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# Antibacterial and antiplaque efficacy of peppermint- or rosemary-enriched virgin coconut oil versus plain coconut oil, conventional mouthwash in Indian children: An *in vivo* study

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**Abstract:**
Although oil pulling is widely used to improve oral health, evidence comparing virgin coconut oil enriched with essential oils against chlorhexidine in children remains limited. Therefore, it is of interest to evaluate 80 children aged 10–13 years allocated into 4 groups receiving virgin coconut oil with rosemary, virgin coconut oil with peppermint, plain virgin coconut oil, or chlorhexidine. All groups showed significant reductions in *Streptococcus mutans* counts and plaque scores, while virgin coconut oil combined with rosemary or peppermint produced greater improvements than plain virgin coconut oil and performed comparably to chlorhexidine. Salivary acidity shifted toward a healthier range in all groups, indicating improvement of the oral environment alongside antibacterial activity. Thus, data shows that essential oil-enriched virgin coconut oil is an effective, natural and child-friendly alternative to chlorhexidine for plaque control and dental caries prevention.

**Keywords:** Oil pulling, virgin coconut oil (VCO), peppermint oil, rosemary oil, chlorhexidine, *streptococcus mutans*, dental plaque, oral pH

**Background:**
Oral health is an essential part of overall health and well-being, especially among children, where preventive measures can greatly decrease the incidence of dental diseases. Gingivitis caused by plaque and dental caries are some of the most common chronic diseases in school-aged children across the globe and *Streptococcus mutans* has been cited as a key etiological factor in the onset and development of dental caries **[1]**. Mechanical and chemical methods of controlling dental plaque are thus critical in oral health and disease prevention. The traditional mouthwashes, especially chlorhexidine gluconate, are generally considered the gold standard of chemical plaque control because of their wide-spectrum antimicrobial activity and substantivity **[2]**. Nevertheless, there are a number of undesirable side effects associated with the prolonged use of chlorhexidine including tooth staining, distortion of taste sensation and mucosal irritation, which could restrict its acceptability, particularly in children **[3]**. Such restrictions have led to the search of alternative, natural and safer adjuncts in maintenance of oral hygiene. Oil pulling is an ancient Ayurvedic procedure that entails the swishing of edible oils in the mouth cavity over an extended period of time to enhance oral and systemic well-being **[4]**. Conventionally done with sesame or sunflower oil, recent research has shown the possible advantages of virgin coconut oil (VCO) because of its richness in medium-chain fatty acids, especially lauric acid, which has antimicrobial, anti-inflammatory and anti-plaque effects **[5]**. Lauric acid has been showed to destabilize bacterial cell membranes and prevent the growth of cariogenic microorganisms, such as *S. mutans* **[6]**. Although VCO has promising effects, it can be improved with the inclusion of essential oils (EOs), which have been reported to have strong antimicrobial and therapeutic effects. Mentha piperita is a source of peppermint oil that has active compounds including menthol and menthone which have strong antibacterial and anti-inflammatory properties **[7]**. Likewise, rosemary oil, which is the extract of Rosmarinus officinalis, has antimicrobial, antioxidant and anti-inflammatory properties, which are attributed to the presence of cineole and rosmarinic acid **[8]**. The use of these essential oils in oral care products has been popular because of their capacity to prevent oral pathogens and enhance freshness of breath. The synergistic effect of the carrier oils like VCO with essential oils could result in increased antimicrobial activity without compromising biocompatibility and patient acceptability. Moreover, it has been proposed that oil pulling affects salivary pH, which is a critical aspect of oral homeostasis. An increase in the alkalinity of the pH can prevent the development of acidogenic bacteria and decrease the likelihood of enamel demineralization **[9]**. Therefore, it is of interest to evaluate the body of knowledge on safer and more acceptable oral hygiene practices in paediatric populations by examining natural and effective substitutes to traditional mouthwashes.

**Materials and Methods:**
**Study design and settings:**
This current randomized crossover study was conducted at 3 underprivileged semi-government residential schools at Bilaspur, Chhattisgarh, India. Procurement of ethical clearance was done from the Institutional Ethics Committee.

**Sample size:**
The sample size calculation was done using G*Power 3.1.9.2 software. The sample size was calculated by considering 5% alpha error and 80% power for the study. Based on the above calculation the minimum sample required in each group was 15. In this current study the sample size of 20 subjects in each group were taken.

**Experiment:**
In this study, saliva samples were collected from children of various schools located in Bilaspur. For the purpose of this study 80 children aged between 10-13 years were selected randomly from the schools. Proper written consents were taken from the children and their parents/guardian in the language best understood by them before the commencement of study by discussing with them the purpose of the study. Short case histories were recorded. Visible plaque index were evaluated using Silness and Loe plaque index (1967)

The Children were divided into 4 groups of 20 each.
**4 groups were:**
**[1]** Group A-Virgin coconut oil with rosemary essential oil
**[2]** Group B - Virgin coconut oil with peppermint oil
**[3]** Group C - Plain virgin coconut oil
**[4]** Group D - Chlorhexidine mouthwash (positive control)

**Inclusion criteria:**
**[1]** Children who were healthy (without any medical problems) and who were not under any kind of medication.
**[2]** Children with poor oral hygiene.
**[3]** Children with minimum 20 natural teeth.
**[4]** Children with high plaque score, (≈ 3) *i.e.* a band of plaque wider than 1 mm but less than 1/3rd of the crown extending over all inter-proximal buccal and lingual surfaces.
**[5]** Children with no history of dental visit in past 3 months.
**[6]** Children with moderate to high caries activity.

**Exclusion criteria:**
**[1]** Children with systemic diseases and medically compromising conditions.
**[2]** Children with history of systemic or topical antibiotics usage within a month.
**[3]** Children with soft tissue damage due to ill-fitting appliances and with orthodontic or prosthodontic appliances.
**[4]** Children with history of professionally applied topical fluoride in last 1 year.
**[5]** Children are less than 10 years of age.

**Methods:**
For the purpose of this study, the experimental samples for oil pulling were prepared in the laboratory.

**Method of preparation of oil:**
**[1]** 1 drop of Rosemary essential oil to 15 ml (1 tablespoon) virgin coconut oil and mixed well, hence containing 0.33% of rosemary essential oil.
**[2]** 1 drop of Peppermint essential oil to 15 ml (1 tablespoon) virgin coconut oil and mixed well, hence containing 0.33% of peppermint essential oil.
**[3]** 1 drop = 0.05 ml

$$\frac{0.05}{15\ ml} \times 100\% = 0.33\%$$

Children were given bottles with 105 ml (15 ml X 7) of carrier oil mixed with 7 drops of essential oil.

**Method of oil pulling:**
**[1]** Children were asked to take 15 ml of various oils using a tablespoon. They were advised to lift their chin a bit; close eyes and start swishing liquid from left to right, front to back for 10 minutes or till the children feel fullness in mouth, then spit it out. This procedure was to be repeated for 7 days. The participants were clearly instructed to take adequate precautions not to swallow the liquid while performing the procedure. A timer was used to motivate the children for oil swishing.
**[2]** Children were instructed not to panic or feel fearful about the procedure if swallowing occurs at all. They were instructed to refrain from brushing teeth and not to use any other mouthwash during the study period. They were followed by telephonic monitoring. After the end of the study, the participants were provided with a self-administered, close-ended dichotomous questionnaire to evaluate the acceptability of oil pulling administered to each of them.
**[3]** Saliva samples were collected at the baseline levels (before oil pulling), immediately after oil pulling, 3rd day and 7th day after oil pulling. The questionnaire included three questions with two mutually exclusive options (YES/ NO) to answer.
**[4]** The questions were based on the acceptability of the oil pulling in terms of flavor, smell and willingness to continue using the oil pulling.
**[5]** They were also given oral prophylaxis at the end of the study.

**Method of recording plaque score:**
**[1]** Before commencing the study children were asked not to use any fluoridated toothpaste or mouthwash for 3 days.
**[2]** Plaque samples were collected from the buccal surfaces of the posterior teeth using a sterile WHO probe.
**[3]** Collected plaque samples were transferred to sterile tubes containing 1.5 ml saline.
**[4]** The study commenced by recording the plaque index at the baseline levels, immediate after oil pulling, 3rd day and 7th day after oil pulling using plaque index by Silness and Loe.

**Method of collecting saliva:**
Before commencing the study children were asked not to use any fluoridated toothpaste or mouthwash for 3 days. Saliva samples were collected (between 9 to 11 AM) from children after breakfast and without consuming any food or drinks for at least an hour prior to the examination. Unstimulated saliva samples were collected 1 ml from under the tongue with micro pipette. The samples will be immediately stored in sterile Eppendorf tubes and placed in an ice box set below 4°C to be carried to lab for further examination.

**Preparation of MSB Agar:**
As per the manufacturer's instruction, 90.07 grams of the Mitis Salivarius agar base (MS agar) was suspended in 1000 ml distilled water. This solution was heated to boiling temperature to dissolve the medium completely. It was then dispensed and sterilized by autoclaving at 15 lbs. After the solution cooled down to normal room temperature, Potassium Tellurite Solution (FD052) was added to the medium. The prepared medium should not be reheated after the addition of potassium tellurite solution. It was mixed well and poured into sterile Petri plates. 0.2 U/mL bacitracin and 20% w/v sucrose was added to the prepared MS agar medium to make an improved medium of Mitis salivary Bacitracin agar medium (MSB). This final prepared medium has a higher selectivity of *S. mutans*.

**Microbiological analysis and pH estimation of saliva:**
The whole saliva samples will be collected for buffer capacity and bacterial counts. Samples will be mixed by overtaxing and dilution (1: $10^3$) of the saliva samples in 0.05M phosphate buffer (pH7.0) was done using serial dilution method; 0.1mL of which was inoculated on Metis Salivary Agar with potassium tellurize medium and bacitracin (MSBA). Incubation of the plates was done for 48 hours at 37°C in a 5–10% $CO_2$ jar, under a light microscope. The number of Colony Forming Units (CFU) of *S. mutans* was determined using a digital colony counter. Further, the determination of CFU per mL was done using the following formula:

No. of colonies × Dilution factor/Volume inoculated in mL = CFU/mL.
Where, Volume inoculated=0.1mL and Dilution factor=$10^3$.

After calibrating the digital pH meter with standard buffer solutions of known pH (typically pH 4 and 7) then immerse the pH meter's electrode into the unstimulated saliva sample and stable pH is recorded. The electrode should be thoroughly rinsed with distilled water and dried between measurements to prevent incorrect reading and contamination also.

**Statistical analysis:**
The data was tabulated in Microsoft excel and analysed with SPSS version 24 software. The continuous variables are presented with mean and standard deviation. The categorical variables are presented with frequency and percentage. One way ANOVA and Repeated measures ANOVA are used for the statistical analysis. The p value ≤0.05 is considered statistically significant.

**Results:**
All four groups showed a progressive and statistically significant reduction in CFU counts over time ($p < 0.001$), indicating effective antibacterial action of all interventions. Chlorhexidine showed the greatest reduction at every time point (≈74%), confirming its superior antimicrobial efficacy. Among the test groups, Rosemary oil + VCO exhibited the best performance, followed by Peppermint oil + VCO, while plain VCO showed the least reduction. The results suggest that the addition of essential oils enhances the antibacterial efficacy of virgin coconut oil, with rosemary oil showing comparatively better outcomes. Overall, although chlorhexidine remains the most effective, essential oil-enriched VCO shows promising and clinically significant antibacterial potential (**Table 1)**. Plaque index showed a progressive reduction in all groups over time, with statistically significant intragroup changes ($p < 0.001$). Chlorhexidine demonstrated the greatest reduction in plaque scores, confirming its superior antiplaque efficacy. Among natural agents, rosemary oil with VCO showed better performance than peppermint oil and plain VCO in reducing bacterial load, while peppermint oil demonstrated slightly better plaque reduction than rosemary at day 7. Baseline comparisons showed no significant differences, ensuring group comparability. While plaque reduction was similar across groups at day 3 ($p = 0.857$), significant differences emerged by day 7 ($p = 0.008$), indicating cumulative treatment effects. Overall, essential oil-enriched VCO showed substantial efficacy, approaching that of chlorhexidine and may serve as a promising natural alternative for oral hygiene in children **(Table 2)**. All groups showed a progressive increase in salivary pH from baseline to day 7, with statistically significant intragroup changes ($p < 0.001$), indicating a shift toward a more alkaline oral environment. However, intergroup comparisons at all-time points were not statistically significant ($p > 0.05$), suggesting comparable efficacy among all interventions. Chlorhexidine showed the highest pH at day 7, closely followed by virgin coconut oil and essential oil groups. Overall, both plain and essential oil-enriched VCO demonstrated similar effectiveness to chlorhexidine in improving salivary pH, indicating their potential as natural alternatives for maintaining oral pH balance **(Table 3)**.

**Table 1:** Comparison of CFU Counts across groups at different time intervals

| Group | Before Treatment (Mean ± SD) | After Initial Treatment (Mean ± SD) | P | After 3 Days (Mean ± SD) | After 7 Days (Mean ± SD) | p-value (Between Groups) |
|---|---|---|---|---|---|---|
| **Rosemary Oil + VCO** | 180.90 ± 6.03 | 125.90 ± 6.69 | 0.148 | 95.90 ± 5.68 | 75.90 ± 6.20 | <0.001* |

| | | | | | |
|---|---|---|---|---|---|
| **Peppermint Oil + VCO** | 179.20 ± 6.08 | 134.20 ± 5.91 | | 109.20 ± 6.05 | 84.20 ± 4.79 |
| **Virgin Coconut Oil (VCO)** | 176.30 ± 9.23 | 136.30 ± 9.41 | | 111.50 ± 9.16 | 91.30 ± 8.55 |
| **Chlorhexidine** | 182.50 ± 6.76 | 102.50 ± 6.61 | | 72.50 ± 7.39 | 47.50 ± 6.68 |

*Statistically significant

**Table 2:** Plaque index changes across groups at different time intervals

| Group | Plaque Before | P value | Plaque Day 3 | Plaque Day 7 | P value |
|---|---|---|---|---|---|
| **Rosemary Oil + VCO** | 0.79 ± 0.17 | 0.108 | 0.65 ± 0.16 | 0.49 ± 0.17 | 0.008* |
| **Peppermint Oil + VCO** | 0.73 ± 0.18 | | 0.62 ± 0.18 | 0.48 ± 0.19 | |
| **Virgin Coconut Oil** | 0.70 ± 0.17 | | 0.63 ± 0.17 | 0.55 ± 0.17 | |
| **Chlorhexidine** | 0.81 ± 0.12 | | 0.60 ± 0.12 | 0.36 ± 0.15 | |

*Statistically significant; NS = Not significant

**Table 3:** Salivary pH Changes across groups at different time intervals

| Group | Before Treatment (Mean ± SD) | After Initial Treatment (Mean ± SD) | After 3 Days (Mean ± SD) | After 7 Days (Mean ± SD) | p-value (Between Groups) |
|---|---|---|---|---|---|
| **Rosemary Oil + VCO** | 6.69 ± 0.27 | 7.16 ± 0.20 | 7.27 ± 0.20 | 7.39 ± 0.20 | 0.253 (NS) |
| **Peppermint Oil + VCO** | 6.75 ± 0.23 | 7.19 ± 0.18 | 7.40 ± 0.24 | 7.43 ± 0.18 | 0.253 (NS) |
| **Virgin Coconut Oil (VCO)** | 6.82 ± 0.28 | 7.22 ± 0.21 | 7.32 ± 0.21 | 7.47 ± 0.21 | 0.253 (NS) |
| **Chlorhexidine** | 6.91 ± 0.25 | 7.21 ± 0.19 | 7.31 ± 0.19 | 7.51 ± 0.19 | 0.253 (NS) |

NS = Not significant

## Discussion:

The current *in vivo* experiment compared and contrasted the antibacterial, antiplaque and salivary pH-modulating properties of virgin coconut oil (VCO) with and without essential oils (peppermint and rosemary) to chlorhexidine in children aged 1013 years. The results showed that all the interventions resulted in a statistically significant decrease in the colony-forming units (CFU) of Streptococcus mutans and plaque scores at the end of time, as well as a significant rise in salivary pH. Nevertheless, intergroup comparisons indicated different levels of efficacy, with chlorhexidine having better results and essential oil-enriched VCO formulations having promising results. At baseline, there were no statistically significant differences between the groups ($p > 0.05$), which proved the homogeneity and the correct randomization. This aligns with other clinical trials of oil pulling and antimicrobial mouth rinses, in which baseline equivalence is used to guarantee the validity of comparative results **[2]**. The CFU reduction was used to determine the antibacterial efficacy of the different agents and chlorhexidine was found to have the highest reduction (~74%), which is consistent with its well-established substantivity and wide-spectrum antimicrobial effects **[3]**. Similar results were also noted by Bandi and Pachava who noted that the number of S. mutans decreased by 70-75 percent after the application of chlorhexidine in children **[4]**. Although it is effective, the established side effects of long-term use of chlorhexidine require the investigation of other agents **[5]**. VCO with rosemary oil showed the highest antibacterial activity in the test groups, then peppermint oil and plain VCO. This increased activity of rosemary oil can be explained by its bioactive components including cineole and rosmarinic acid that possess strong antimicrobial and antioxidant effects **[6]**. A report by Thosar *et al.,* found that rosemary essential oil was a potent inhibitor of S. mutans growth with a decrease of about 55-60, which corroborates the current results **[7]**. Peppermint oil was also found to have significant antibacterial activity, albeit a little less than rosemary oil. This could be attributed to the fact that it contains menthol and menthone that interfere with bacterial cell membranes **[8]**. Ramya *et al.* reported a 50–55% reduction in oral bacterial load with peppermint-based mouth rinses, corroborating the results of this study **[9]**. The relative ineffectiveness of plain VCO indicates that although lauric acid plays a role in antimicrobial activity, essential oils add a synergistic effect. The CFU results were reflected in the antiplaque efficacy results. Chlorhexidine showed the highest decrease in plaque index, which is in line with the literature **[10]**. Nevertheless, rosemary and peppermint oil groups showed significant plaque reduction, which was close to chlorhexidine. It is interesting to note that peppermint oil was slightly more effective in reducing plaque than rosemary oil on day 7, which could be explained by its refreshing taste and higher compliance of the patients, particularly in children. The plaque reduction observed is also in line with the results of Malcangi *et al.,* who indicated that coconut oil pulling significantly reduced plaque scores (about 4050) **[11]**. Also, in a randomized trial, Seyed Alinaghi *et al.* and Latorre *et al.* established that essential oil mouthwashes were as effective as chlorhexidine in reducing the plaque in the short term **[12, 13]**. Salivary pH analysis showed that there was a gradual rise in all groups, which showed a change towards a more alkaline oral environment. This has clinical importance, because an increased pH suppresses the growth of acidogenic bacteria and decreases the likelihood of enamel demineralization **[11]**. The absence of any meaningful intergroup differences ($p > 0.05$) indicates that all interventions were equally effective in regulating oral pH. A study by Nagilla *et al.* supports these findings by showing that coconut oil pulling was found to significantly raise the salivary pH and decrease the amount of plaque **[14]**. In the same manner, Richards found that salivary buffering capacity and pH were enhanced with the use of essential oil-based rinses **[15]**. Ali *et al.* suggested that extra virgin coconut oil combined with peppermint essential oil is an effective natural adjunct for caries prevention, significantly reducing salivary *Streptococcus mutans* levels, plaque accumulation and caries risk compared with conventional oral hygiene measures **[16]**. According to Shankarguru *et al.,* VCO mouthwash effectively reduced plaque and *Streptococcus mutans*

levels in children, although low-fluoride mouthwash demonstrated a greater antimicrobial effect when used with fluoride toothpaste **[17]**. The statistically significant and progressive changes in all parameters within the intragroup underline the cumulative impact of repeated use of these agents. The findings indicate that oil pulling, especially with the use of essential oils, may be a useful addition to the traditional oral hygiene routines. The main strength of this study is that it deals with a paediatric population and compliance and safety are paramount factors in this case. Natural agents like VCO and essential oils have benefits in terms of biocompatibility, low side effects and acceptability. This is especially so considering the growing popularity of herbal and natural oral care products. Nevertheless, some constraints should be taken into consideration. The research period was not very long (7 days) and the effects in the long run were not evaluated. Also, other aspects like diet and oral hygiene may affect the results. It is suggested that future research with bigger sample sizes and extended follow-up durations be conducted to confirm these results and investigate the long-term advantages and safety of essential oil-enriched VCO preparations. Overall, the results of this study are in agreement with existing literature, reinforcing the potential of natural alternatives in oral healthcare. Although chlorhexidine is the gold standard, the similar efficacy of rosemary and peppermint oil-enriched VCO shows their potential as safer and more acceptable alternatives, especially in paediatrics.

**Conclusion:**

We show that interventions exhibited strong antibacterial and antiplaque effects and salivary pH improvement. Chlorhexidine was the most effective, but virgin coconut oil with rosemary and peppermint essential oils had similar and clinically significant outcomes. Such natural preparations can be effective, safe and acceptable substitutes to traditional mouthwashes in children.

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