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ANTIBACTERIAL EFFECT OF VIRGIN COCONUT OIL VERSUS CHLORHEXIDINE ON CARIOGENIC ORAL BACTERIA (IN-VITRO STUDY)

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ABSTRACT

Aim: This study aimed to assess and compare the antibacterial efficacy of Virgin Coconut Oil and chlorhexidine against the cariogenic oral bacteria Streptococcus mutans and Lactobacillus species, in a controlled in-vitro environment.

Materials and methods: This study evaluated and compared the antimicrobial efficacy of chlorhexidine digluconate and Virgin Coconut Oil against the cariogenic oral bacteria Streptococcus mutans and Lactobacillus species. The relative antibacterial activity of these agents was assessed through inoculation of the test bacteria onto their respective culture media, followed by agar diffusion testing. The diameters of the inhibition zones were measured in millimiters, and the mean values were statistically analyzed.

Results: The results showed that the mean diameter of the inhibition zone for chlorhexidine was 23.9 mm, while for Virgin Coconut Oil it was 18.2 mm against Streptococcus mutans. Against Lactobacillus species, the inhibition zone diameter was 16 mm for chlorhexidine and 18.7 mm for Virgin Coconut Oil. The difference in inhibition zone diameters between chlorhexidine and Virgin Coconut Oil was statistically significant ($p < 0.05$) for both Streptococcus mutans and Lactobacillus species.

Conclusion: Within the limitations of this in vitro study, Virgin Coconut Oil showed significant antibacterial activity against the cariogenic oral pathogens Streptococcus mutans and Lactobacillus species so VCO could be used as a natural antibacterial alternative to synthetic chlorhexidine against oral bacteria.

KEYWORDS: Virgin Coconut Oil, Chlorhexidine, Streptococcus mutans, Lactobacillus, Agar diffusion test

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INTRODUCTION

Oral health is critically influenced by the balance of bacterial flora within the mouth. Imbalance in this microbiota can lead to a range of oral disorders, encompassing dental caries, gingivitis, and periodontitis [1].

Streptococcus mutans and Lactobacillus species are the primary bacteria implicated in the development of dental caries (tooth decay). Streptococcus mutans is particularly notorious for its ability to produce acids from fermentable carbohydrates, leading to the demineralization of tooth enamel. Lactobacillus contributes to caries progression by maintaining an acidic environment conducive to enamel degradation. Both bacteria thrive in the biofilm (dental plaque) on the tooth surface [2].

Chlorhexidine is widely used antiseptic in dentistry, as it is highly effective against a broad spectrum of bacteria, including S. mutans and Lactobacillus. It works by disrupting the bacterial cell membrane, leading to cell death. Chlorhexidine is considered a gold standard in preventing dental caries and managing oral biofilm due to its potent antibacterial properties and ability to reduce plaque formation [3]. However, it has side effects, including tooth staining and alteration of taste [4]

With rising concerns about antibiotic resistance, natural alternatives like virgin coconut oil (VCO) are gaining attention due to their potential antibacterial properties. VCO, rich in lauric acid, has shown antimicrobial activity against several pathogens, but its specific effects on oral bacteria need more comprehensive study [5]. There is a growing public and scientific interest in natural products for health care, driven by concerns about the side effects of synthetic chemicals, antibiotic resistance, and the desire for more holistic health approaches. Virgin Coconut Oil (VCO), known for its antimicrobial properties, has gained attention as a potential natural alternative to conventional oral antiseptics like chlorhexidine [6]. While chlorhexidine is effective, it is not without drawbacks. Prolonged use can lead to adverse effects such as tooth staining, taste disturbances, and, in some cases, mucosal irritation. These side effects can reduce patient compliance and limit the long-term use of chlorhexidine. Exploring VCO, which is generally regarded as safe and free from these side effects, could provide a more patient-friendly alternative [7]. This study is conducted in a controlled laboratory environment, focusing on bacterial cultures. While in-vitro studies provide important initial data, the conditions do not fully replicate the complex environment of the human oral cavity, where factors such as saliva, food particles, and other oral microbiota interact. Therefore, the results may not directly translate to the clinical effectiveness of VCO in human subjects without further in-vivo studies or clinical trials. The aim of this study is to evaluate and compare the antibacterial effects of Virgin Coconut Oil (VCO) and chlorhexidine on cariogenic oral bacteria, specifically Streptococcus mutans and Lactobacillus species, in an in-vitro setting.

MATERIAL AND METHODS

Sample size calculation

A statistical power analysis was conducted to determine the appropriate sample size for a two-sided hypothesis test examining differences in bacterial growth inhibition between the test groups. Using an alpha level of, a beta level of, and an effect size of 1.16 based on a previous study, the total required sample size was calculated to be samples. To account for potential variability and procedural errors during testing, the sample size was increased to samples. The sample size was increased to account for possible variability and procedural errors during testing to be (64) samples (i.e., (32 for each antimicrobial) - (16 samples per

group). Sample size calculation was performed using R statistical analysis software version 4.4.1 for Windows [8].

Bacterial strains preparation and tested antimicrobials

In this in-vitro study, the antimicrobial properties of Virgin Coconut Oil and Chlorhexidine were assessed against Streptococcus mutans and Lactobacillus species. The bacterial strains of Streptococcus mutans and Lactobacillus were obtained from a microbiological resources center (Cairo MERCIN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt). The antimicrobial agents evaluated in this study were Chlorhexidine digluconate and virgin coconut oil.

The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Dentistry, Cairo university by approval no (291124) and is organized and operated according to the declaration of helsinki for human subject research (2013).

Methods

Agar diffusion test

The antimicrobial efficacy of Virgin Coconut Oil (Infinity Clinica Pharma, cairo , Egypt) and Chlorhexidine (Serva Electrophoresis, GmbH-69115 Heidelberg, Germany) was evaluated using the agar diffusion test method. Bacterial inocula were swabbed onto the surface of Mueller-Hinton agar plates. Wells were created in the agar, and 50 µL of each test solution (virgin coconut oil, chlorhexidine digluconate) was added to the respective wells. The plates were incubated at 37°C for 24 hours, and the diameter of the zones of inhibition around each well was measured in millimeters.

Antibacterial solutions preparation: A 0.1% solution of chlorhexidine digluconate was prepared, and virgin coconut oil was tempered to be in a liquid state at room temperature

Antimicrobial test by inhibition zone determination

The antimicrobial efficacy of Virgin Coconut Oil and chlorhexidine was evaluated using the agar diffusion test method. Six sterile glass Petri dishes containing selective agar media were prepared. After solidification, six equidistant wells were created in the agar, and 100 µL of each test solution was added to the respective wells. The plates were then incubated at 37°C for 24 hours (Bacteriological Incubator, Stericox India Private Limited) to allow for the diffusion of the antimicrobials through the agar. Distilled water was used as a negative control.

Following incubation, the plates were observed for zones of inhibition around the wells containing the tested antimicrobials. Inhibition zones measured in millimeters using digital clipper.

Statistical analysis of data collected

The collected data were analyzed using a two-way ANOVA to determine the effect of the antimicrobials and bacterial strain, as well as their interaction, on the inhibition zone. Additionally, a one-way ANOVA was used to test for significant differences among the antimicrobials.

RESULTS

For the effect of the tested antibacterial on the *Streptococcus mutans* bacterial strains, results showed that the mean diameter and standard deviation of inhibition zone (mm) for chlorhexidine (CHX) was 23.9 (2.04) mm, and Virgin Coconut Oil (VCO) was 18.2 (1.3) mm, for MS while the result for chlorhexidine (CHX) was 16 (2.6) mm and VCO was 18.7 (1.8) mm for Lactobacillus.

One-way ANOVA showed that the tested antimicrobials had a statistically significant difference in their effects against Streptococcus *mutans* (Table1).

TABLE (1) The mean diameter and standard deviation of inhibition zone (mm) for chlorhexidine and VCO for both S.mutans and lactobacilli

	Chlorhexidine (CHX)	**Vergin coconut oil (VCO)**	***P*-value**
Streptococcus mutans(MS)	**23.9 (2.04)**	**18.2 (1.3)**	**<0.001**
Lactobacillus	**16(2.6)**	**18.7(1.8)**	**<0.001**

DISCUSSION

This study aimed to assess and compare the antimicrobial properties of Virgin Coconut Oil and chlorhexidine digluconate against the predominant cariogenic oral bacteria, Streptococcus mutans and Lactobacillus species.

The results of this study showed that both Virgin Coconut Oil and chlorhexidine exhibited antibacterial activity against Streptococcus mutans and Lactobacillus spp.

Chlorhexidine exhibited greater antimicrobial efficacy against Streptococcus mutans compared to Virgin Coconut Oil, consistent with previous studies (Janakiram et al., 2020) (Hendry et al., 2009). Chlorhexidine is a widely used and highly effective antimicrobial agent widely used in dentistry due to its broad-spectrum activity and substantivity in the oral cavity. (Janakiram et al., 2020) However, Virgin Coconut Oil showed comparable antibacterial effects against Lactobacillus species. (Hendry et al., 2009)

Virgin Coconut Oil has been suggested as a natural, safe, and cost-effective alternative to synthetic antimicrobials for oral hygiene (Janakiram et al., 2020). Several studies have reported the antimicrobial properties of Virgin Coconut Oil against various oral pathogens, including Streptococcus mutans and Lactobacillus spp. (Nunes et al., 2021) (Zhao et al., 2017) (Janakiram et al., 2020)(Al-Maweri et al., 2019).

The antibacterial properties of Virgin Coconut Oil are attributed to its high content of medium-chain fatty acids, particularly lauric acid, which has been shown to disrupt bacterial cell membranes and inhibit bacterial growth. (Kemung et al., 2020)

These findings suggest that Virgin Coconut Oil could be a potential alternative to chlorhexidine for certain applications, such as the prevention and management of caries and gingivitis, especially in situations where chlorhexidine may not be suitable or preferred, such as during pregnancy or for long-term use.

Though the results of this in-vitro study are promising, further clinical studies are warranted to fully evaluate the efficacy and safety of Virgin Coconut Oil as an antimicrobial agent for oral health applications.

CONCLUSION

In conclusion, in this in-vitro study Virgin Coconut Oil showed significant antibacterial activity against the cariogenic oral pathogens Streptococcus mutans and Lactobacillus species so VCO could be used as a natural antibacterial alternative to synthetic chlorhexidine against oral bacteria.

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