



Evaluation of the Antimicrobial Effect of Olive Oil Extract on Oral Microflora

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ABSTRACT

Purpose: The aim of this study was to evaluate the antimicrobial effect of Extra virgin olive oil extract as mouthwash in the oral cavity of children against streptococcus mutans and Compare the efficiency between olive oil and the “gold standard” chlorhexidine. **Materials and Methods:** A total of 60 Egyptian children from both sex were included in this study. Children age ranging from 6 to 12 years old in a good physical condition. Children randomly were distributed into two Groups A and B, each includes of 30 children in each group. **Group A(study group)** : olive oil(Extra virgin): consisted of thirty children, each participant was instructed to rinse with 2ml of olive oil extract twice a day each rinse for about 1 minute for 3 weeks. **Group B (Control group):** chlorhexidine mouthwash (0.2%): consisted of thirty children, each participant was instructed to rinse with 2ml of chlorhexidine mouthwash (0.2%) twice a day each rinse for about 1 minute for 3 weeks. **Results:** There was a significant reduction in mean *Streptococcus mutans* count in the 2 groups after the study. **Conclusion:**olive oils can be used as valuable preventive agents in maintaining and improving oral health in low socioeconomic status population.

INTRODUCTION

Dental caries is a multifactorial dental disease that occurs in the oral cavity and affects populations across developed and developing nations. Studies show that dental caries is still a major health problem in most industrialized countries affecting 60–90% of school-children and the vast majority of adults. The two main etiological factors, management and frequency of free sugar intake, and regular removal of dental biofilm, should be taught at a young age and be applied throughout life into old age. This is because the overall salivary

KEYWORDS

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bacterial load in saliva decreased with increasing salivary glucose concentration. Recent evidence, coming from systematic reviews, supports the efficacy of mechanical and chemical plaque control in the reduction of gingivitis and caries especially if fluoridated mouthwashes are used^(1,2).

On exposure to saliva, a proteinaceous surface coating called pellicle is formed almost immediately on all solid substrates. This conditioning layer changes the properties of the substrate. The nature of the chemical groups exposed at the surface mainly defines the adhesion forces. Most studies focus on the adhesion of oral bacteria to salivary agglutinin that is adsorbed to the oral pellicle on tooth surfaces⁽³⁾.

In the literature, fluoridated and non-fluoridated chlorhexidine forms are the gold standard, in spite of side effects, in fighting dental plaque formation and caries progression^(3,4). Recently, effort has been ushered to replace chlorhexidine by natural essential oils in order to show the side effects of chlorhexidine. Olive oil has demonstrated excellent antibacterial and antifungal action, extra virgin olive oil is an integral ingredient of this traditional diet and for centuries Mediterranean people have appreciated his nutritional, medical, and cosmetics benefits. Indeed, virgin olive oil has been used as a folk remedy for combating diseases due to its pharmacological properties including cardioprotective, hypotensive, antihyperglycemic, antimicrobial, and anti-inflammatory effects. Therefore, this study aims at evaluating the antimicrobial action of extra virgin olive oil extract as mouthwash in oral cavity of children and at comparing such an effect to chlorhexidine⁽⁴⁾.

MATERIALS AND METHODS

Materials used: Olive oil extract (Grocery & Gourmet Foods, company, Spain) as mouth wash placed in hermetically sealed bottles and kept at room temperature, Chlorhexidine mouthwash

(0.2%) (Oraxine ,medical cosmetic products Ltd, KSA) and Mitis Salivarius Bacitracin *MSB*: Base enriched with sucrose to selectively isolated oral streptococci and inhibit any other micro-organisms.

Case Selection: 60 children were selected from the Outpatient clinic of Pediatric Dentistry Department, Faculty of Oral and Dental Medicine, Al-Azhar University (Girls'branch) . **Inclusion criteria:** Children age ranging from 6 to 12 years old in a good physical condition with no history of recent antibiotic administration (last 2 weeks) or anti-microbial mouth rinse (last 12 hours), Children with no orthodontic appliance and Children with low caries index (DMF&def: ≤ 4), DMF for permanent dentition, def for mixed dentition.

Exclusion criteria: Children using antibiotics, medications or mouthwashes, at the time of the study, Children with oral or systemic diseases and Children undergoing any dental treatment.

Methods: Children randomly were distributed into two Groups A and B, each of 30 children **Group A (study group):** consisted of thirty children, each participant was given a new bottle of specific effective amount of olive oil extract(extra virgin) (90ml) to be used as a mouthwash. Children were instructed to: Rinse with 2ml of olive oil extract by using the cap of olive oil bottle which was equivalent to the required amount (2ml), Stop eating or drinking for at least an hour after rinsing, Use toothbrush without any dental tooth paste after rinsing, Rinse twice a day each rinse for about 1 minute and Participants were advised to use the formations for three weeks. **Group B (Control group):** consisted of thirty children, each participant was given a new bottle of (90ml) chlorhexidine mouthwash (0.2%). Children were instructed to :Rinse with 2ml of olive oil extract by using the cap of olive oil bottle which was equivalent to the required amount (2ml), Stop eating or drinking for at least an hour after rinsing, Use toothbrush without any dental tooth paste after

rinsing, Rinse twice a day each rinse for about 1 minute and Participants were advised to use the formations for three weeks^(5,6).

Collection of saliva sample

Non stimulated saliva samples were collected from each child by asking him to spit in a labeled sterile container (3ml on the average). Each subject was refrained from tooth brushing in the morning and from eating or drinking at least 1hours before sampling time.

Baseline sample (S1): The initial sample was taken before using the mouth wash by asking the child to spit in a labeled sterile container.

Second sample (S2): The second sample was taken after using specific mouth wash for 3 weeks by asking the child to spit in a labeled sterile container.

Procedures of sampling occurred in complete aseptic conditions because any contamination could affect the bacteriological counts. Two saliva samples (**S1 and S2**) are taken for each individual as shown in the following table:

	Group 1	Group 11
Description	Initial sample	After 3 weeks
Sample	S1	S2

Preparation of the media

The selective medium Mitis salivarius bacitracin (MSB) was prepared as following⁽⁷⁾:

- 90 grams of dehydrated mitis salivarius agar were solved in one liter of distilled water.
- The medium was heated to dissolve the components and then autoclaved at 121⁰ C for 15 minute.
- The medium was left to cool to 55⁰ C then 1 ml of 1 % sterilized potassium tellurite and 1 ml of 200 units 1 ml sterilized bacitracin was

added. Sterilization of tellurite and bacitracin was performed by filtration.

- Each plate was poured with approximately 20ml of the medium and allowed to dry 24 hours at room temperature under 5 – 10% CO₂ tension (incubator).
- Plates were stored in the refrigerator at 4°C until use.

Microbial Analysis

Non stimulated saliva was collected in the sterile saliva containers, was taken to the microbiological lab immediately after collection. One milliliter of the saliva sample was transferred to calibrated sterile centrifuging tubes containing four milliliters of Brain Heart Infusion broth (BHI) by means of a sterile disposable syringe. The salivary sample was vortexed to uniformly mix the saliva and BHI broth using a cyclomixer . Using an inoculation loop (four millimeter inner diameter), ten micro liter of the vortexed 1:5 dilution sample was streaked on Mitis Salivarius Bacitracin Agar (MSB) selective for Streptococcus mutans. The MSB agar plates were incubated for 48 hours at 37°C in 5% of CO₂ in Nitrogen. Following incubation, counts were made of colonies with magnifying glass based on the morphological characteristics for Streptococcus mutans on the MSB agar. Two to three colonies of MSB culture medium were selected for the evaluation of cell morphology by Gram staining and Streptococcus mutans colonies were examined systematically and the number of the colony forming units (CFU / ML) of the original saliva were calculated.

Statistical Analysis

Statistical analysis was then performed using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA) to compare the mean Colony forming unit (CFU) of Streptococcus

mutans recorded pre and post operatively. As data was parametric, paired t-test for this comparison.

The percentage of change (decrease) post-operatively was calculated by the following formula:

$$\text{Value after} - \text{value before} \times 100 / \text{Value before}$$

Unpaired t-test was used to compare the percent change (decrease) in both study groups (olive oil and chlorhexidine) while ANOVA (ANalysis Of Variance) with post-hoc Tukey HSD (Honest Significant Differences) tests were calculated to compare between the expression of the different groups.

The level of significance was set at $P < 0.05$.

RESULTS

The results of this study revealed that the use of olive oil mouth-rinse in children as antimicrobial agent achieved statistically significant decrease in mean Log₁₀ CFU of *Streptococcus mutans* after 3 weeks and The effect of 0.2% Chlorhexidine was higher than the effect of olive oil in bacterial count reduction.

The antimicrobial activity of olive oil mouth wash was effective against Gram positive than Gram negative bacteria but non-significant chlorhexidine were effective against Gram negative than Gram positive bacteria but non-significant.

Table(1): Colony forming unit of *Streptococcus mutans* X 10⁴ in olive oil and Chlorhexidine groups pre and post- operatively, and significance of the difference between groups.

	Pre-operative		Post-operative	
	Olive oil group	Chlorhexidine group	Olive oil group	Chlorhexidine group
Mean	3762.67	4206.11	2.61	0.04
SD	1227.33	1240.47	0.79	0.01
Min	1200.00	1500.00	2.20	0.02
Max	7600.00	9100.00	6.30	0.07
t value	1.2071		13.74	
p-value	0.2336 ^{ns}		<0.0001*	

Significance level $p < 0.05$, *significant, ns=non-significant

Table(2): Percent change in Colony forming unit (CFU) of *Streptococcus mutans* in olive oil and Chlorhexidine groups post- operatively, and significance of the difference between groups (unpaired t-test).

Percent change after treatment	OLIVE OIL GROUP	Chlorhexidine GROUP
Mean	-99.904	-99.997
SD	0.071319	0.007
Min	-99.9939	-100.000
Max	-99.6417	-99.968
t value	7.24	
p-value	<0.0001*	

Significance level $p < 0.05$, *significant

DISCUSSION

Dental caries continues to plague most of the world's population. Thus, more effective public health and preventive measures are needed to address this worldwide problem⁽⁸⁾. Recently, Extra-virgin olive oil has demonstrated excellent biological properties in several medical treatments, on the other hand it eradicates *staphylococcus aureus*, *helicobacter pylori*, *candida albicans*, *lactobacillus* and *streptococcus pyogenes*⁽⁹⁾. Higher consumption of extra-virgin olive oil is associated with a lower risk of osteoporosis-related fractures in middle-aged and elderly Mediterranean population at high cardiovascular risk⁽¹⁰⁾.

EVOO manifests antidiabetic properties through inhibition of carbohydrate-hydrolysing enzymes⁽¹¹⁾. The main derivative compound of EVOO is, Oleuropein, a secoiridoid derived from olive tree, inhibits the proliferation of human colorectal cancer cell through downregulation of HIF-1 α ⁽¹²⁾. It also poses promising results in treating breast cancer⁽¹³⁾ and other cancers⁽¹⁴⁾. For the anti-inflammatory effect, extra-virgin olive oil, via Oleuropein and hydroxytyrosol, prevents macrophage activation⁽¹⁵⁾ as well as mast cell degranulation induced by immune and non-immune pathways⁽¹⁶⁾. Therefore EVOO was chosen in this study.

This study aimed to evaluate the antimicrobial action of EVOO extract as mouthwash in oral cavity of children in relation to the gold standard chlorhexidine. All collected saliva samples from each group were immediately submitted to the Culture and Sensitivity Unit at Regional Center for Mycology and Biotechnology at Al-azhar University. After dilution, the sample was inoculated in plate count agar media (Also known as: *Trypticase Glucose Yeast Agar*; *Standard Methods Agar*). Each sample was cultured in triplicate. The plates were incubated at 37°C for 24-48 hours. Then, colony forming units of saliva sample were determined in the 2 groups (olive oil and chlorhexidine). This was

in accordance with different studies⁽¹⁷⁻²⁰⁾. The study of the levels of *S.mutans*, in saliva is one of the most common methods for identifying subjects at risk of dental caries⁽²¹⁾. In this study non stimulated saliva samples were preferred because it is easier and it reflects accurately the caries experience and risk in every individual, this was in accordance with previous study⁽²²⁾.

On contrast paraffin wax stimulated saliva was used by another study, where all participants were asked to chew the paraffin wax and expectorated the stimulated saliva into sterile container⁽¹⁷⁾.

Mitis Salivarius Bacitracin media, a selective medium for *Streptococcus mutans*, was assigned for detection and counting of colonies of *S.mutans* because it is selective for such colonies. The addition of bacitracin to the media allows *Streptococcus mutans* to grow and form colonies and inhibit the growth of most other oral bacteria⁽²³⁾. In this study, we used *Mitis Salivarius Bacitracin* for easily comparing our results to those in literature (which used to reference MSB as the mainstay method for detection of *Streptococcus mutans* isolation)^(24,25).

In the present study the children's age (6-12) were decided as they can easily rinse their mouth without swallowing the mouthwash to avoid swallowing reflex. This agrees with another study where mouth rinses should be recommended only for children who have demonstrated mastery of their swallowing reflex⁽¹⁹⁾. In the current study, the mean of decayed and filled teeth (DMF) and (def) in these children were ≤ 4 , this indicates that the selected children are of low caries index, this agrees with previous study⁽²⁶⁾.

In the present study, Saliva was collected in the morning at least 1 hour before meal due to possible fluctuations in saliva microbial counts, which usually occur throughout the day children were asked to rinse their mouth with 2ml of specific mouth wash for about 1 minute for 3 weeks under their parents' supervision. Saliva samples were collected from each child by asking him to spit

in a labeled sterile container (3 ml in average) to get similar volume of all samples. Each subject was refrained from tooth brushing in the morning and from eating or drinking at least 1 hour before sampling time. The initial sample was taken before using the mouth wash and the second sample was taken after using specific mouth wash for 3 weeks. This goes in accordance with another study⁽²⁰⁾.

In our study children were distributed into two groups (**A and B**). **In group A (EVOO group)**, the mean number of CFU decreased from $3762.67 \pm 1227.33 \times 10^4$ preoperatively to $2.61 \pm 0.79 \times 10^4$ postoperatively. Thus, EVOO, if used as a mouth wash, has an excellent antimicrobial action on eradicating *Streptococcus mutans* in vivo. Similarly, our study also in agreement with previous study⁽²⁷⁾ which has reported that the growth inhibitory effect of some vegetable oils on *Streptococcus mutans* and *Lactobacillus casei*. On contrast, other study⁽²⁸⁾ had identified that no strong antimicrobial activities of virgin olive oils on different *Streptococci mutans* in a Turkish sample. **In group B (Chlorhexidine group)**, The mean number of CFU decreased from $4206.11 \pm 1240.47 \times 10^4$ preoperatively to $0.04 \pm 0.01 \times 10^4$ postoperatively. Our systematic reviews and meta-analyses confirm the CHX antibacterial effect on *Streptococcus mutans*, these results are consistent with those reported by other studies^(29,30).

Interestingly, EVOO is nearly as efficient as the “gold standard” chlorhexidine in eradicating colonies of *Streptococcus mutans*. However, six children have (20%) clinically demonstrated yellowish dental staining in group B while patients in group A showed no clinical signs of buccal pigmentations or dental discoloration. Also, three children (10%) have reported altered taste sensation after rinsing with chlorhexidine. However, no similar findings were reported in group A. This goes hand in hand with the previous studies which reported that chlorhexidine alters taste sensation and produces brown dental staining^(31,32,33). Previous study has concluded that chlorhexidine gel can be successfully

formulated and will inhibit plaque growth to some degree⁽³¹⁾, but not to the same extent, as a CHX mouthwash. When CHX DF/gel is used in a non-brushing model, it is significantly less effective in plaque inhibition compared to CHX mouthwash. Based on a study where CHX gel was applied with a finger after brushing, it is significantly more effective on plaque scores and the gingival index. The only brushing study also with a long follow-up showed that there is no significant difference between CHX DF and CHX mouthwash. However, as a corollary, significantly more tooth discoloration was observed with the CHX mouthwash. Although, CHX mouthwash is the first product of choice.

The antimicrobial effect of EVOO can be attributed to the antimicrobial action of the polyphenols with special reference to hydroxytyrosol, oleuropein and tyrosol. The reduction in the CFU of *Streptococcus mutans* over the study interval can predict anticariogenic effect of EVOO in pediatric populations. However, this cannot be generalized given the small-scale sample of the study and the single institutional experience we report. Studies on too large a scale, within multinational multi-centric facilities, may approve our findings⁽³⁴⁾.

Our study coincides with the previous study, which proved that the antimicrobial effect of extra virgin olive oil and honey on various oral microorganism and found that they had greater effect on oral bacteria.⁽²²⁾ This study concluded that olive oil extract possesses in vivo antimicrobial activity against *S. mutans* present in the oral cavity and might be used as an alternative measure to prevent dental caries and it is also supported by previous study where many different types of olive oils against several micro-organisms were examined where the antimicrobial activity was higher in extra virgin olive oils, followed by virgin olive oils and pomace olive oils, which is in accordance with their decreasing content in phenolic compounds⁽³⁵⁾. The results of this study are in agreement with another study where Extra virgin olive oil is

very effective on oral pathogenic microorganisms as EVOO had antimicrobial, immunomodulatory and antioxidative effect also mouth rinsing with olive oil is presumed to prevent oral malodor and plaque formation⁽³⁶⁾, but there was disagreement of another study where the antibacterial activity of olive leaf extract with large variety of bacteria his Results indicated that olive oil extract did not present broad-spectrum antibacterial activity, but had appreciable activity on *H. pylori* and *C. jejuni*⁽⁹⁾. Also the current study is in accordance with another study which confirmed a bactericidal effect with all types of olive oils against the cariogenic bacteria *Streptococcus mutans*, also approved that an olive oil extract decrease both bacterial growth and adhesion⁽²¹⁾.

CONCLUSIONS

Within the limitations of this study, it can be concluded that :

1. Olive oil is safe and natural. It is a non-toxic substance and not caused allergy upon ingestion due to its anti-allergic and antiinflammatory effects.
2. Olive oil is an antimicrobial agent as it significantly reduced the salivary levels of *S.mutans* of children when compared by a potent antiseptic like 0.2%chlorhexidine.
3. Olive oil is a subject of recent dentistry research due to its antimicrobial properties, it has been called a *natural antibiotic*.

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