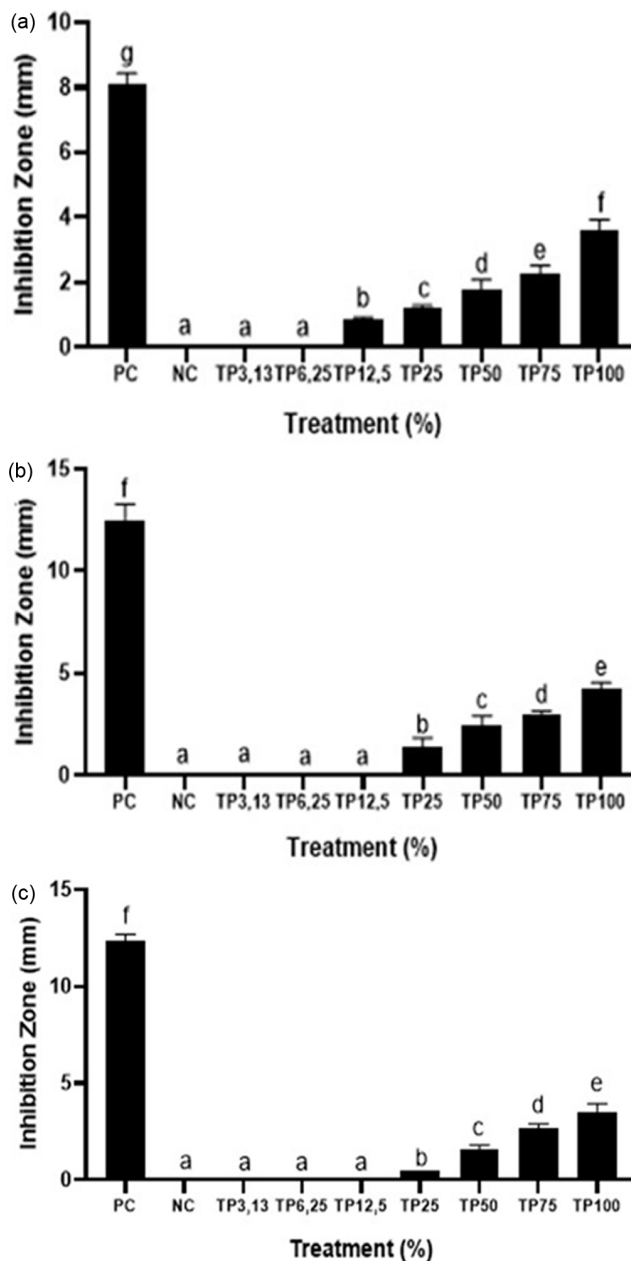


Fig. 2 — Effect of various concentrations of TPME against microorganisms. (a) *S. mutans*, (b) *C. albicans*, and (c) *P. gingivalis*. *Data are presented as mean \pm standard deviation; tests were conducted in three replicates. Different letters indicate significant differences between treatments based on the Kruskal-Wallis Test and the Mann-Whitney test.

Table 3 — Inhibitory zone measurements of TPME against *S. mutans*, *C. albicans*, and *P. gingivalis*

Treatment (%)	<i>S. mutans</i> (mm)	<i>C. albicans</i> (mm)	<i>P. gingivalis</i> (mm)
Positive control	8.10±0.35 ^g	12.50±0.75 ^f	12.35±0.40 ^f
Negative control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
TP 3.13	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
TP 6.25	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
TP 12.5	0.85±0.10 ^b	0.00±0.00 ^a	0.00±0.00 ^a
TP 25	1.25±0.15 ^c	1.45±0.25 ^b	0.45±0.05 ^b
TP 50	1.75±0.20 ^d	2.55±0.40 ^c	1.60±0.30 ^c
TP 75	2.30±0.25 ^e	3.05±0.35 ^d	2.75±0.20 ^d
TP 100	3.65±0.30 ^f	4.30±0.45 ^e	3.55±0.40 ^e

efficacy down to a 25% concentration. Against *S. mutans*, TPME also exhibited its strongest inhibition at 100% concentration (3.63 mm) and inhibitory activity was observed down to 12.5%. In the case of *P. gingivalis*, the most significant inhibition was observed at 100% concentration (3.55 mm; $p < 0.05$), with detectable inhibitory activity beginning at 25% concentration. Table 3 summarises the inhibitory zone measurements for each examined microorganism.

Discussion

Brushing teeth with toothpaste is the most common oral hygiene practice in many countries. The effectiveness of a toothpaste is largely determined by its ability to eliminate harmful oral microorganisms that contribute to dental plaque formation. Toothpaste Mix Extract (TPME) is expected to enhance the antimicrobial and therapeutic properties of toothpaste. The antioxidant and antimicrobial activities of TPME were evaluated. Based on the results, TPME has antioxidant capacity as assessed by DPPH, ABTS, NO, and H₂O₂ scavenging assays. Antioxidant activity can be evaluated using several methods, including DPPH and ABTS assays²². The DPPH method operates on the principle that antioxidants interact with DPPH by transferring electrons or hydrogen atoms, thereby neutralising the free radicals present in DPPH²⁴. Fig. 1a shows that the DPPH scavenging activity increases with higher concentrations of the extract. This is due to the higher concentration of antioxidant compounds, which results in a more pronounced inhibition of DPPH by the extract²⁵. This implies that when the antioxidant content of the toothpaste is high, the mixed herbal formulation has the potential to reduce oxidative damage in oral tissues, enhance gum health and healing, and exert antibacterial effects through plant-derived compounds^{26,27}.

The IC₅₀ value is a common method for assessing antioxidant activity, with a lower IC₅₀ indicating a

stronger antioxidant activity²⁷. Antioxidant activity is deemed potent if the IC₅₀ is less than 50 µg/mL, strong if between 50-100 µg/mL, moderate if between 101-150 µg/mL, and weak if greater than 150 µg/mL. The DPPH IC₅₀ value for the toothpaste extract is 95.08±3.15 µg/mL, indicating strong antioxidant activity. These findings suggest that the extracts in the toothpaste formulation possess notable antioxidant properties. Comparatively, a study investigating a combination of *Caesalpinia sappan* (secang) and ginger reported the highest antioxidant activity, with an IC₅₀ of 90.14 µg/mL²⁸. In a related investigation, Yang *et al.* reported that cinnamon extract exhibited antioxidant potential, with increasing concentrations resulting in enhanced DPPH radical scavenging activity. A study on black cumin showed DPPH scavenging activity with an IC₅₀ value of 104.76 µg/mL²⁹. Additionally, royal jelly showed antioxidant activity through the DPPH method, with an inhibition value of 24.90±6.41%³⁰.

The ABTS assay is based on the principle of testing the antioxidant potential of compounds to reduce free radicals³¹. This current study showed that sample concentration is proportional to scavenging activity (Fig. 1b). Table 1 shows the IC₅₀ value of 548.90±55.10. This IC₅₀ indicates weak antioxidant activity. The IC₅₀ value affects the ABTS scavenging activity. A lower IC₅₀ value indicates stronger scavenging activity, while a higher IC₅₀ value reflects weaker scavenging activity³². Previous studies have reported that ginger extract exhibits strong antioxidant activity when evaluated using the ABTS method³³. Similarly, a study by Gulcin *et al.*²³ demonstrated that cinnamon extract also possesses antioxidant activity, as assessed by the ABTS method.

The nitric oxide (NO) assay was utilised to evaluate the inhibitory capacity of the plant extract against nitric oxide radical generation³⁴. Fig. 1c demonstrates that as the concentration of TPME increases, its NO scavenging activity also increases. The IC₅₀ value obtained for the NO method is 133.62±3.63. This result indicates that the NO method has moderate antioxidant activity. The H₂O₂ scavenging test measures a compound's ability to capture hydrogen peroxide (H₂O₂). Fig. 1d shows the same results as for DPPH, ABTS, and NO: the higher the concentration, the greater TPME's scavenging activity. The H₂O₂ method shows an IC₅₀ value of 229.96±9.28. This result implies that the H₂O₂ method has weak antioxidant activity. The previous research

reported that the ginger extract exhibited NO and H₂O₂ scavenging activity. The values of H₂O₂ and NO were 72.5-75.6 and 18.78-32.14%, respectively³⁵. Dinagaran *et al.* reported that black cumin extract possesses antioxidant properties, as evidenced by its effectiveness in the radical scavenging assay for H₂O₂ and NO³⁶. The highest H₂O₂ and NO scavenging (1,9% and 1,6%, respectively) is observed at the highest concentration. These findings align with earlier research that showed the highest concentration of TPME exhibited the greatest scavenging activity (Fig. 1c).

Antimicrobial efficacy was assessed using the disk diffusion assay. The results indicated a concentration-dependent increase in antibacterial activity, as evidenced by progressively larger inhibition zones surrounding the disc at higher toothpaste concentrations. In another study by Teke *et al.*³⁷, the antimicrobial efficacy of commercial toothpastes, including Mericle, Oral-B, and Colgate Protection Max, against *P. gingivalis* was evaluated, yielding inhibition zones of 5.6 mm, 7.3 mm, and 13.9 mm, respectively. These results also imply that a polyherbal toothpaste mix demonstrate potential antimicrobial activity comparable to some commercial toothpaste formulations. Inhibition occurs because the polyphenolic compounds in the extracts contained in the toothpaste products inhibit microbes by binding to polyamide polymer proteins in microbes³⁸. As for flavonoid compounds, the possible mechanism involves their association with the lipid bilayer. The non-specific interactions between flavonoids and the phospholipid components of bacterial membranes can alter membrane characteristics, leading to decreased fluidity and compromised integrity, which, in turn, suppress microbial growth³⁹. Therefore, the phytochemicals present in toothpaste can increase their antibacterial activity. A study on the extract used in the toothpaste, black cumin, reported an inhibition zone of 12-13 mm against *S. mutans*⁴⁰. Ginger extract demonstrated antibacterial activity against *P. gingivalis*, producing an inhibition zone of 24.53 mm at 100% concentration⁵⁸. Similarly, cinnamon extract exhibited antimicrobial activity against *C. albicans*, with inhibition zones ranging from 39.30 to 42.18 mm at concentrations of 20-50%.

Flavonoids that are contained in herbal extracts have antioxidant activity, as well as antibacterial activity. Flavonoids exert antibacterial effects by interfering with nucleic acid synthesis, energy metabolism, and the function of the cytoplasmic membrane. Antioxidant

flavonoids provide protective effects against reactive oxygen species (ROS) in the body. Consequently, TPME may serve dual functions, acting as both an antimicrobial agent to help control bacterial plaque formation and an antioxidant to mitigate excess ROS.

Conclusion

This study demonstrates that a polyherbal toothpaste mix containing royal jelly, secang wood, black cumin, ginger, and cinnamon exhibits promising antioxidant and antimicrobial activities, suggesting potential as a natural oral health care product. To further establish its therapeutic potential, future research is necessary to assess its minimum inhibitory concentration (MIC), antibiofilm efficacy, formulation stability, safety profile, and clinical effectiveness through randomised controlled trials and *in vivo* studies.

Acknowledgement

All authors thank the Ministry of Education and Culture of the Republic of Indonesia (Grant Number: 180/E5/PG.02.00.PL/2023) and Aretha Medika Utama for their support of this research. We thank Fadhilah Haifa Zahiroh, Faradhina Salfa Nindya, Dwi Nur Triharsiwi, Annisa Firdaus Sutendi, Adilah Hafizha Nur Sabrina, and Nindia Salsabila Mia Dewi from the AMUBBRC, Bandung, West Java, Indonesia, for their assistance in laboratory work and methodological support.

Conflict of interest

There are no financial or other conflicts of interest to disclose on the part of the author or editor.

References

- 1 Benahmed A G, Gasmı A, Dadar M, Arshad M and Björklund G, The role of sugar-rich diet and salivary proteins in dental plaque formation and oral health, *J Oral Biosci*, 2021, **63**(2), 134–141, doi: 10.1016/j.job.2021.01.007.
- 2 Ren Z, Cui T, Zeng J, Chen L, Zhang W, *et al.*, Molecule targeting glucosyltransferase inhibits *Streptococcus mutans* biofilm formation and virulence, *Front Microbiol*, 2016, **60**(1), 126–135, doi: 10.1128/aac.00919-15.
- 3 How K Y, Song K P and Chan K G, *Porphyromonas gingivalis*: An overview of periodontopathic pathogen below the gum line, *Front Microbiol*, 2016, **7**, 53, doi: 10.3389/fmicb.2016.00053.
- 4 He H, Duan J, Xu J, Ma M, Chai B, *et al.*, *Candida albicans* biofilm inactivated by cold plasma treatment *in vitro* and *in vivo*, *Plasma Process Polym*, 2020, **17**(4), e1900068, doi: 10.1002/ppap.201900068.
- 5 Xiang Z, Wakade R S, Ribeiro A A, Hu W, Bittinger K, *et al.*, Human tooth as a fungal niche: *Candida albicans* traits in dental plaque isolates, *mBio*, 2023, **14**(1), e02769-22, doi: 10.1128/mbio.02769-22.

- 6 Gutiérrez-Venegas G, Gómez-Mora J A, Meraz-Rodríguez M A, Flores-Sánchez M A and Ortiz-Miranda L F, Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque, *Heliyon*, 2019, **5**(12), e03013, doi: 10.1016/j.heliyon.2019.e03013.
- 7 Vyas T, Bhatt G, Gaur A, Sharma C, Sharma A, *et al.*, Chemical plaque control – A brief review, *J Family Med Prim Care*, 2021, **10**(4), 1562–1568, doi: 10.4103/jfmpc.jfmpc_2216_20.
- 8 Fitria K T, Elisma M S and Halid I, Bacterial inhibition effect of essential oil toothpaste against dental plaque of children with autism syndrome disorder (ASD), *J Int Dent Med Res*, 2023, **16**(2), 655–660.
- 9 Qi F, Huang H, Wang M, Rong W and Wang J, Applications of antioxidants in dental procedures, *Antioxidants*, 2022, **11**(12), 2492, doi: 10.3390/antiox11122492.
- 10 Puttipan R, Chansakaow S, Khongkhunthian S and Okonogi S, *Caesalpinia sappan*: A promising natural source of antimicrobial agent for inhibition of cariogenic bacteria, *Drug Discov Ther*, 2018, **12**(4), 197–205, doi: 10.5582/ddt.2018.01035.
- 11 El-Refai A A, Ghoniem G A, El-Khateeb A Y and Hassaan M M, Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents, *J Nanostruct Chem*, 2018, **8**, 71–81, doi: 10.1007/s40097-018-0255-8.
- 12 Yanakiev S, Effects of cinnamon (*Cinnamomum* spp.) in dentistry: A review, *Molecules*, 2020, **25**(18), 4184, doi: 10.3390/molecules25184184.
- 13 Dhanik J, Arya N and Nand V, A review on *Zingiber officinale*, *J Pharmacogn Phytochem*, 2017, **6**(3), 174–184.
- 14 Natto Z S, Herbs and oral health in oral health care – an important issue of the modern society, *IntechOpen*, 2022, **1**, 1–10, doi: 10.5772/intechopen.103715.
- 15 Vaou N, Stavropoulou E, Voidarou C, Tsigalou C and Bezirtzoglou E, Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives, *Microorganisms*, 2021, **9**(10), 2041, doi: 10.3390/microorganisms9102041.
- 16 Varban R, Benedec D, Socaci S, Hanganu D, Varban D, *et al.*, The chemical composition and the antibacterial activity of essential oils obtained from three varieties of *Mentha × piperita* f. *citrata*, *Farmacia*, 2020, **70**(3), 440–446, doi: 10.31925/farmacia.2022.3.9.
- 17 Siregar I D, Kusuma H S W, Widowati W, Marpaung H H, Ferdinand S, *et al.*, Antioxidant and antityrosinase activities of ethanolic *Pachyrhizus erosus* peel and tuber extract, *Maj Kedokt Bandung*, 2019, **51**(2), 75–81, doi: 10.15395/mkb.v51n2.
- 18 Hasanah H, Syukri D and Ismed I, Effect of maltodextrin concentration on antioxidant activity and stability of natural coloring powder of secang wood (*Caesalpinia sappan* L) in various conditions of pH and temperature, *Andalas Int J Agric Nat Sci*, 2023, **3**(2), 1–9.
- 19 Lister I N, Ginting C N, Girsang E, Armansyah A, Marpaung H H, *et al.*, Antioxidant properties of red betel (*Piper crocatum*) leaf extract and its compounds, *J Nat Remedies*, 2020, **20**(4), 198–205, doi: 10.18311/jnr/2019/23633.
- 20 Widowati W, Wargasetia T L, Zakaria T M, Marthania M and Akbar R A, Antioxidant activity of TEMON (*Clitoria ternatea* and *Citrus* sp.) as an infused herbal tea, *Trad Med J*, 2022, **27**(1), 32–39, doi: 10.22146/mot.71628.
- 21 Utami S, Sachrowardi Q R, Damayanti N A, Wardhana A, Syarif I, *et al.*, Antioxidants, anticollagenase and antielastase potentials of ethanolic extract of ripe sesoot (*Garcinia picrorrhiza* Miq.) fruit as antiaging, *J Herbmед Pharmacol*, 2018, **7**(2), 88–93, doi: 10.15171/jhp.2018.15.
- 22 Sugiaman V K, Djuanda R, Naliani S, Alfiyola E and Winardi J, Antibacterial differences effect between onion extract and lemon juice on *in vitro* growth of *Enterococcus faecalis*, *J Int Dent Med Res*, 2023, **16**(1), 111–116.
- 23 Webber D M, Wallace M A and Burnham C D, Stop waiting for tomorrow: Disk diffusion performed on early growth is an accurate method for antimicrobial susceptibility testing with reduced turnaround time, *J Clin Microbiol*, 2022, **60**(5), e03007-20, doi: 10.1128/JCM.03007-20.
- 24 Emmulo E, Ceccantoni B, Bellincontro A and Mencarelli F, Use of water and ethanol extracts from wine grape seed pomace to prepare an antioxidant toothpaste, *J Sci Food Agric*, 2021, **101**(14), 5813–5818, doi: 10.1002/jsfa.11232.
- 25 Gulcin İ and Alwasel S H, DPPH radical scavenging assay, *Processes*, 2023, **11**(8), 2248, doi: 10.3390/pr11082248.
- 26 Munteanu I G and Apetrei C, Analytical methods used in determining antioxidant activity: A review, *Int J Mol Sci*, 2021, **22**(7), 3380, doi: 10.3390/ijms22073380.
- 27 Kanouté A, Dieng S N, Diop M, Dieng A, Sene A K, *et al.*, Chemical vs natural toothpaste: Which formulas for which properties? A scoping review, *J Public Health Afr*, 2022, **13**(3), 1945, doi: 10.4081/jphia.2022.1945.
- 28 Olugbami J O, Gbadegesin M A and Odunola O A, *In vitro* evaluation of antioxidant potential of *Anogeissus leiocarpus*, *Afr J Med Med Sci*, 2014, **43**(1), 101–109.
- 29 Onger O D, Pharmacological properties of ginger combinations, *IntechOpen*, 2022, **1**, 1–12, doi: 10.5772/intechopen.107214.
- 30 Yang C H, Li R X and Chuang L Y, Antioxidant activity of various parts of *Cinnamomum cassia*, *Molecules*, 2012, **17**(6), 7294–7304, doi: 10.3390/molecules17067294.
- 31 Soleimanifar M, Niazmand R and Jafari S M, Evaluation of oxidative stability and antioxidant properties of black cumin seed oil, *J Food Meas Charact*, 2019, **13**, 383–389, doi: 10.1007/s11694-018-9953-7.
- 32 Balkanska R, Correlations of physicochemical parameters and antioxidant activity of royal jelly, *Int J Curr Microbiol Appl Sci*, 2018, **7**(4), 3744–3750, doi: 10.20546/ijemas.2018.704.421.
- 33 Theafelicia Z and Wulan S N, Comparison of antioxidant testing methods on black tea, *J Teknol Pertan*, 2023, **24**(1), 35–44.
- 34 Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, *et al.*, Determination of antioxidants by DPPH scavenging activity of *Ficus religiosa*, *Molecules*, 2022, **27**(4), 1326, doi: 10.3390/molecules27041326.
- 35 Makanjuola S A, Influence of extraction solvent on antioxidant properties of tea and ginger, *Food Sci Nutr*, 2017, **5**(6), 1179–1185, doi: 10.1002/fsn3.509.
- 36 Chaves N, Santiago A and Alías J C, Quantification of antioxidant activity of plant extracts, *Antioxidants*, 2020, **9**(1), 76, doi: 10.3390/antiox9010076.
- 37 Tanveer S, Aamir S, Masood S B and Muhammad S, Radical scavenging linked antioxidant comparison and quantification of conventional and supercritical fluid ginger extracts, *J Nutr Food Sci*, 2016, **6**(4), 1–7, doi: 10.4172/2155-9600.1000511.

- 38 Dinakaran S, Sridhar S and Eganathan P, Chemical composition and antioxidant activities of black seed oil, *Int J Pharm Sci Res*, 2016, **7**(11), 4473–4479, doi: 10.13040/IJPSR.0975-8232.7(11).4473-79.
- 39 Teke G N, Enongene N G and Tiagha A R, *In vitro* antimicrobial activity of commercial toothpastes, *Int J Curr Microbiol Appl Sci*, 2017, **6**(1), 433–446, doi: 10.20546/ijcmas.2017.601.052.
- 40 Yuan G, Guan Y, Yi H, Lai S, Sun Y, *et al.*, Antibacterial activity and mechanism of plant flavonoids, *Sci Rep*, 2021, **11**(1), 10471, doi: 10.1038/s41598-021-90035-7.