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ORIGINAL ARTICALE

EFFECTIVENESS OF EXTRA VIRGIN COLD PRESSED COCONUT OIL WITH PEPPERMINT ESSENTIAL OIL AS A NATURAL ORAL HYGIENE CLEANER ON *SALIVARY STREPTOCOCCUS MUTANS* LEVELS: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Background: This study aimed to assess the effectiveness of extra virgin coconut oil combined with peppermint essential oil for pulling and brushing teeth with oil to reduce new carious lesions, salivary *Streptococcus mutans* levels, and plaque accumulation in high-risk patients with caries compared with conventional toothpaste.

Materials and Methods: A study was conducted on 40 high-risk patients, with Group C1 using the Coco Pull blend for oral care and Group C2 using fluoride toothpaste and chlorhexidine mouthwash. Caries risk was assessed via Cariogram software, and salivary profiles were measured. a caries risk assessment was performed at baseline (T0), one month (T1), and three months (T2) to determine the change in the percentage of actual chances of avoiding new lesions.

Results: This study revealed substantial antimicrobial efficacy in both groups, with the Coco Pull blend demonstrating superior results ($p<0.001$), as evidenced by notable differences in the green, red, and light-blue sectors of the Cariogram at various intervals.

Conclusion: Extra virgin coconut oil with essential peppermint oil, when used for oil pulling and tooth brushing, was effective as a natural cleaning agent for oral hygiene in reducing salivary *Streptococcus mutans* levels, dental caries, and plaque accumulation in patients at high risk for caries.

Keywords*:* Coconut oil, Peppermint essential oil, Tooth brushing, Oil pulling, Streptococcus mutans.

INTRODUCTION

Oral health plays a crucial role in overall well-being, as the mouth harbors billions of microorganisms, some of which can contribute to systemic diseases like cardiovascular disease and diabetes. A delicate balance exists between the host and its oral microbiome, influenced by factors such as diet, hygiene, and immune response. When this balance is disrupted, harmful bacteria can thrive, leading to conditions like dental caries. Maintaining good oral hygiene is essential to preventing dysbiosis and supporting general health. [1,2]

A holistic approach is essential for understanding dental caries, as multiple factors contribute to its development. [3] The Cariogram, an interactive educational tool, visually represents caries risk by displaying the probability of avoiding new cavities and the influence of various contributing factors.

It uses a pie chart with five colored segments to illustrate these interactions. *Streptococcus mutans*, a key bacterial species in caries formation, exhibits genetic diversity affecting its virulence.

Its ability to produce acid, tolerate acidic environments, and adhere to surfaces plays a crucial role in caries progression. Inhibiting its acid production is vital for prevention. [4,5]

Managing dental caries effectively requires assessing individual risk and adapting treatment plans accordingly.

Regular monitoring is essential to track changes in oral health over time.

The primary method of plaque removal is brushing with toothpaste, but many oral hygiene products lack rigorous risk assessments, potentially exposing users to harmful ingredients that could cause local or systemic adverse effects.[6]

Fluoride, sodium lauryl sulfate (SLS), and triclosan have been linked to various oral health issues, including mucosal irritation and allergic reactions. While fluoride is an effective cariostatic agent, excessive use can cause fluorosis, posing public health concerns. This has driven the search for alternative agents with fewer side effects. Natural products, with their broad biological activities and favorable safety profiles, have gained attention as potential cariostatic agents. Their accessibility, affordability, and biocompatibility make them promising alternatives, especially in regions where conventional treatments are less effective. [7]

Ayurvedic medicine, a 3,000-year-old holistic health system, emphasizes prevention and balance between mind, body, and spirit. Practices like oil pulling are believed to detoxify the body by eliminating toxins through the tongue. The coconut tree, known as the "tree of life," provides valuable resources, with virgin coconut oil (VCO) offering greater health benefits than refined copra oil. Essential oils, such as peppermint, have long been used for oral health, with menthol providing antimicrobial and pain-relieving properties. [8]

Randomized clinical trials are ideal for study design, but evidence on the efficacy of extra-virgin cold-pressed coconut oil with peppermint essential oil in oral hygiene remains limited. This trial evaluated their impact on salivary *Streptococcus mutans* levels in high-caries-risk patients compared to conventional fluoride toothpaste and chlorhexidine mouthwash, testing the null hypothesis that the natural regimen would not influence caries risk.

MATERIALS AND METHODS

1. Materials

Dental hygiene products utilized in this study

Three categories of dental hygiene products were used, which were further classified into two groups:

- Coco Pull a healthy blend of organic oil (as a mouthwash by oil pulling and dental cleaning agent by brushing with oil).
- Fluoride toothpaste (Colgate Total, 1450 ppm) and chlorhexidine (hexitol) mouthwash were used (CHX) (Table 1).

2. Methods:

2.1 Trial Registration and Study Design.

2.2 Trial Registration

The protocol for this study was registered in the protocol registration and results system database (www.clinicaltrials.gov) under the identification number (NCT05803109) on 07/04/2023. The research procedures involving human subjects adhered to the ethical standards set by the Research Ethics Committee of the Faculty of Dentistry, Cairo University (Approval no. 4722), and it followed the Declaration of Helsinki and its subsequent amendments.

2.2 Informed consent

Before the clinical trial, patients were thoroughly briefed on the study's objectives, methods, safety measures, potential benefits, and duration. They engaged in informed discussions with the researcher, and consent was obtained from all participants, approved by the Research Ethics Committee (REC) of Cairo University's Faculty of Dentistry.

2.4 Sample Size Calculation

On the basis of a previous study by Fouad et al. (2022) [9], subjects per group were required to detect a true difference of five between the experimental and control means with 80% power and a Type I error probability of 0.05. To account for potential attrition, the sample size was increased by 20%, resulting in a total of 40 patients (20 per group). Calculations were conducted via PS power and sample size calculations (version 3.1.6) for Windows.

2.5 Study Setting

This double-blind, parallel-arm, randomized controlled trial was conducted at the Conservative Dentistry Department outpatient clinic, Faculty of Dentistry, Cairo University, from January to December 2023, with patient recruitment from January to July 2023**.**

2.6 Eligibility criteria

Inclusion criteria

Age: 20–50 years, high caries risk assessment, nonsmoking, no antibiotic therapy during the study or in the preceding month, both male and female participants, and regular oral hygiene practices.

Exclusion criteria

A compromised medical history, low caries risk assessment, severe or active periodontal disease, hypersensitivity to study drugs or chemicals, antibiotic use within the past month, tobacco use, and significantly reduced salivary flow (xerostomia < 0.5 ml/min, score 3 on the cariogram were reported).

2.7 Allocation:

2.7.1 Sequence generation:

An external contributor used the Random Sequence Generator from Randomness and Integrity Services Ltd. to assign numbers (1–20) to either the intervention or the comparator in the study.

2.7.2 Allocation concealment:

The assigned random numbers were stored in a sealed, opaque envelope, and the contributor did not participate in later trial stages. The operator and co-supervisor recorded the randomisation outcomes digitally, with all records maintained by the main supervisors.

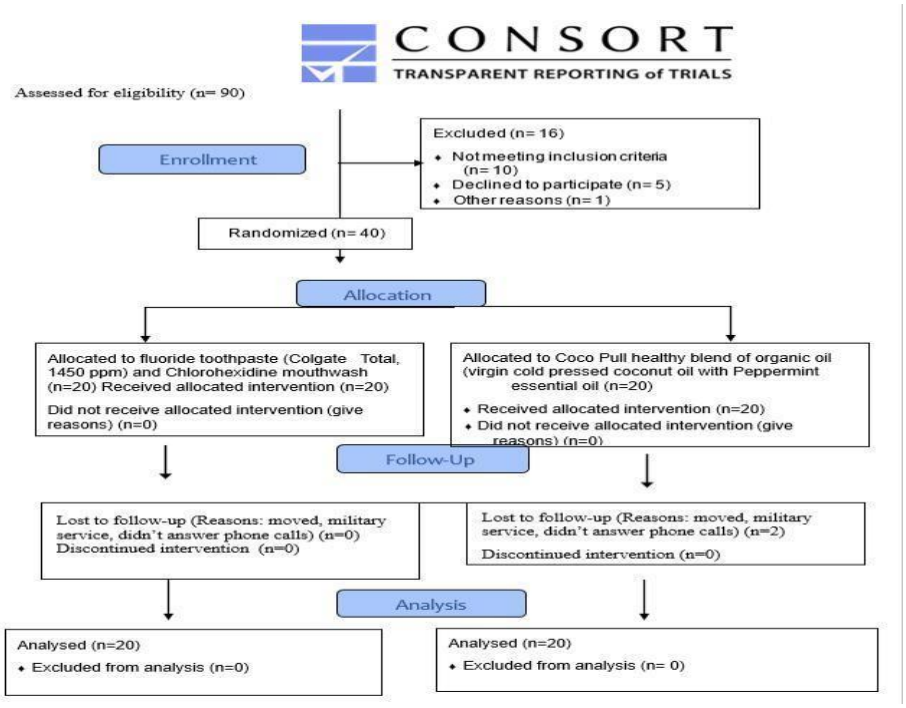

Figure 1. CONSORT 2010 Flow Diagram

2.8 Interventions

Intervention Group C1.

Patients were instructed to use a packet of the Coco Pull healthy blend of organic oil, which is a mixture of Virgin Cold-Pressed Coconut Oil and Peppermint Essential Oil (AVIVA-PURE-CocoPull-Organic in the USA). They were instructed to swish this oil in their mouth, pull it between their teeth for five minutes, and then spit out the oil. The participants were subsequently instructed to brush their teeth with the same oil for two minutes. This routine was repeated twice daily for three months. (Table 1)

Table 1. Tooth cleaning agents used in this trial, categorization, active ingredients, manufacturers and lot numbers

Teeth cleaning products	Category	Active Ingredients	Manufacturer and Lot number
Coco Pull healthy blend of organic oil	Ayurvedic, Natural product	virgin cold-pressed coconut oil with Peppermint essential	(AVIVA-PURE-CocoPull-Organic- made in USA) Xooo VMGWFP
Fluoride toothpaste (Colgat Total, 1450 ppm)	Synthetic chemical compound	Glycerin, Water, Hydrated Silica, Sodium Lauryl Sulfate, Arginine, Flavor, Zinc Oxide, Cellulose Gum, CI 77891, Poloxamer 407, Tetrasodium Pyrophosphate, Zinc Citrate, Benzyl Alcohol, Xanthan Gum, Cocamidopropyl Betaine, Sodium Fluoride, Sodium Saccharin, Phosphoric Acid, Sucralose	Colgate Palmolive China Co Ltd. 338 Qing Nian Road China
Chlorohexidine mouthwash (Hexitol)	Synthetic chemical compound	Chlorohexidine Hydrochloride	Arab Drug Company "ADCO" for pharmaceutical and chemical industries, Cairo, Egypt LOT #: 930626

Comparator Group C2.

Patients were guided to clean their teeth via fluoride toothpaste (Colgate Total, 1450 ppm) for 2 min. This routine was followed twice daily for three months. Additionally, they were advised to rinse with 10 ml of 0.12% chlorhexidine for 1 minute each day,30 minutes after brushing their teeth, right before bedtime, and only one week per month [9](Table 1).

2.9 Follow-up strategy

Over three months, patients provided saliva samples at baseline (T0) and at one-month (T1) and two-month (T2) intervals for microbiological analysis and caries risk assessment via Cariogram software. (Fig. 2A, 2 B) Patients were divided into two groups based on their oral hygiene regimen: the intervention group used oil pulling followed by brushing with oil, while the control group used fluoride toothpaste and chlorhexidine mouthwash. Both groups followed identical oral hygiene instructions using the modified Bass technique with medium-hardness toothbrushes.

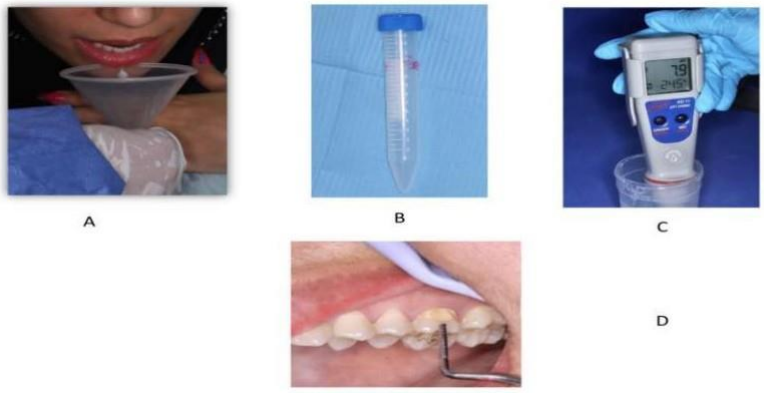

Figure 2.A,B,C Microbiological analysis and caries risk assessment via Cariogram software.D.Measure the plaque index

Participants were guided to maintain a noncariogenic diet, reducing sugar intake to below 10% of total dietary energy. They avoided sugary foods, particularly at bedtime, and refrained from using oral hygiene products from other suppliers. Regular assessments monitored changes in caries risk throughout the study.

2.10 Outcome assessment

2.10.1 Cariogram (chance to avoid new lesions) Green sector.

At each of the three visits, participants' caries risk profiles were assessed via Cariogram software (Cariogram, internet Version 2.01. April 2, 2004, Copyright: D. Bratthall, Sweden). At baseline, ten caries-related factors were entered into the software for each group: (1) caries history; (2) related health conditions (immunologic diseases, endocrinopathies, hematologic conditions, systemic infections, diabetes mellitus, and heart disease); (3) diet content; (4) meal frequency; (5) *Streptococcus mutans levels*; (6) plaque volume; (7) fluoride treatment; (8) saliva buffering capacity; (9) saliva production rate; and (10) clinical assessment. These factors determine the probability of caries prevention in the future. The region was classified as low risk because of Egypt's water fluoridation program.

2.10.2 Bacterial colonies were counted, and the plaque index was measured to assess the cariogram (bacteria) Red sector.

Inoculation of saliva samples.

One milliliter of collected saliva was diluted tenfold with normal saline. A total of 25 µL of the diluted sample was applied to a freshly prepared M. salivarius agar plate via an automatic micropipette. The sample was transferred from the test tube to the agar medium and evenly distributed across the agar surface with a sterile glass rod to ensure uniform bacterial growth and a smooth surface, avoiding scratches or indentations [10].

A microaerophilic environment with a 5–10% CO2 concentration was created via a candle jar for the agar plates, which were then incubated at 37°C for 48 h. Subsequently, the jar was sealed and placed in a precision dual-illumination incubator (Germany). After a 48-h incubation, the plates were removed from the incubator. An examiner blinded to the treatment details of the 40 patients counted *S. mutans* colonies.

The total colony-forming units (CFUs) were calculated for each salivary sample corresponding to each mouthwash. *S. mutans* colonies typically exhibit characteristics such as 0.5 mm raised, convex, and undulated colonies that are light blue with rough margins and a granular frosted glass appearance. Colonies were quantified as CFUs per millilitre [10].

The Silness and Loe scale was used to measure the plaque index of six specific teeth by evaluating the amount of plaque in the cervical region. (Fig. 2D)

In this study, dietary content and frequency were assessed with a score of 1 in accordance with the study's inclusion criteria. Fluoride intake was quantified on the basis of the fluoride supplements consumed by each group.

All participants in the study received a score of three for the "caries experience" factor, indicating a more severe status than typical for that age group. The "clinical judgment" factor was assigned a score of 1 for all participants to mitigate potential bias. Additionally, the "related diseases" factor was scored zero, indicating that none of the participants exhibited any major general diseases associated with dental caries, according to the inclusion criteria, and all represented the assessment of the cariogram (circumstances): Yellow sector.

2.10.3 Buffering capacity and salivary secretion rate measurement to assess the cariogram (susceptibility) Light blue sector

The buffer capacity of unstimulated saliva samples was assessed via the Ericsson method (1959). In this method, 3 ml of 5 mmol/L HCl was added to 1 ml of stimulated saliva, and the mixture was stirred thoroughly. The sample was then left to stand for 10 minutes. The final pH was subsequently measured via a calibrated Adwa (AD-11) digital pH meter (Adwa Hungary Kft, Szeged, Hungary). (Fig. 2C) [11] The scoring was as follows: 0 = adequate/normal buffer capacity, saliva pH > 6; 1 = reduced buffer capacity, saliva pH 4.5–5.5; 2 = low buffer capacity, saliva pH < 4.0.

The salivary secretion rate of stimulated saliva was categorized as follows: normal (0), 0.9–1.1 mL/min (1), and 0.5–0.9 mL/min (2). Rates less than 0.5 mL/min, which is indicative of xerostomia, were excluded from this study.

All the collected data were subsequently analyzed via a standardized procedure in the Cariogram program. These data were then input into the program to generate a pie chart. The software produces a pie chart that illustrates the percentages of "Bacteria" (depicted in red), "Diet" (dark blue), "Circumstances" (yellow), and "Susceptibility" (light blue). These four sectors collectively determine the percentage of the "probability of avoiding caries' (green sector). The magnitude of the green sector was inversely proportional to the risk of caries, indicating superior oral hygiene and dental health.

2.11 Statistical analysis

The data were analyzed using MedCalc software version 22, assessing normality via the Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous data were normally distributed and presented as means and standard deviations. Intergroup comparisons used an independent t-test (**p** ≤ 0.05), while intragroup comparisons applied repeated measures ANOVA (**p** ≤ 0.016 for follow-up periods, **p** ≤ 0.005 for *S. mutans* acidogenic assay after Bonferroni correction). Categorical data were analyzed using chi-square tests, with intergroup significance set at **p** ≤ 0.05 and intragroup significance at **p** ≤ 0.016. All tests were two-tailed, with an 80% study power and a 95% confidence level.

RESULTS

Cariogram (chance to avoid new lesions): Green sector

Intergroup comparisons revealed no statistically significant difference at baseline (p = 0.7612), whereas after 1 and 3 months, there was a statistically significant difference (p < 0.0001). Intragroup comparisons within C1 and C2 revealed statistically significant differences between the different follow-up periods (P < 0.001) (Table 2 and Fig 3).

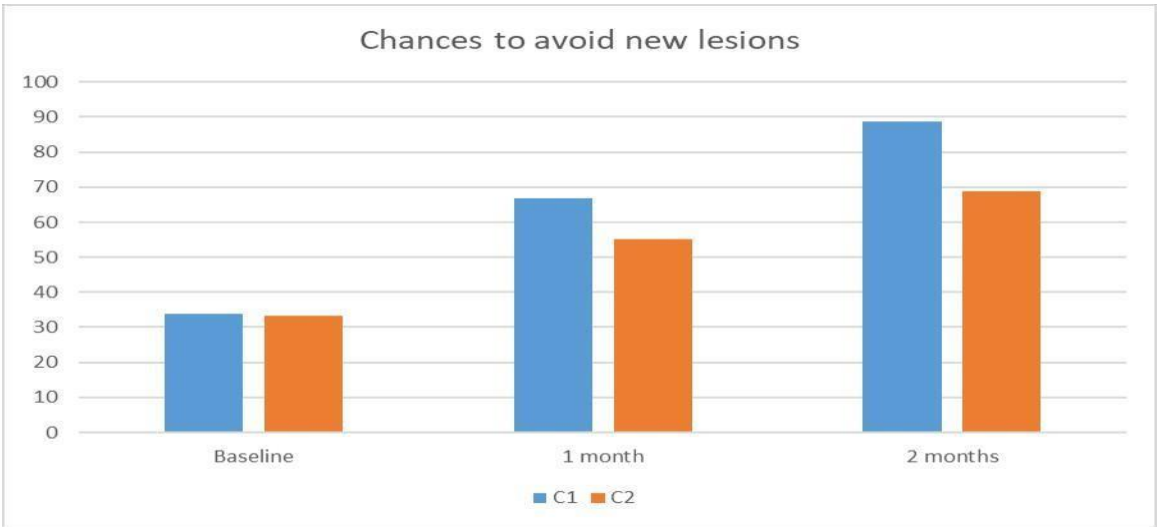

Figure 3. Bar chart showing the effect of the intervention on the percentage of chances of avoiding new lesions in the green sector at each follow-up

Table 2. Means and standard deviations of the cariogram sectors of the two groups and comparisons between them (also a comparison between different follow-up periods of all groups)

	C1		C2		
	Mean	SD	Mean	SD	P value
Green sector					
Baseline	33.85[c]	6.43	33.20[c]	6.99	P = 0.7612
1 month	66.80[b]	4.21	55.28[b]	4.01	P < 0.0001*
3 months	80.70[a]	5.42	68.94[a]	4.60	P < 0.0001*
P value	P<0.001*		P<0.001*		
Red Sector					
Baseline	22.25[a]	3.86	24.25[a]	3.13	P = 0.0798
1 month	8.25[b]	0.44	8.28[b]	0.57	P = 0.8532
3 months	2.55[c]	1.19	2.65[c]	0.93	P = 0.7688
P value	P<0.001*		P<0.001*		
Light blue sector					
Baseline	25.10[a]	3.35	22.95[a]	4.05	P = 0.0752
1 month	9.20[b]	2.26	17.44[b]	2.91	P < 0.0001*
3 months	6.50[c]	2.56	13.53[c]	2.94	P < 0.0001*
P value	P<0.001*		P<0.001*		
Yellow Sector					
Baseline	9.60	1.57	10.00	2.64	P = 0.5637
1 month	9.90	1.41	9.61	1.69	P = 0.5592
3 months	10.35	2.21	9.65	1.73	P = 0.2717
P value	P = 0.399		P = 0.845		

	C1		C2		
	Mean	SD	Mean	SD	P value

*means that those that do not share a letter vertically are significantly different; * corresponds to a statistically significant difference*

Cariogram (bacteria): Red sector

Intergroup comparisons revealed no statistically significant differences at baseline or at 1 and 3 months (P > 0.05). Intragroup comparisons within C1 and C2 revealed statistically significant differences between the different follow-up periods (P < 0.001) (Table 2).

Cariogram (susceptibility): Light blue sector

Intergroup comparisons revealed no statistically significant difference at baseline (p = 0.0752), whereas after 1 and 3 months, there was a statistically significant difference (P < 0.0001). Intragroup comparisons within C1 and C2 revealed statistically significant differences between the different follow-up periods (P < 0.001) (Table 2).

Cariogram (circumstances): Yellow sector.

Intergroup comparisons revealed no statistically significant differences at baseline or at 1 and 3months (P > 0.05). Intragroup comparisons within C1 and C2 revealed no statistically significant differences between the different follow-up periods (P > 0.016) (Table 2).

DISCUSSION

Despite modern healthcare advancements, the precise cause of dental caries remains uncertain. However, it is widely accepted that dysbiotic changes in the oral biofilm, influenced by fermentable sugars and carbohydrates, contribute to its development. Prevention relies on daily plaque removal through brushing, flossing, and rinsing, with fluoridated toothpaste being the most common method. While oral care products often contain antimicrobials like chlorhexidine and fluoride, these

agents struggle to effectively combat *Streptococcus mutans* due to its complex biological pathways. Fluoride, in particular, does not alter biofilm composition or bacterial virulence [12,13].

Even with regular fluoride use, excessive sugar intake—more than six times daily—can lead to cavities. Prolonged exposure to high concentrations of chlorhexidine (CHX) and fluoride (1000–2000 µg/ml) poses risks, including fluorosis, weakened bones, and developmental neurotoxicity [14].

Virgin coconut oil (VCO) is extracted from fresh coconut kernels without heat or chemical refining, preserving antioxidants, vitamins, and polyphenols. It contains lauric acid (LA), which converts to monolaurin in the body, enhancing its antimicrobial effects. Medium-chain fatty acids and monoglycerides in VCO disrupt bacterial membranes, making it effective against various microbes.[15]

Essential oils, valued for their aroma, include phytoncides—natural plant compounds that protect against pathogens. These volatile substances exhibit antimicrobial, antibacterial, antifungal, anti-inflammatory, stress-relieving, and analgesic properties [16]

This study incorporated peppermint essential oil into virgin coconut oil (VCO), utilizing its antioxidants to prevent rancidity and enhance quality. VCO serves as a carrier oil, reducing cytotoxicity, potentially boosting antimicrobial effects, and providing a refreshing taste and aroma. Oil pulling with VCO helps reduce plaque and gingival inflammation, while coconut derivatives like coconut milk and water may support enamel remineralization due to their high calcium bioavailability. [17,18]

Participants used Coco Pull, an organic blend of virgin cold-pressed coconut oil and peppermint essential oil, by swishing it for five minutes, pulling it between their teeth, and brushing with it for two minutes. The control group followed a conventional regimen with fluoride toothpaste (Colgate Total, 1450 ppm) and 0.12% chlorhexidine rinse, used 30 minutes after brushing to minimize interactions with dentifrice ingredients like MFP and SLS. This protocol served as a comparator, given its established broad-spectrum antimicrobial efficacy, fluoride's role in remineralization, and its lack of systemic adverse effects.

Participants received standardized oral hygiene instructions and medium-hard toothbrushes (Colgate Classic Deep Clean). They followed a modified Bass brushing technique for two minutes, supported by a demonstration video to ensure consistency. To minimize confounding factors, they adhered to a noncariogenic diet by reducing daily sugar intake in both quantity and frequency throughout the three-month study.

This study used the Cariogram to assess caries risk by visually representing an individual's risk based on etiological factors. It categorized patients into low-, moderate-, or high-risk groups, enabling tailored prevention strategies. Key Cariogram sectors showed significant differences between and within groups over time, with the Coco-Pull blend (oil pulling and brushing with oil) demonstrating superior efficacy ($P<0.001$). [19]

The red sector, reflecting bacterial activity, was assessed using bacterial count and plaque index scores. In the control group using fluoride toothpaste and chlorhexidine, a significant reduction was observed over baseline, one-month, and three-month intervals, indicating a decline in *Streptococcus mutans* levels and plaque accumulation [19] .

The reduction in bacterial activity is due to fluoride in toothpaste and chlorhexidine (CHX). Combining mechanical and chemical oral hygiene methods is highly effective—mechanical cleaning removes most plaque, leaving a thin, disorganized layer that can be further diminished with chemical agents [20].

Chlorhexidine (CHX) has been shown to have effective antiplaque activity owing to its dual binding to bacterial cell membranes and salivary components. Several studies have examined the effects of chlorhexidine mouthwash on plaque levels [9].

Fouad et al. [9]found that chlorhexidine (CHX) and fluoride toothpaste significantly reduced plaque scores. CHX has broad-spectrum antibacterial properties, acting as a bacteriostatic agent at low concentrations by increasing membrane permeability, and as a bactericidal agent at higher concentrations by precipitating cytoplasmic proteins. Sodium fluoride (NaF) disrupts key enzymes in *Streptococcus mutans*, inhibiting its metabolic processes. [21]

In the intervention group, the Coco Pull blend of virgin cold-pressed coconut oil and peppermint essential oil significantly reduced *Streptococcus mutans* levels and plaque index scores over baseline, one-month, and three-month intervals. These results align with in vivo studies by Raju et al. (2024) [22] and Peedikayil et al. (2015) [23], which observed similar reductions following oil pulling.

Supporting in vitro studies by Ng et al. (2024)[23] and Desam et al. (2019) [25]further demonstrated the antimicrobial activity of VCO and peppermint EO against *S. mutans* biofilms, likely due to their synergistic antibacterial properties.

Virgin coconut oil (VCO) combats bacteria due to its high lauric acid content, which disrupts bacterial cell membranes, leading to cell death. Peppermint essential oil (EO) enhances this effect with antimicrobial, anti-inflammatory, and stress-relieving properties. The Coco-Pull group showed a significant plaque reduction, attributed to oil pulling and mechanical brushing, which emulsify the oil and reduce bacterial coaggregation. [26]

VCO also undergoes saponification in the mouth, forming a soap-like substance that minimizes plaque adhesion(Fig. 5b), offering an alternative to sodium lauryl sulfate (SLS), which can cause contact dermatitis. [26,27] The light blue Cariogram sector, reflecting saliva-related factors, showed improvement in both groups, with the Coco-Pull group demonstrating higher saliva secretion rates, likely due to VCO's polyphenols and peppermint EO's antioxidant effects.

Additionally, peppermint EO aromatherapy reduces sympathetic nervous system activity and salivary cortisol, further enhancing saliva production. This aligns with a review by Kontogiannopoulos et al. (2023)[28], which highlighted natural compounds' ability to alleviate salivary dysfunction through various molecular pathways.

Prolonged chlorhexidine (CHX) use can lead to adverse effects, including calculus buildup, tooth stains, xerostomia, taste disruption, and, in rarer cases, oral paresthesia and mucosal desquamation.[29] This contributed to lower salivary secretion rates in the fluoride toothpaste and CHX group. Regarding salivary pH, the Coco-Coco-Pull group exhibited a higher pH after two months. CHX may alter the oral microbiome, reducing bacterial diversity and increasing Firmicutes while decreasing Bacteroidetes, ultimately leading to saliva acidification. [29] The yellow sector, representing past caries experience and systemic diseases, remained unchanged across all participants to ensure standardization and minimize confounding factors. No significant differences were observed in this sector for either group over time.

CONCLUSIONS

Within the limitations of the present study, the following conclusions were drawn.

1. Extra virgin coconut oil combined with essential peppermint oil, which is utilized through oil pulling and tooth brushing, is effective as a natural cleansing agent for routine oral hygiene. This approach effectively reduces salivary *Streptococcus mutans* levels, dental caries, and plaque accumulation in high-caries-risk patients, presenting minimal adverse effects as an alternative to current synthetic preventive measures.

Limitations of the study:

Further scientific studies are needed

DECLARATIONS

Data availability

The corresponding author (asmaa.reda@dentistry.cu.edu.e.g.)can provide access to the datasets utilized or examined in this study upon a reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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