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Original Article

Antimicrobial Effects of *Syzygium aromaticum* and *Salvadora persica* against Common Peri-implantitis Pathogens *In Vitro*

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Abstract

Background and objectives: Clove essential oil (CEO) derived from *Syzygium aromaticum* and miswak (*Salvadora persica*) contains bioactive compounds with antimicrobial properties. Due to the growing interest in alternatives to conventional antibiotics, this study aimed to evaluate the *in vitro* antimicrobial efficacy of CEO, miswak, and their combination against key peri-implantitis pathogens.

Methods: The antimicrobial activities of CEO, miswak, and their combinations were tested against *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia.* Disc diffusion and serial dilution methods were used to measure the inhibition zones and minimum inhibitory concentrations, respectively. Doxycycline served as a standard antibiotic for comparison, while ethanol was used as a negative control. Data were analyzed using one-way analysis of variance and Tukey's honestly significant difference test, with significance set at $\alpha = 0.05$.

Results: CEO exhibited inhibition zones of 10–16 mm, comparable to that of doxycycline (13–16 mm), whereas miswak (6–13 mm vs. 1–14 mm) and the CEO–miswak combination (8–14 mm vs. 0–14 mm) showed lower activity. Mean minimum inhibitory concentration values were lowest for doxycycline (1.73 ± 0.46 μg/mL), followed by CEO (2.37 ± 0.24 μg/mL) and CEO–miswak combination (2.92 ± 0.12 μg/mL). Statistical analysis showed that the CEO–miswak combination was less effective than CEO ($p = 0.0326$) and doxycycline ($p = 0.0001$), but not different from miswak ($p = 0.9836$). CEO showed slightly greater activity than miswak ($p = 0.0605$).

Conclusions: Among the natural extracts tested, CEO exhibited superior antimicrobial efficacy, whereas miswak was less effective. The combination of CEO with miswak did not enhance antimicrobial efficacy, suggesting antagonistic interactions between their bioactive compounds.

Introduction

Peri-implantitis, characterized by inflammation of the soft and hard tissues around dental implants, results from excessive plaque accumulation.[1] This condition arises from the colonization of diverse biofilm-forming microbiota in the tissues surrounding the implants, often leading to implant loss and breakdown of the supporting tissues.[2,3]

Peri-implantitis, a major cause of implant failure, is typically managed with antibiotic therapy.[2,3] Given the limitations of current treatments, including the reduced efficacy of antibiotics due to antimicrobial resistance, alternative approaches are critically needed.[4] In search of alternatives to conventional antibiotics, this study focuses on *Syzygium aromaticum* and *Salvadora persica*, which have been identified for their potent antimicrobial proper-

Keywords: Antimicrobial activity; Clove essential oil; Miswak; Pathogens; Peri-implantitis; *Syzygium aromaticum*; *Salvadora persica*.

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ties. Among the plant products listed (clove, peppermint, miswak, lemongrass, and black cumin seeds), *Syzygium aromaticum* (clove) and *Salvadora persica* (miswak) are of particular interest due to their reported antimicrobial properties, which are specific to peri-implant pathogens.[5–7]

Syzygium aromaticum (clove), known for its aromatic properties, contains compounds such as eugenol, α-humulene, and β-caryophyllene. These compounds exhibit notable antimicrobial, antiparasitic, and antioxidant activities.[5] Clove essential oil (CEO) is a complex mixture of secondary metabolites obtained from clove buds using various extraction methods.[8] Studies on CEO have reported antibacterial, antioxidant, and antifungal activities attributed to high percentages of eugenol, which is effective against oral organisms, including gram-positive and gram-negative bacteria, as well as fungi.[8–12]

Salvadora persica (miswak) is a plant rich in flavonoids, saponins, tannins, vitamin C, tannic acid, and benzyl isothiocyanate (BITC), which collectively prevent and treat various oral infections.[13] BITC exerts antimicrobial effects by interfering with bacterial enzyme function and disrupting microbial cell membranes.[13,14] The bark and roots of the plant contain chlorides, fluorides, ash, and alkaloids, which contribute to its antimicrobial effects.[13–15] Methanol and aqueous extracts of miswak have been reported to have inhibitory effects on conditions such as periodontitis and the accumulation of dental plaque.[16]

This study aimed to address the growing issue of antibiotic resistance by assessing the antimicrobial potency of natural agents, including CEO and miswak, against pathogens associated with peri-implantitis. These agents are of growing interest as alternative treatment modalities. Previous studies have demonstrated the potential of these extracts against different oral pathogens; however, limited research exists on their comparative efficacy or potential synergistic effects in treating peri-implantitis-associated microorganisms.[17] Therefore, this study aimed to evaluate the *in vitro* antimicrobial efficacy of CEO, miswak, and their combination against key peri-implantitis pathogens, comparing their effectiveness with that of doxycycline, a commonly used antibiotic in peri-implantitis treatment.

Materials and methods

After recognizing the potential of CEO and miswak, we conducted an *in vitro* study to assess their antimicrobial properties. This study involved a comprehensive methodology that included the preparation of natural extracts, the selection of bacterial strains, and the execution of antimicrobial assays. The study was conducted at Ibn Sina National College for Medical Studies and received approval from the Institutional Research Review Board (approval no. IRRB-03-28012024).

Preparation of plant extracts

CEO

We obtained CEO from iHerb, a certified supplier in Saudi Arabia, and stored it in amber glass bottles at 4°C. This process was performed in a dark, humidity-controlled environment to prevent the degradation of bioactive compounds.

Miswak extract

Fresh *Salvadora persica* (miswak) sticks were collected from Saudi Arabia in February 2024. The sticks were cleaned with distilled water and shade-dried at ambient temperatures (25–28°C) for 48 h. The dried sticks were ground into a fine powder. Miswak powder (50 g) was mixed with 500 mL of 70% ethanol in a sealed conical flask. The mixture was shaken at 150 rpm for 48 h and filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness at 40°C under reduced pressure (300 mbar) using a rotary evaporator to obtain a crude extract, which was then reconstituted in 70% ethanol at a final concentration of 100 mg/mL. The extract was stored in amber glass containers at 4°C in a cool, dark environment, protected from light and humidity, until further use.

Mixture of CEO and miswak

Equal volumes of CEO and reconstituted miswak extract (both in 70% ethanol) were combined to create a mixture with comparable solvent bases and equal effective concentrations of both extracts.

After the plant extracts (CEO, miswak extract, and a mixture of the two) were prepared, specific bacterial strains were selected to evaluate their antimicrobial responses to these extracts.

Bacterial strains and culture conditions

To ensure the relevance and applicability of our findings in mimicking clinical conditions, we selected clinically isolated strains that were prevalent in peri-implantitis. The strains used were *Fusobacterium nucleatum* (F. *nucleatum*) (ATCC 22586, United Kingdom), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) (ATCC 43718, United Kingdom), *Porphyromonas gingivalis* (*P. gingivalis*) (ATCC 33277, United Kingdom), and *Prevotella intermedia* (*P. intermedia*) (ATCC 25611, United Kingdom). Bacterial strains were cultured in selective growth media specific to each organism under anaerobic conditions at 37°C. After incubation for 48 h, the bacterial suspensions were prepared by diluting the cultures in sterile saline. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL), to ensure a uniform inoculum density for subsequent antimicrobial testing.

Antimicrobial assays

Disc diffusion method for zones of inhibition determination

The zone of inhibition is the region where the growth of microbes is halted or suppressed by the agent. The effectiveness of the test compound against a particular microorganism can be evaluated. This study examined the antimicrobial effects of CEO, miswak extract, and their combination on the zones of inhibition using the disc diffusion method on Mueller–Hinton agar plates. Each plate was uniformly inoculated with bacterial suspensions prepared to a 0.5 McFarland standard (~1.5×10^8 CFU/mL) using sterile cotton swabs to obtain a lawn culture. Sterile paper discs (6 mm in diameter) were impregnated with CEO, miswak extract, or their combination at concentrations of 50, 100, and 200 µg/disc. Vehicle control discs containing 70% ethanol were included to rule out solvent effects. Sterile paper discs (6 mm in diameter) were impregnated with 100 µL of CEO, miswak extract, or their combination and placed on the agar surface. The control discs were soaked in ethanol (negative control) and doxycycline (positive control). Doxycycline was chosen for this study because, among the various antibiotics used for peri-implantitis treatment, it effectively reduces bacterial loads, inhibits tissue-destructive enzymes (matrix metalloproteinases), and has anti-inflammatory properties that promote periodontal healing. These characteristics make doxycycline a preferred option in clinical practice for managing peri-implantitis. The plates were incubated under anaerobic conditions

at 37°C for 48 h. To minimize spatial bias, the discs were randomly assigned to different positions on the agar plates within each treatment group (CEO, miswak, and their combination). Following incubation, the diameters of the zones of inhibition around each disc were measured in millimeters using a digital caliper (Mitutoyo, Japan). To ensure unbiased data collection, measurements were performed by an independent investigator who was blinded to the treatment groups. The procedure was performed in triplicates for each treatment (n = 3) to ensure reproducibility.

Serial dilution method for minimum inhibitory concentration (MIC) determination

The MIC is defined as the minimum antimicrobial concentration that prevents noticeable microbial growth in simulated media after a fixed incubation period. This is determined by placing a known number of microorganisms into several test tubes and then adding increasing concentrations of a specific antibiotic. The MIC for drug–pathogen pairing is defined as the lowest antibiotic concentration that inhibits the growth of bacteria. In this study, two-fold serial dilutions of CEO, miswak extract, and their combinations were prepared using Mueller–Hinton broth. CEO and reconstituted miswak extract were tested at concentrations ranging from 3.125 to 200 µg/mL. A 96-well microtiter plate was filled with 100 µL of each dilution, and bacterial suspensions of the four peri-implantitis pathogens were added to achieve a final concentration of approximately 5×10^5 CFU/mL. Wells containing only broth (negative control) and those containing broth with bacteria (positive control) were included. Solvent controls (70% ethanol) were also incorporated. The microtiter plates were sealed and incubated at 37°C for 24 h in an anaerobic incubator. Ten microliters of the well contents were subcultured onto Mueller–Hinton agar plates, incubated at 37°C under anaerobic conditions, and monitored for 48 h for colony formation to confirm bacterial viability in wells without visible growth, to obtain the minimal bactericidal concentration. The entire procedure was performed in triplicate for each treatment group to ensure reproducibility.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows (version 29.0.2.0; IBM Corp., Armonk, NY, IBM Corp.). All results are expressed as the mean ± standard deviation (SD) of three biological replicates (n = 3) for the zones of inhibition and MIC. Differences in antimicrobial efficacy between CEO, miswak, and their mixtures were assessed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test post hoc test at $\alpha = 0.05$.

Results

Zone of inhibition analysis

Antimicrobial activity of CEO

The antibacterial properties of CEO were tested against *F. nucleatum, A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia* (Fig. 1). The mean ± SD of the zone of inhibition for *F. nucleatum* was 16 ± 0.5 mm. The inhibition zones of CEO were comparable to those of the positive control (doxycycline) (Figs. 2a–d). No inhibition zones were observed for the negative control samples.

Antimicrobial activity of miswak extract

Miswak demonstrated moderate activity against *F. nucleatum*, with a mean ± SD inhibition zone of 13 ± 0.7 mm. Its activity was limited to *A. actinomycetemcomitans* (6 ± 0.4 mm), *P. gingivalis* (9 ± 0.5 mm), and *P. intermedia* (7 ± 0.6 mm) (Figs. 3a–d). No inhibition zones were observed in the negative control group.

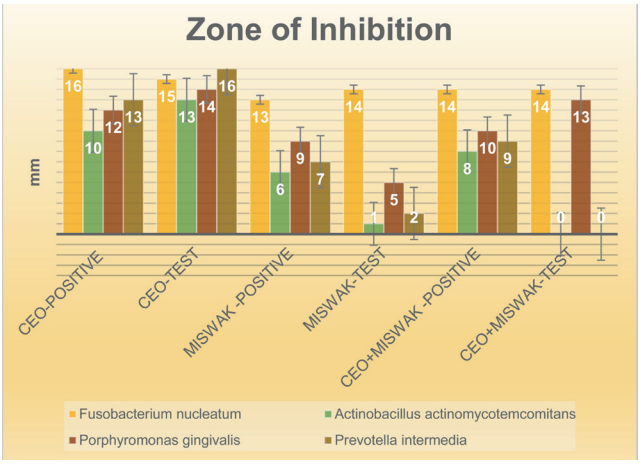

Fig. 1. Zone of Inhibition after incubation for 48 h with different treatment groups against different microorganisms. CEO, clove essential oil.

Antimicrobial activity of CEO and miswak combination

The combination of CEO and miswak was effective against *F. nucleatum*, with a mean ± SD inhibition zone of 14 ± 0.6 mm. For other microorganisms, the efficacy of the combination was lower than that of CEO alone (Figs. 4a–d). No inhibition zones were observed for the negative control samples.

The observed inhibition zones suggest that CEO was the most effective treatment, while miswak was the least effective. *F. nucleatum* was the most susceptible species, whereas *A. actinomycetemcomitans* and *P. intermedia* were the least susceptible. To further quantify this efficacy, the MIC was analyzed.

MIC analysis

Table 1 and Fig. 5 shows the MIC values (mean ± SD) of doxycycline, CEO, miswak, and their combinations against the four peri-implantitis-associated bacterial strains. Doxycycline consistently displayed the lowest MIC values across all strains (1.67–1.80 µg/mL), indicating that it had the strongest antimicrobial activity against the tested strains. CEO showed moderate activity with MIC values ranging from 2.30 to 2.45 µg/mL, whereas miswak exhibited higher MICs (3.30–3.60 µg/mL), indicating weaker efficacy. The combination of CEO and miswak produced MICs between those of CEO and miswak (2.85–2.98 µg/mL), suggesting no synergistic effect.

Statistical analysis

Table 2 shows the intergroup comparison of MIC values for doxycycline, CEO, miswak, and their combination against each peri-implantitis-associated bacterial strain using a one-way ANOVA. For all four organisms, the *p*-values were below 0.05 (*F. nucleatum*, 0.026; *A. actinomycetemcomitans*, 0.016; *P. gingivalis*, 0.032; *P. intermedia*, 0.006), indicating statistically significant differences among treatments. Doxycycline consistently exhibited the lowest MIC, reflecting its high antimicrobial potency. CEO showed intermediate activity, whereas miswak showed the highest MICs, suggesting weaker efficacy. The combination of CEO and miswak

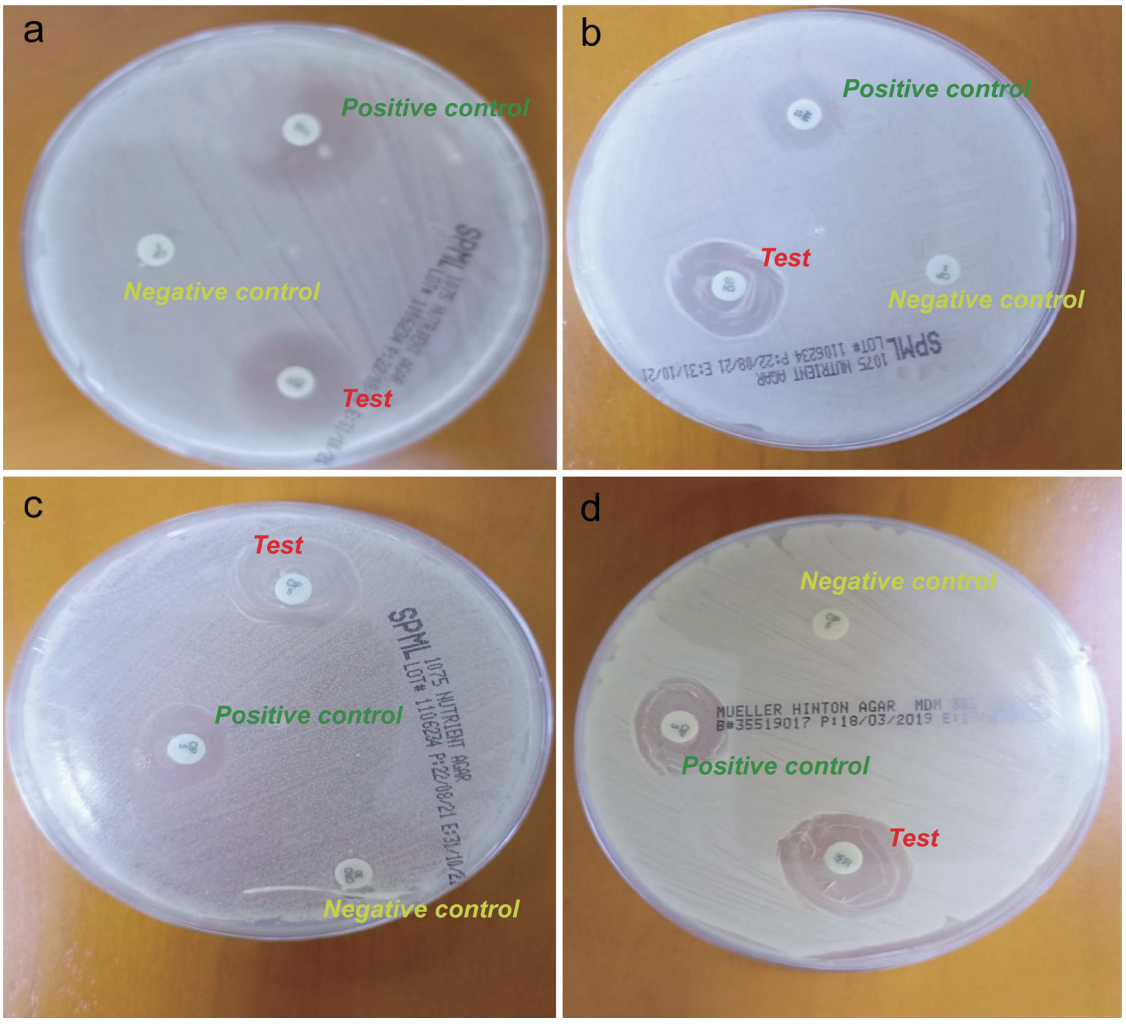

Fig. 2. Zones of inhibition at 48 h post-incubation with clove essential oil for all four microorganisms. (a) Zones of inhibition at 48 h post-incubation with clove essential oil for *Fusobacterium nucleatum*. (b) Zones of inhibition at 48 h post-incubation with clove essential oil for *Aggregatibacter actinomycetemcomitans*. (c) Zones of inhibition at 48 h post-incubation with clove essential oil for *Porphyromonas gingivalis*. (d) Zones of inhibition at 48 h post-incubation with clove essential oil for *Prevotella intermedia*.

produced MICs between CEO and miswak, confirming that no synergistic effect was observed.

Table 3 presents the intragroup analysis comparing the MIC values of different bacterial strains within each treatment group using one-way ANOVA. For all four treatment groups, doxycycline, CEO, miswak, and CEO + miswak, the *p*-values were above 0.05 (doxycycline: 0.987; CEO: 0.975; miswak: 0.964; CEO + miswak: 0.991), indicating no statistically significant differences in susceptibility among the tested organisms for a given treatment. This suggested that each treatment exhibited relatively consistent antimicrobial activity against *F. nucleatum, A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia.* The results showed that, although the absolute MIC values differed between treatments, the relative effectiveness of each treatment was uniform across these peri-implantitis-associated pathogens.

Tukey's HSD post-hoc analysis (Table 4) revealed clear differences in the antimicrobial efficacy among the tested treatments. The combination of CEO and miswak was slightly less effective than that of CEO alone, with a statistically significant mean difference of −0.5625 ($p = 0.0326$). Compared to doxycycline, the combination was significantly less effective (mean difference −1.1875, $p = 0.0001$). These results indicate that CEO demonstrates higher antimicrobial activity than miswak and that the combination does not enhance efficacy beyond that of CEO alone.

Discussion

This study was conducted to demonstrate the effectiveness of CEO and miswak extracts as safe herbal alternatives for the antimicrobial treatment of peri-implantitis pathogens. Among the three treatments, CEO exhibited the strongest antimicrobial activity, followed by the CEO-miswak mixture, whereas the miswak extract exhibited the least efficacy. In this study, *F. nucleatum*, *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* were selected as the key pathogens because of their established roles in periodontal and peri-implant disease pathogenesis.[17]

Considering the critical role of microbial pathogens in the progression of peri-implantitis, it is imperative to develop alter-

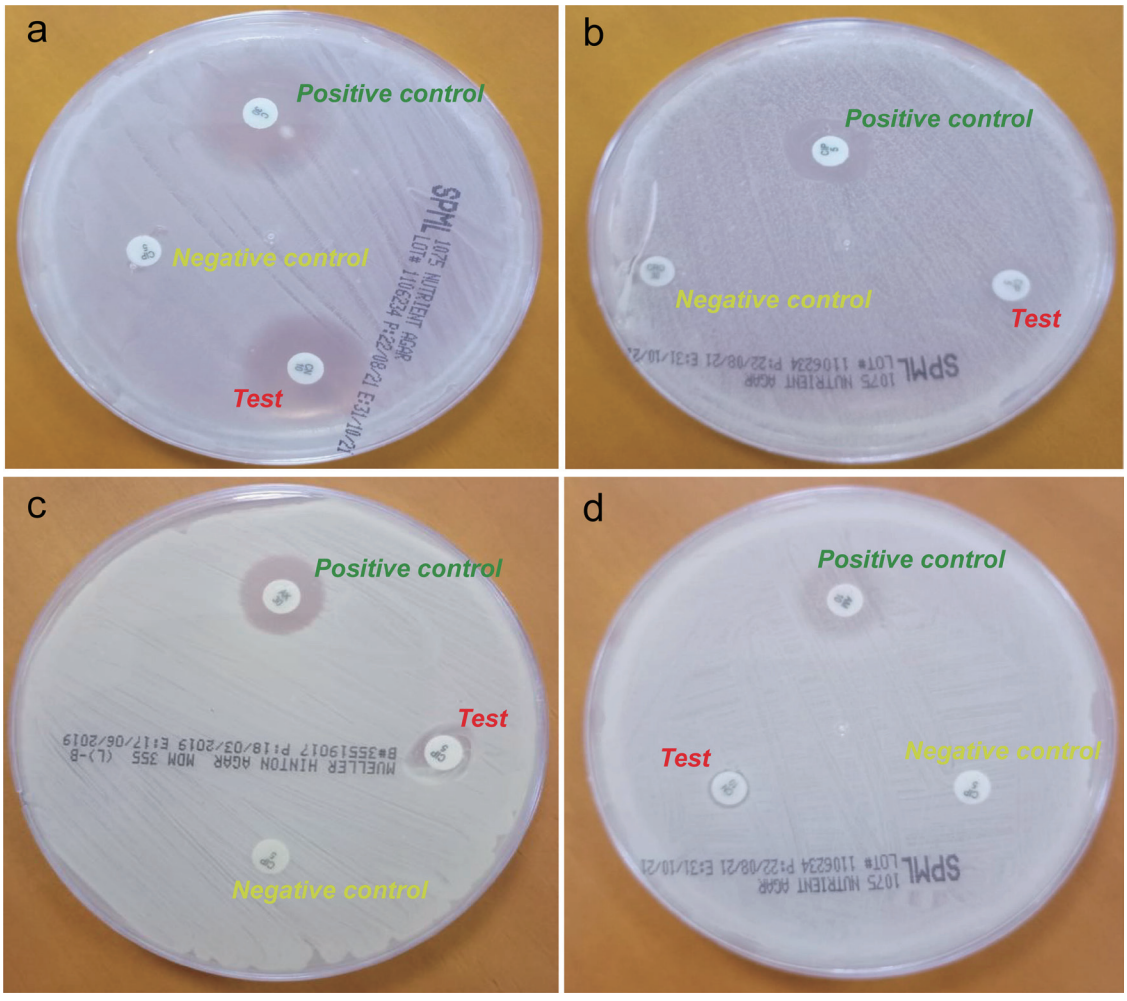

Fig. 3. Zones of inhibition at 48 h post-incubation with miswak for all four microorganisms. (a) Zones of inhibition at 48 h post-incubation with miswak extract for *Fusobacterium nucleatum*. (b) Zones of inhibition at 48 h post-incubation with miswak extract for *Aggregatibacter actinomycetemcomitans*. (c) Zones of inhibition at 48 h post-incubation with miswak extract for *Porphyromonas gingivalis*. (d) Zones of inhibition at 48 h post-incubation with miswak extract for *Prevotella intermedia*.

native therapeutic strategies. In two separate studies, Nugraha *et al*.[18,19] investigated natural substances, specifically *Moringa oleifera* (drumstick tree) and *Coffea canephora* (Robusta green coffee beans), as alternatives to address the growing issue of antibiotic resistance in peri-implantitis treatment. These promising findings, coupled with the rise in peri-implantitis cases and growing concerns over antibiotic resistance, have driven interest in exploring natural antimicrobial agents, such as CEO and miswak, which offer promising, safe, and cost-effective options for managing peri-implantitis. Thus, this study aimed to evaluate the antimicrobial efficacy of these natural agents against selected peri-implant pathogens, potentially supporting their use in future therapeutic strategies for the treatment of peri-implantitis.

CEO

In vitro studies have demonstrated that CEO has a broad range of properties, including antibacterial, antiviral, antifungal, antioxidant, anticancer, anesthetic, and analgesic effects.[9–12,20–24] Furthermore, the significant anti-inflammatory effects of CEO stem from its ability to suppress the activity of cyclooxygenase-2 and lipoxygenase enzymes, making it a promising candidate for managing inflammation in peri-implantitis.[11,12] The antimicrobial activity of CEO is due to eugenol, which disrupts bacterial membranes, inhibits ATPase and other enzymes, and generates reactive oxygen species, reinforcing its potential as a therapeutic agent for managing peri-implantitis.[12] Expanding its broad-spectrum antimicrobial properties, further investigation is needed into how CEO specifically affects bone health, which is a key issue in the treatment of peri-implantitis. Karmakar *et al*.[25] reported that dried clove buds, which are rich in the volatile phenolic compound eugenol, effectively prevented bone loss. This property could be particularly beneficial in the treatment of peri-implantitis.[25] Eugenol increases the permeability of bacterial cell membranes, leading to the leakage of intracellular proteins and ultimately causing bacterial cell death. This antimicrobial mechanism, coupled with its ability to prevent bone resorption, suggests that eugenol may play a valuable role in managing peri-implantitis.

In this study, CEO exhibited significant antibacterial activity against all four peri-implantitis pathogens, with inhibition zone diameters ranging from 10–16 mm, comparable to those of doxy-

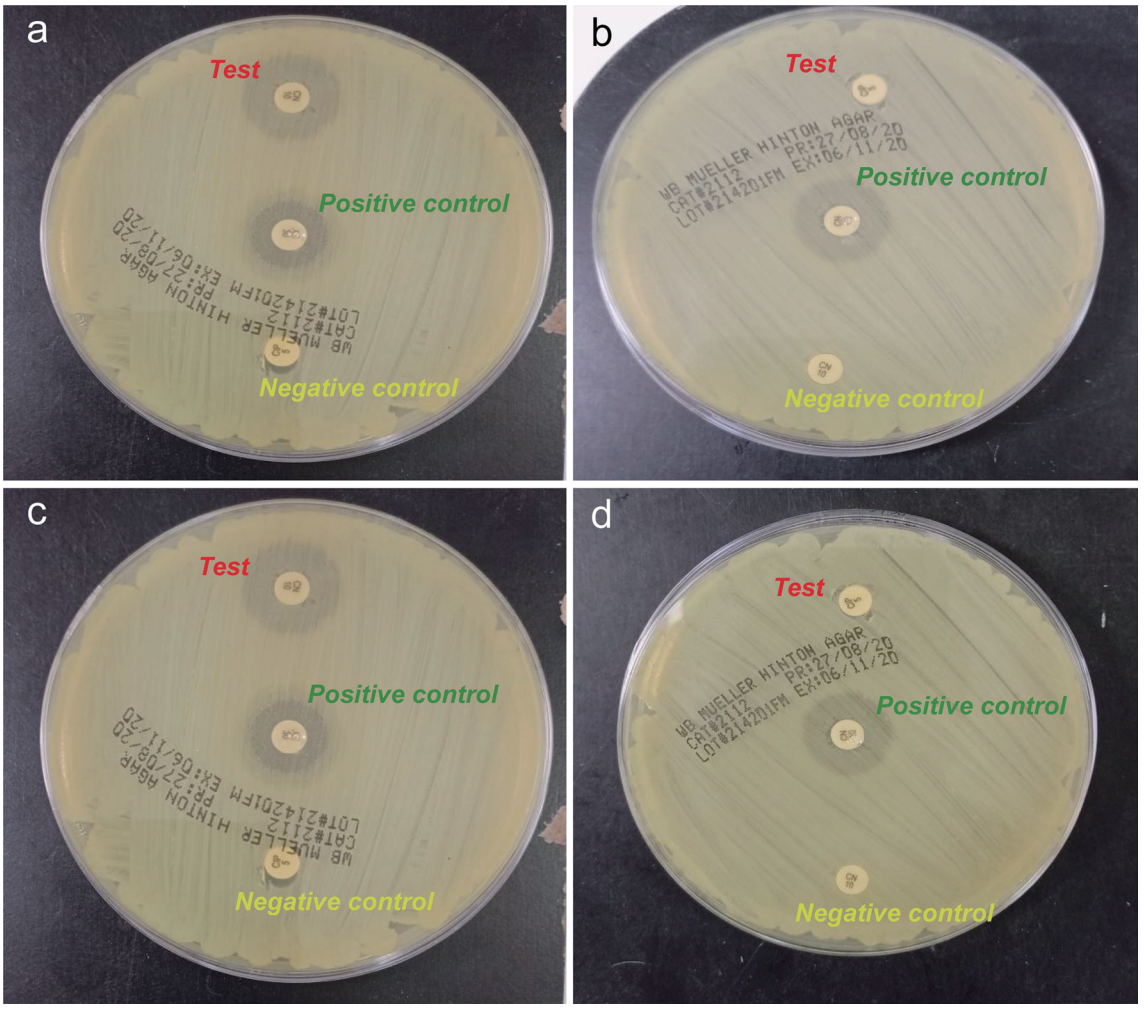

Fig. 4. Zones of inhibition at 48 h post-incubation with the clove essential oil (CEO) and miswak extract mixture for all four microorganisms. (a) Zones of inhibition at 48 h post-incubation with clove essential oil and miswak extract mixture for *Fusobacterium nucleatum*. (b) Zones of inhibition at 48 h post-incubation with clove essential oil and miswak extract mixture for *Aggregatibacter actinomycetemcomitans*. (c) Zones of inhibition at 48 h post-incubation with clove essential oil and miswak extract mixture for *Porphyromonas gingivalis*. (d) Zones of inhibition at 48 h post-incubation with clove essential oil and miswak extract mixture for *Prevotella intermedia*.

cycline (13–16 mm). The MIC of CEO ranged from 2.30 to 2.45 µg/mL, with the largest inhibition zone observed against *F. nucleatum* (16 mm in diameter). Notably, CEO consistently demonstrated lower MIC values (2.30–2.45 µg/mL), indicating its superior efficacy in inhibiting microbial growth, particularly against *P. gingivalis* and *F. nucleatum*. These findings differed significantly from those reported by Cai *et al*.,[26] who reported much higher MIC values for crude clove extract, indicating stronger antimicrobial activity against *P. intermedia* and *P. gingivalis*, with MICs of 156 and 625 µg/mL, respectively. Differences in reported MIC values highlight the need for standardized methods in clove extract preparation and antimicrobial testing.

The significant anti-inflammatory properties of CEO were further complemented by its antibacterial activity, suggesting a dual

Table 1. Minimum inhibitory concentration (MIC) of different treatment groups among different organisms

Bacterial strain	Doxycycline (test) µg/mL	Clove essential oil (CEO) µg/mL	Miswak µg/mL	CEO + miswak µg/mL
Fusobacterium nucleatum	1.75 ± 0.21	2.30 ± 0.20	3.30 ± 0.40	2.85 ± 0.15
Aggregatibacter actinomycetemcomitans	1.73 ± 0.87	2.40 ± 0.25	3.60 ± 0.50	2.95 ± 0.10
Porphyromonas gingivalis	1.67 ± 0.74	2.35 ± 0.22	3.55 ± 0.45	2.90 ± 0.12
Prevotella intermedia	1.80 ± 0.04	2.45 ± 0.28	3.45 ± 0.42	2.98 ± 0.14
Mean ± standard deviation of different treatment groups	1.73 ± 0.46	2.37 ± 0.24	3.50 ± 0.44	2.92 ± 0.12

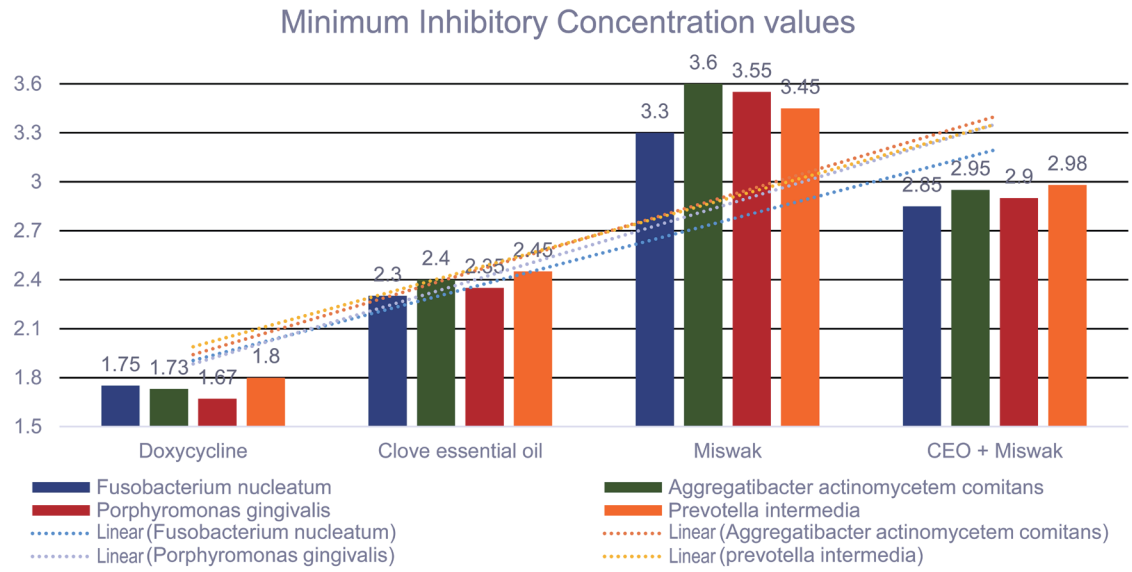

Fig. 5. Minimum inhibitory concentration (MIC) values of natural extracts (clove essential oil (CEO), miswak, and their combination) and doxycycline against peri-implantitis-associated microorganisms.

mechanism of action that could be beneficial in peri-implantitis treatment. Several studies have highlighted the antibacterial effects of CEO. Uju *et al*.[27] reported that crude clove extracts inhibited the growth of periodontal pathogens, including *Streptococcus mutans*. Utami *et al*.[28] found that eugenol, a component of CEO, inhibited *A. actinomycetemcomitans* at low concentrations. Zhang *et al*.[8] reported that eugenol not only damaged the cell membranes of *P. gingivalis* but also inhibited biofilm formation and downregulated genes encoding virulence factors.[27,28] However, research has not yet explored the impact of CEO on *F. nucleatum* and *P. intermedia*, indicating potential avenues for future studies. Nonetheless, based on these findings, CEO has the potential to serve as an adjunct treatment for peri-implantitis, particularly in patients who cannot tolerate antibiotics.

Miswak

In this study, miswak demonstrated antibacterial activity against all four tested peri-implantitis pathogens, with inhibition zone

Table 2. Intergroup analysis (comparison of different treatment groups against the same organism) - one-way ANOVA

Bacterial strain	Treatment group	Mean MIC (µg/mL)	Standard deviation (SD)	*p*-value
Fusobacterium nucleatum	Doxycycline (test)	1.75	0.21	0.026*
	Clove essential oil (CEO)	2.3	0.2	
	Miswak	3.3	0.4	
	CEO + Miswak	2.85	0.15	
Aggregatibacter actinomycetemcomitans	Doxycycline (test)	1.73	0.87	0.016*
	CEO	2.4	0.25	
	Miswak	3.6	0.5	
	CEO + Miswak	2.95	0.1	
Porphyromonas gingivalis	Doxycycline (test)	1.67	0.74	0.032*
	CEO	2.35	0.22	
	Miswak	3.55	0.45	
	CEO + Miswak	2.9	0.12	
Prevotella intermedia	Doxycycline (test)	1.80	0.04	0.006*
	CEO	2.45	0.28	
	Miswak	3.45	0.42	
	CEO + Miswak	2.98	0.14	

*Statistically significant ($p < 0.05$). ANOVA, analysis of variance; MIC, minimum inhibitory concentration.

Table 3. Intragroup analysis (comparison of different organisms against the same treatments) - one-way ANOVA with a significance level of 0.05

Treatment group	Bacterial strain	Mean MIC (µg/mL)	Standard deviation (SD)	*p*-value
Doxycycline (test)	*Fusobacterium nucleatum*	1.75	0.21	0.987
	Aggregatibacter actinomycetemcomitans	1.73	0.87	
	Porphyromonas gingivalis	1.67	0.74	
	Prevotella intermedia	1.8	0.04	
Clove essential oil (CEO)	*Fusobacterium nucleatum*	2.3	0.2	0.975
	Aggregatibacter actinomycetemcomitans	2.4	0.25	
	Porphyromonas gingivalis	2.35	0.22	
	Prevotella intermedia	2.45	0.28	
Miswak	*Fusobacterium nucleatum*	3.3	0.4	0.964
	Aggregatibacter actinomycetemcomitans	3.6	0.5	
	Porphyromonas gingivalis	3.55	0.45	
	Prevotella intermedia	3.45	0.42	
CEO + Miswak	*Fusobacterium nucleatum*	2.85	0.15	0.991
	Aggregatibacter actinomycetemcomitans	2.95	0.10	
	Porphyromonas gingivalis	2.90	0.12	
	Prevotella intermedia	2.98	0.14	

ANOVA, analysis of variance, MIC, minimum inhibitory concentration.

diameters ranging from 6–13 mm. The MIC of miswak ranged from 3.3 to 3.6 µg/mL, with the lowest activity observed against *F. nucleatum* (MIC = 3.3 µg/mL). These results indicate the relatively low antimicrobial efficacy of miswak compared with that of CEO. However, our findings contrast with those of Al-Sieni, who reported mild to high antibacterial activity of miswak against *F. nucleatum* with an MIC of 50 µg/mL.[29] Additionally, other studies have demonstrated increased bactericidal activity of miswak against various oral pathogens.[30,31] Sekar *et al*.[30] reported notable inhibition of *P. gingivalis* by miswak *in vitro*. Similarly, Saquib *et al*.[31] found that ethanol-extracted miswak exhibited significant inhibitory effects against *P. gingivalis* and *A. actinomycetemcomitans*, with synergistic effects when combined with antibiotics. The antibacterial activity of miswak extract is due to its antioxidant and anti-inflammatory properties.[7,14,32] Additionally, the anti-inflammatory activity of miswak is linked to its ability to suppress proinflammatory cytokines by modulating key inflammatory pathways.[33] These combined properties not only help neutralize oxidative stress but also enhance its bactericidal efficacy, as demonstrated in various studies.[30–33]

Another proposed mechanism underlying the antibacterial activity of miswak extract is the action of BITC. This bioactive compound disrupts bacterial membranes. Electron microscopy studies on periodontal bacteria have shown that miswak, specifically BITC, can induce membrane protrusions similar to the effects of antimicrobial peptides.[13,34] By compromising the bacterial cell wall, BITC allows further penetration of other bioactive compounds, disrupting bacterial redox systems and membrane potential. BITC has similar effects on mitochondrial membranes, impairing bacterial cell physiology.[13,34]

Although miswak demonstrated weaker antimicrobial activity than CEO, particularly against *A. actinomycetemcomitans* and *P. gingivalis*, its selective efficacy against *F. nucleatum* (with inhibition zones of 13 mm) highlights its potential use in specific clinical contexts where targeted action against pathogens is required. This selective efficacy aligns with previous studies reporting moderate activity of miswak against oral pathogens.[30,32,34]

Given the promising results observed with CEO and miswak in-

Table 4. Tukey HSD post hoc test results showing comparisons between groups with a significance level of 0.05

Comparison	Mean difference	*p*-value	Confidence interval (lower)	Confidence interval (upper)	Significant (reject null)
CEO+Miswak vs. CEO	−0.5625	0.0326	−1.0818	−0.0432	Yes
CEO+Miswak vs. Doxycycline (test)	−1.1875	0.0001	−1.7068	−0.6682	Yes
CEO+Miswak vs. Miswak	−0.0625	0.9836	−0.5818	0.4568	No
CEO vs. Doxycycline (test)	−0.625	0.0174	−1.1443	−0.1057	Yes
CEO vs. Miswak	0.5	0.0605	−0.0193	1.0193	No
Doxycycline (test) vs. Miswak	1.125	0.0002	0.6057	1.6443	Yes

CEO, clove essential oil; HSD, honestly significant difference.

dividually, investigating their combined effects was a natural next step in exploring their potential synergistic actions.

CEO and miswak combination

The combination of CEO and miswak demonstrated antibacterial activity against all four bacteria, with inhibition zone diameters ranging from 8–14 mm. The MIC of this combination ranged from 2.85 to 2.98 µg/mL across all pathogens. Unexpectedly, the combined extract exhibited lower antibacterial activity than CEO alone. This trend was evident in the higher MIC values of the combination compared with those of CEO, although it was still lower than that of miswak alone. Additionally, more pathogens were susceptible to CEO than to the combination extract. The unexpectedly high MIC values of the CEO–miswak combination suggested a potential antagonistic interaction between its bioactive compounds. The decrease in both bioavailability and antibacterial activity of the CEO–miswak combination might have resulted from chemical interactions, such as the reaction of eugenol in CEO with BITC in miswak, potentially leading to the formation of fewer active complexes. The decreased antibacterial activity of the combined extracts, as observed in our study, has been reported in a few studies where combinations of natural extracts led to the neutralization of bioactive compounds or the formation of inactive complexes.[35,36] For example, Uduwana *et al*.[36] reported antagonism among flavonoid compounds in green tea, honey, and lemon. The potent antioxidants in each extract may react with one another, generating weaker antioxidants. Furthermore, polymerization of bioactive compounds may diminish their bactericidal efficacy. Moreover, the accidental neutralization of free radicals might further reduce the antioxidative effectiveness of the combined extracts.[36] Although our results showed that combining CEO and miswak did not improve antimicrobial efficacy, further research should investigate the biochemical interactions between their bioactive compounds. Understanding whether these interactions are truly antagonistic or whether other factors contribute to the lack of observed synergy is crucial. Consequently, further research is needed to explore the molecular interactions between CEO and miswak compounds using techniques such as high-performance liquid chromatography or mass spectrometry to better understand their antagonistic effects.

Comparing natural products and conventional antibiotics

Research has extended beyond the individual benefits of CEO and other plant extracts, revealing promising synergistic effects that enhance the effectiveness of traditional antibiotics. Building on the specific findings concerning CEO and miswak, we observed a general trend of lower MIC and greater susceptibility of microorganisms to antibiotics than to plant extracts. Similar findings were reported by Hassan *et al*.,[37] who found that antibiotics exhibited significant antimicrobial activity, whereas natural extracts demonstrated comparatively lower efficacy. In our study, CEO exhibited antimicrobial activity comparable to that of doxycycline in some cases, despite being a natural product. This comparison not only highlights CEO's potential but also serves as a stepping stone for evaluating the broader application of plant extracts in combating microbial infections. However, doxycycline remains the gold standard owing to its consistently low MIC values. These results indicate that local administration of CEO, along with reduced systemic doses of doxycycline, may mitigate adverse effects commonly associated with higher doses of antibiotics. These findings support the idea that plant-based extracts are safer alternatives to synthetic antibiotics, offering benefits such as cost-effectiveness and fewer side effects. In addition to their antimicrobial properties, plant extracts have therapeutic benefits such as cost-effectiveness, availability, ease of administration, and fewer side effects.[38] Given these inherent benefits, researchers are increasingly investigating how these plant extracts can complement conventional antibiotics to increase their efficacy. Recent studies have explored the synergistic effects of combining plant extracts with antibiotics, suggesting a promising strategy to address the increasing incidence of antibiotic resistance.[39] For example, CEO has been studied for its synergistic interactions with conventional antibiotics such as ampicillin, gentamicin, vancomycin, and β-lactam antibiotics.[11,40] This synergistic effect may be attributed to eugenol, a key component of CEO, which disrupts bacterial membranes, thereby enhancing the penetration of antibiotics. Time-kill studies confirmed these interactions, highlighting the combination of CEO or eugenol with antibiotics. Although the combination of plant extracts with antibiotics is an under-researched area, they should be used with caution, as noted by Kahlout *et al*.,[39] who reported antagonistic activity when combining plant extracts with antibiotics. To date, no studies have investigated the synergistic effects of CEO or miswak in combination with doxycycline. Future research could explore this avenue through time-kill studies to assess the potential interactions between CEO and doxycycline, as well as between miswak and doxycycline. These findings have significant implications for reducing antibiotic dosages and minimizing the associated adverse effects. Cytotoxicity assays using human gingival fibroblasts are planned in future studies to evaluate the safety of CEO and miswak. However, biofilm formation assays were not performed in this study, which is a limitation. These assays are suggested for future investigations to better mimic *the in vivo* conditions.

Conclusions

Although less potent than doxycycline, CEO exhibited promising antimicrobial activity, indicating its potential as a complementary treatment in situations where antibiotics are not preferred. Miswak has demonstrated selective efficacy, particularly against *F. nucleatum*; however, its effectiveness against other pathogens is limited. Given the significant individual effects of the combination of CEO and miswak, we hypothesized that combining them would enhance their antimicrobial efficacy. However, these results did not demonstrate a synergistic effect, indicating that the combination did not exceed the benefits observed when used alone. This lack of synergy underscores the complexity of interactions between natural compounds. This study provides preliminary evidence to support the use of CEO and miswak in peri-implantitis management. Optimization of natural antimicrobial treatments could offer safer and more effective alternatives for managing peri-implantitis and combating antimicrobial resistance.

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Conflict of interest

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Author contributions

Study concept and design (MS, RH), acquisition of data (RA, OSM, AA), analysis and interpretation of data (MS, RH, RA, TA, SH), drafting of the manuscript (MSM, MS), critical revision of the manuscript for important intellectual content (RH, RA, SH), administrative, technical, or material support (OSM, AA, MSM), and study supervision (TA, MS, AA). All authors have made significant contributions to this study and have approved the final manuscript.

Ethical statement

The study was conducted at Ibn Sina National College for Medical Studies and received approval from the Institutional Research Review Board (approval no. IRRB-03-28012024).

Data sharing statement

The data used in support of the findings of this study are available from the corresponding author at mshammas@ibnsina.edu.sa upon request.

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