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# Evaluation of the Antimicrobial Effect of Anise Extract on Cariogenic Oral Microflora

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## *ABSTRACT*

**PURPOSE:** To measure the antimicrobial effect of Anise extract as mouth rinse in children against streptococcus mutans and lactobacilli. **Material and method:** A total of 60 Egyptian children from both sexes ranging from 6 to 12 years old for mixed dentition in a good physical condition. Children were randomly distributed into three groups (A, B, C) (20 children each). Group A: Patients were instructed to rinse with 40ml of anise extract (anise 1.6g/ 40ml) three times/ day, each rinse for 1 minute for 1 week. Group B: Patients were instructed to rinse with 40ml of anise extract (anise 10g/ 40ml) three times/ day, each rinse for 1 minute for 1 week. Group C (control group): Patients were instructed to rinse with 40ml of distilled water three times/ day, each rinse for 1 minute for 1 week. **Results:** There was statistical significant reduction in streptococcus mutans and lactobacilli count in Group B (Anise 10g/ 40ml group). **Conclusion:** Anise extract can be used as a preventive therapy at home to maintain oral hygiene as it is natural, safe and has no side effects.

***KEYWORDS***

*Anise extract,*
*Distilled water,*
*Streptococcus mutans,*
*Lactobacilli, Mouth wash.*

## INTRODUCTION

Dental caries is a chronic multifactorial disease resulting in the wearing of tooth structure and may lead to tooth loss if not treated promptly. It has a lot of impact on humans and on the Community as a whole in the form of discomfort, pain, functional impairment, aesthetic concerns and financial burden for treatment[1, 2].

- Paper extracted from Master thesis titled "Evaluation of the antimicrobial effect of anise extract on cariogenic microflora"

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Dental plaque occurs when acidogenic increase due to high frequency of intake of carbohydrate. The metabolism of bacteria will cause acidification of the dental smear layer activate acid induced demineralization of enamel and dentin[3, 4].

Streptococcus mutans is the main pathogen cause dental caries. S.mutans can deplete multiple sugars to form acids that cause disintegration of minerals from enamel and eventually lead to dental caries. Streptococci have the central role in initiation of dental caries while Lactobacilli aid in caries progression and root caries lesions. Hence decontamination of bacterial base of caries is one of the ways to remove this widespread infection [5, 6].

Because of side effect with chemicals in mouth washes the need of natural herbal product increase around the world. There has been change in thinking with a growing demand to phytochemicals present in herbs could gain an effective alternative to chemicals in mouth wash. Researches have been focused on natural mouth washes due to their proud spectrum of antibacterial activities, safe and low cost [7].

Pimpinella anisum is an annual, herbaceous plant, its height up to 0.5-m high. Fine hairs cover most of the plant. It has an erect cylindrical stalk with deep penetrating, its root spindle in shape. The plant has two distinctive, bright green leaf patterns which alternate with yellowish-white flowers in typical umbrella shaped clusters. When the flowers turn into seed-like fruits, they appear to be oval-pear shaped, compressed at the side. They appear to be grayish green to dull yellowish-brown in color [8].

Recently anise recommended as a replacement of antibiotics. Anise essential oils contain anethole as active ingredient, eugenol, methylchavicol, anisaldehyde and estragole. Pimpinella anisum has antimicrobial activity by degradation of bacterial cell wall, alteration of permeability of cell membrane and coagulation of cytoplasm. Anethole and its isomers are the most commonly determined essential oil component they exhibited antibacterial activity against broad spectrum bacteria and fungi [9]. Therefore this study was conducted to evaluate the antibacterial action of anise extract as a mouth wash in oral cavity of children and at comparing such an effect to distilled water.

## MATERIAL AND METHODS

Prepared anise extract (isis for herbal product) was used as a mouth wash and Placebo mouth wash (Bravo Pharma group), Distilled water colored with food dye. Both mouth rinses were put into hermetically sealed plastic bottles, Mitis Salivarius Bacitracin *MSB* (Difico laboratories, Detroit, Mich-USA) Base enriched with sucrose to selectively isolated oral streptococci and inhibit any other microorganisms, Rogosa agar (Unipath, Basingstoke, UK) medium for lactobacilli culture.

### Case selection:

60 Egyptian children from both sexes were included in the study their life time ranged from (6-12) years old. They were chosen from orphanage, where research approval was obtained before research implementation from Ethical Research Committee of Faculty of Dental Medicine for Girls, Al-Azhar University. All children were examined clinically for dental caries.

### Inclusion criteria:

Children with no systemic disease, no history of recent antibiotic administration (last 2 weeks), antimicrobial mouth-rinse (last 12 hours), topical fluoride treatment within 4 weeks prior to baseline, children with no untreated active carious lesion, no orthodontic appliance or with low caries index (DMF& def<4) were selected for the present study.

### Exclusion criteria:

Children using antibiotics, medications or mouthwashes at the time of the study, children with oral or systemic disease, children undergoing any dental treatment.

Sixty children were randomly distributed into three groups (A, B& C) (20 children each). Group A: each participant was given a new bottle of specific effective amount of anise extract (1.6g /40ml) to be used as a mouthwash. Children were instructed to rinse with 40ml of anise extract. Group B: each participant was given a new bottle of specific effective amount of anise extract (10g /40ml) to be used as a mouthwash. Children were instructed to rinse with 40ml of anise extract. Group C (Control group): each participant was given a new bottle of (40ml) distilled water. Children were instructed to rinse with 40ml of distilled water. All groups instructed to rinse three times a day with specific mouth wash for each group about one minute and participants were advised to use the mouth wash for seven days.

### Collection of saliva samples

Prior to the start of the experiment, samples of unstimulated saliva were taken when the child spit suitable amount of unstimulated saliva in a sterile container in the morning before breakfast. Initial salivary samples were collected to establish base levels (S1) using selective culture media, the salivary samples (S2) were collected immediate after rinsing with mouthwash when the child spit suitable amount of unstimulated saliva in a sterile container. The salivary samples (S3) were collected after one week of using the mouthwash when the child spit suitable amount of unstimulated saliva in a sterile container in the morning before breakfast.

### Statistical analysis

Logarithmic transformation ($Log_{10}$ transformation) of Colony Forming Unit (CFU) counts was performed due to the high range of bacterial counts. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution except for percentage changes in bacterial counts which showed non-normal (non-parametric) distribution.

## RESULTS

When comparing three groups statistical analysis showed that anise 10g have statistical significant change in mean $Log_{10}$ CFU of S.mutans and Lactobacilli after 7 days. Distilled water and anise 1.6g show no statistical significant change in mean $Log_{10}$ CFU of S.mutans and Lactobacilli after 7 days. (Table 1, 2)& (Fig. 1, 2)

**Table (1)** Descriptive statistics and results of repeated measures ANOVA test for the changes by time in $Log_{10}$ CFU of S. mutans counts within each group.

| Time | Anise 1.6 g | | Anise 10 g | | Distilled water | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| **Pre-operative** | 6.6 | 1.24 | 6.96 | 0.26 | 7.59 | 0.91 |
| **Immediate post-operative** | 6.25 | 1.36 | 6 | 0.71 | 7.74 | 0.83 |
| **7 days** | 6.33 | 0.97 | 2.5 | 0.23 | 7.96 | 0.94 |
| ***P*-value** | 0.192 | | <0.001* | | 0.069 | |

*P-value <0.05: significant (*); non-significant >0.05 (ns)*

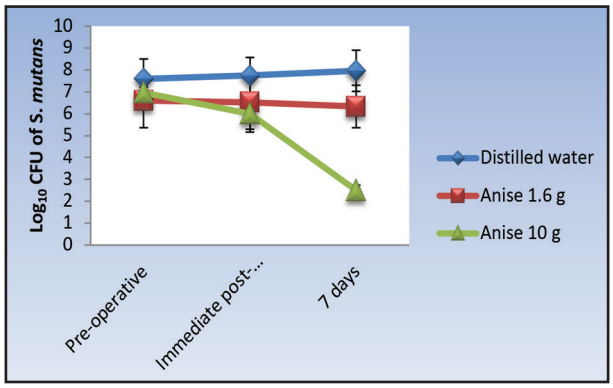


Figure (1) Line chart representing mean and standard deviation values for the changes in $Log_{10}$ CFU of S. *mutans* counts within each group.

**Table (2)**: *Descriptive statistics and results of repeated measures ANOVA test for the changes by time in $Log_{10}$ CFU of Lactobacilli counts within each group.*

| Time | Anise 1.6 g | | Anise 10 g | | Distilled water | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| **Pre-operative** | 7.53 | 0.89 | 7.03 | 0.27 | 8.1 | 1.21 |
| **Immediate post-operative** | 7.42 | 0.88 | 5.86 | 0.23 | 8.55 | 1.49 |
| **7 days** | 7.07 | 0.95 | 2.79 | 0.65 | 8.6 | 1.55 |
| ***P*-value** | 0.175 | | <0.001* | | 0.113 | |

*P-value <0.05: significant (*); non-significant >0.05 (ns)*

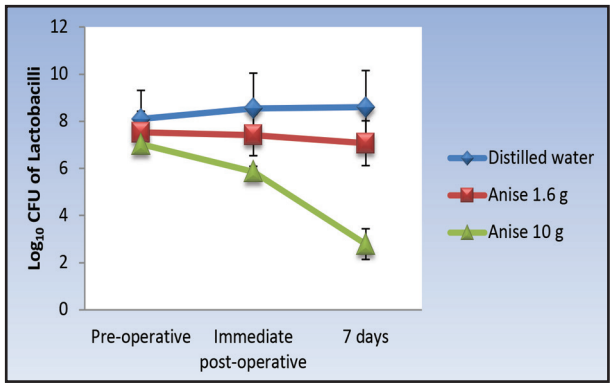


Figure (1) Line chart representing mean and standard deviation values for the changes in $Log_{10}$ CFU of Lactobacilli counts within each group.

## DISCUSSION

In the present study streptococcus mutans and lactobacilli were selected due to S. mutans have the main part in the initiation of dental caries because these can adhere to the enamel smear layer and to other plaque bacteria. Streptococcus mutans responsible for carbohydrate metabolism and produce acids and to make acidic environment creating the risk for cavities. Streptococcus mutans able to form glucan extracellular polysaccrides from glucose by action of glucosyltransferases. The major role of glucan is cell-cell adhesion and cell-surface adhesion [10].

Lactobacilli acidogenic bacteria contribute in dental caries. Their adherence to tooth surface very weak so they can not initiate dental caries. They colonize at low PH lesion and produce acids. They contribute caries progression and cause root caries[11].

In the present study anise was used for preparation of mouth wash because it contains anethole as active ingredient, eugenol, methylchavicol, anisaldehyde and estragole. These essential oils exhibited antibacterial activity against broad spectrum of gram negative and gram positive bacteria and fungi. Anise have antibacterial action by degradation of bacterial cell membrane, increase cytoplasmic permeability inactivation of extracellular enzyme, reduction of intracellular ATP, coagulation of cytoplasm and interruption of electron flow and active transport. So that this study aimed to evaluate the antimicrobial action of anise extract as mouthwash on the level of salivary mutans streptococci and lactobacillus in oral cavity of children [12, 13].

In the present study distilled water was used for preparation of mouth wash as alcohol used in oral care product can be irritating to cheek, teeth and gum. Excessive use of mouth wash that contains alcohol lead to weaken immune system and natural ability to fight bacteria and illness. Also this method is safe, economic, easy in preparation and can use as safe at home [14, 15].

The result of the present study in agreement with previous study that investigate the antibacterial effect of Pimpinella anisum (10g/40ml distilled water) and some antibiotics (vancomycin, bacitracin, ciprofloxacin) by disc microdilution method and minimum inhibitory concentration. Khodor et al showed that Pimpinella anisum have high antibacterial effect against streptococcus mutans and lactobacilli. They confirmed that the potential use of Pimpinella anisum as broad spectrum antibiotic agent and the medicinal importance of this plant through antibacterial activity antibacterial activity that surely enhance their application among other uses as substitute for antibiotics [16].

The result of the present study in agreement with previous study that compare antibacterial effect of saliva officinalis, pimpinella anisum and satureja hortensis against streptococcus mutans, lactobacilli and actinomyces. Kermanshah et al showed that all extract inhibited the growth of all bacteria. They confirmed that Pimpinella anisum useful plant at traditional medicine, the main agent of the plant trans-anethole (94%). Pimpinella anisum have good inhibitory effect against S.mutans, lactobacilli and actinomyces [17].

On the other hand, the present study was in disagreement with another study investigate the antibacterial properties of aqueous and methanolic extract of anise against gram positive lactobacilli and staphylococci. The methanolic extract show variable antibacterial activity against all test bacteria but aqueous extract show resistance against all tested bacteria. This difference in result may be due to present study boiling anise (10g/40ml) for three minutes at 100° while previous study boiling anise (10g/20ml) for 2 hours at 70° C [18].

On the other hand, the present study was in disagreement with previous study in which antibacterial activity of anise and cumin were investigated against gram positive bacteria (streptococci and staphylococci) using disk diffusion method. The study showed that anise oil produced weak antimicrobial activity effect against tested organisms. This different may be due to different extraction procedure occurred and concentration of anise used in the experiment [19].

## CONCLUSIONS

1. The daily use of a mouth rinse of anise could reduce the salivary levels of S.mutans and lactobacilli, which are the most virulent cariogenic pathogens in the oral cavity.
2. Anise is safe, economical, easy to prepare and can be used as a home care product.

## REFERENCES


1. Pitts N. Global oral health inequalities: dental caries task group- research agenda. Adv Dent Res. 2011; 23: 211-20.
2. Hooley M and Kilpatrick N. Body mass index and dental caries in children and adolescents. Syst Rev. 2012; 1:57.
3. Marsh PD. Are dental diseases examples of ecological catastrophes? Microb. 2003; 149: 279-94.
4. Filoche S, Wong L and Sissons CH. Oral biofilms: emerging concepts in microbial ecology. J Dent Res. 2010; 89: 8-18.
5. Long Chen. A novel antimicrobial peptide against dental-caries-associated bacteria. Anaerobe. 2017; 47: 165-72.
6. Sabzghabaee AM. Clinical evaluation of the essential oil of "Satureja Hortensis" for the treatment of denture stomatitis. Den Res J. 2012; 9: 198-202.
7. Awadalla HI. A pilot study of the role of mouth wash use in oral health. Int J Dent Hyg. 2011; 9: 110-6.
8. Ntalli NG. Synergistic and antagonistic interactions of terpenes against Meloidogyne incognita and the nematicidal activity of essential oils from seven plants indigenous to Greece. Pest Manag Sci. 2011; 67: 341-51.
9. Radaelli M, da Silva BP, Weidlich L, Hoehne L, Flach A, da Costa LA, et al. Antimiocrobial activities of six essential oils commonly used as condiment in Brazil against Clostridium perfringens. J Braz Miocrobiol. 2016; 47: 424-30.
10. Sakamoto M, Umeda M, Benno Y. Molecular analysis of human oral microbiota. J Periodont Res. 2005; 40: 277-85.
11. Tanzer, J.M.; Livingston, J.; Thompson, A.M. The microbiology of primary dental caries in humans. J Dent Educ. 2001; 65: 1028-37.
12. Arzuhan Sihoglu Tepe. Traditional use, biological activity potential and toxicity of Pimpinella Species. Ind Crop Prod. 2015; 69: 153-66.
13. Asokan S and Kumar RS. Effect of oil pulling on halitosis and microorganisms causing halitosis: a randomized controlled pilot trial. J Indian Soc Pedod Prev Dent. 2011; 29: 90-4.
14. Romeo J, Warnberg J. Moderate alcohol consumption and the immune system: a review. Br J Nutr. 2007; 98: 111-5.
15. Awadalla HI, Ragab MH, Bassuoni MW, Fayed MT, Abbas MO. A pilot study of the role of anise use in oral health. Int Dent Hyg. 2011; 9: 110-6.

16. Khodor MH, Jasim AS, Malik A, Zahra HA. Isolation and identification of some bacterial isolates from mercury and photo filling of teeth and study the antibacterial effect of pimpinella anisum. J Uni Thi-Qar. 2013; 3: 8-3.
17. Kermanshah H, Kamangar SS, Arami S, Kamalinegad M, Karimi M, Mirsalehian A, et al. The effect of hydro alcoholic extract of seven plants on cariogenic bacteria an in vitro evaluation. Oral Health Dent Manag. 2014; 13: 2-20.
18. Hasimi N, Tolan V, Kizil S, Kilinç E. Determination of essential oil composition, antimiocrobial and antioxidant properties of anise and cumin seed. J Agric sci. 2014; 3: 19-26.
19. Bajaj N, Tandon S. The effect of triphala and chlorhexidine mouthwash on dental plaque, gingival inflammation and miocrobial growth. Int J Ayurveda res. 2011; 2: 29-36.