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In Vitro Antimicrobial Effectiveness of Vinegar against Oral Microorganisms: Part I

Fahiem Mohammed Mohammed El-Shamy[1], Gurpreet Singh[2], Husham Elraih[3], Iti Gupta[2], Amr Sharawy[4]

Contributors:

[1]Lecturer, Department of Dental Biomaterials, Faculty of Dentistry, Mansoura University, Egypt; [2]Assistant Professor, Department of Endodontics, Faculty of Dentistry, Jazan University, KSA; [3]Lecturer, Department of Microbiology, Faculty of Dentistry, Jazan University, KSA; [4]Associate Professor, Department of Dental Biomaterials, Faculty of Dentistry, Cairo University, Egypt.

Correspondence:

Dr. El-Shamy FM. Department of dental biomaterials, Faculty of Dentistry, Mansoura University, Egypt, ZIP/Postal Code: 45142, KSA. Tel.: 00966561311593. Email: fahiemm@yahoo.com

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Abstract:

Background: To evaluate the antimicrobial effectiveness of vinegar against oral microorganisms *in vitro.*

Materials and Methods: Vinegar was tested for their antimicrobial activity against five oral microorganisms; *Streptococcus mutans, Lactobacillus salivarius, Staphylococcus aureus, Enterococcus faecalis,* and *Candida albicans* by standard agar-disk diffusion assay. Oradex mouth rinses containing 0.12% chlorhexidine gluconate was served as positive control. The testing materials under study were placed in the wells, prepared in the agar. The dishes were incubated for 24 h at 37°C. The growth inhibition zones were recorded and compared for every material and bacterial strain.

Results: Both vinegar and chlorhexidine were effective in inhibiting the growth of all tested microorganisms. Vinegar was statistically significant effective against *S. mutans, S. aureus, E. faecalis,* and *C. albicans* as compared to oradex mouthrinse ($P < 0.01$). Regarding *Lactobacillus,* chlorhexidine was statistically highly significant than vinegar ($P < 0.01$).

Conclusion: This scientific study reported the antibacterial activity of vinegar against four out of five microorganisms was found to be superior to the chlorhexidine, which is actually considered as one of the most effective antimicrobial agents.

***Key Words*:** Antimicrobial properties, chlorhexidine gluconate, mouth rinses, vinegar, zones of inhibitions

Introduction

Mouthwashes as an adjunct to oral hygiene measures were first propagated by ancient Egyptians and Romans. Within the 16th century, a mix of alum, vinegar myrrh, and wine was reported to be used for washing the mouth after meals. Vinegar is a liquid consisting of about 5-20% acetic acid (CH_3COOH), water and different trace chemicals, which can include flavorings. The acetic acid is produced by the fermentation of ethanol by microorganisms.[1] Vinegar was thought to be helpful for treating infections in the past. Hippocrates (46-377 BC) prescribed it for curing pleurisy, fever, ulcers, and constipation; it had been utilized by the ancient Egyptians to kill microorganisms. When combined with honey to form oxymel, it was a standard cough drug in the ancient world.[2]

The most commonly used preventive and therapeutic mouth rinses in children are sodium fluoride and chlorhexidine, respectively, for their anticariogenic effect as dental caries is the most prevalent chronic childhood disease. Chlorhexidine mouth rinse is considered as the "gold standard" because of its bacteriostatic and bactericidal properties at low and high concentrations, respectively;[3] however, it's not suggested for routine home care use owing to its staining and metallic taste.[4]

Mouth rinse with sodium fluoride remains the foremost widely suggested routine home care oral hygiene agent in children because of its anticariogenic properties. Yet, its extensive use in very young children is not advisable owing to the risk of ingestion and fluoride toxicity.[5]

In the 21st century, more awareness about the ill effects of chemical use has led to the search for natural preparations; substances, such as garlic (*Allium sativum*), lime (*Citrus aurantifolia*), green tea (*Camellia sinensis*), and alum (potassium aluminum sulfate), that had been used since ancient times for their therapeutic properties. Recent literature has brought light to the benefits of various natural substances as mouth rinses.[6]

Vinegar is currently used as a change of state ingredient. Traditionally, it was an easily obtainable delicate acid; it had an excellent sort of industrial, medical, and domestic uses; a number of that (such as its use as a general social unit cleanser) is still promoted nowadays.[7]

Researchers at the Food Biotechnology Department, Instituto de la Grasa (CSIC) in Seville, Spain conducted research on the antimicrobial activity of several food products including vinegar. The following microorganisms were used in the study: *Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis, Escherichia coli* 0157:H7, *Shigella sonnei,* and *Yersinia* sp. Vinegar (5% acetic acid) showed bactericidal activity against all strains tested,[8] which was attributed to its acidity. Various studies have compared the natural against the normal mouth rinses and reportable varied degrees of efficaciousness, particularly against *Streptococcus mutans*;[4,5,9,10] however, there are very few studies were done regarding the antimicrobial effect of natural mouth rinses.

This study was conducted to evaluate and compare the antimicrobial efficacy of vinegar to that of chlorhexidine against selected oral microorganisms.

Materials and Methods

Test microorganisms

In the current study, four bacteria and one fungus were used to assess the antimicrobial activity of vinegar: *S. mutans, Lactobacillus salivarius, S. aureus, Enterococcus faecalis,* and *Candida albicans* which were obtained from the Microbiology Laboratory, Faculty of Dentistry, Jazan University.

Determination of antimicrobial activity

Vinegar (Apple vinegar, Zer, Turkey) with 5% acidity and Oradex mouth rinse containing 0.12% chlorhexidine (Watsons Sdn Bhd., Malaysia) were tested for antimicrobial activity using agar-disk diffusion methods at full strength.[11] This method will determine the ability of the testing antimicrobial agent to inhibit the formation of new microbial colonies through the formation of inhibition zone. Each bacterium was suspended in 2 ml of peptone water, whereas *C. albicans* was suspended in 2 ml of sterile saline. The suspension turbidity was adjusted to a 0.5 McFarland standard using turbidimeter.

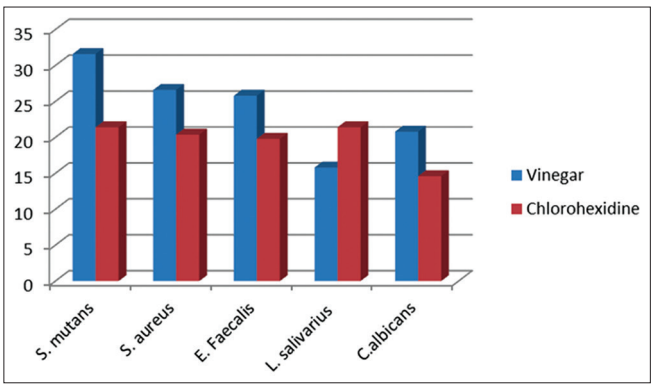
25 Petri dishes (five for each microorganism) formerly sterilized were prepared. A sterile cotton swab was dipped into the inoculum suspension, and a lawn was made on Mueller-Hinton agar (*S. aureus, L. salivarius,* and *S. sobrinus*) and Sabouraud dextrose agar (*C. albicans*) plates. For every dish, two wells were made with a metal tube with four millimeters of diameter to receive the testing materials. The materials were applied in the wells. The agar plates were incubated at 37°C for 24 h. After 24 h, the formed inhibition zones around the wells containing the materials were recorded. All examinations were made three times, and the outcomes were the mean estimation of the three records. Results were expressed as mean±standard deviation. Statistical analysis was performed using SPSS program (statistical package for social sciences).

Results

The zones of inhibition produced by the testing materials against tested microorganisms were shown in Figure 1. The mean values of the microbial inhibition zones are shown in Graph 1 and Table 1. The difference was found to be highly significant ($P < 0.01$).

The mean inhibition zones of vinegar and chlorhexidine were as follows (*S. mutans*; 31.60 ± 0.5 and 21.40 ± 0.9, *S. aureus*; 26.5 ± 0.5 and 20.4 ± 0.9 and for *E. faecalis;* 25.80 ± 0.4 and 19.80 ± 0.4) respectively. Vinegar showed highly statistically significant antibacterial efficacy against these bacteria ($P < 0.01$).

Regarding *L. salivarius* the mean inhibition zones of vinegar and chlorhexidine were 15.80 ± 0.4 and 21.40 ± 0.5, respectively.



Graph 1: Means of inhibition zone diameter (mm) of testing materials against selected bacteria.

A highly significant difference was found between both the materials ($P < 0.01$), with higher chlorhexidine antibacterial efficacy as compared to vinegar.

The antifungal effect of vinegar was found to be (20.80 ± 0.4) against the *C. albicans* which was higher than chlorhexidine (14.60 ± 0.5). The difference was highly significant between the testing materials ($P < 0.01$).

Discussion

Maintenance of good oral hygiene is the key to prevent the oral diseases. This study was primarily conducted to evaluate *in vitro* effect of vinegar in inhibition of *S. mutans, L. salivarius, S. aureus, E. faecalis,* and *C. albicans* and to evaluate its effectiveness as compared to mouthwash containing 0.12% chlorhexidine gluconate.

Dental plaque consists of various microbial communities that is embedded in a matrix of host and bacterial polymers and grows as a biofilm.[12,13] Matured dental plaque, which comprised a high microbial density community with a variety of different microorganisms[12] is considered as a major etiological factor in the formation, development and progression of periodontitis and gingivitis.[14] A number of studies have shown a high positive correlation between the development of gingivitis and the quantity of supragingival plaque and also between removal of bacterial plaque and the resolution of gingival inflammation.

In our study, we included four bacterial species; *S. mutans, E. faecalis, L. salivarius* and *S. aureus,* which are significant oral pathogens that are in agreement with other reported findings elsewhere.[12] In addition, Drake *et al.*[15] revealed that denture plaque containing *C. albicans* plays a main role in the pathogenesis of denture stomatitis. Consequently, it was also decided for assessment of the antifungal activity of the materials arranged in this study.

Chlorhexidine formulations are thought-about to be the standard antigingivitis and antiplaque mouth rinses due to their extended broad spectrum antimicrobial activity and

Table 1: Antimicrobial activity of vinegar and chlorhexidine against various microorganisms (mean of zones of inhibition±standard deviation) in mm.

Subjects	*S. mutans*	*S. aureus*	*L. salivarius*	*E. faecalis*	*C. albicans*
Vinegar	31.60±0.548	26.60±0.548	15.80±0.447	25.80±0.447	20.80±0.447
CHX	21.40±0.894	20.40±0.894	21.40±0.548	19.80±0.447	14.60±0.547
P	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01

S. mutans: Streptococcus mutans, S. aureus: Staphylococcus aureus, L. salivarius: Lactobacillus salivarius, E. faecalis: Enterococcus faecalis, C. albicans: Candida albicans. CHX: Chlorhexidine

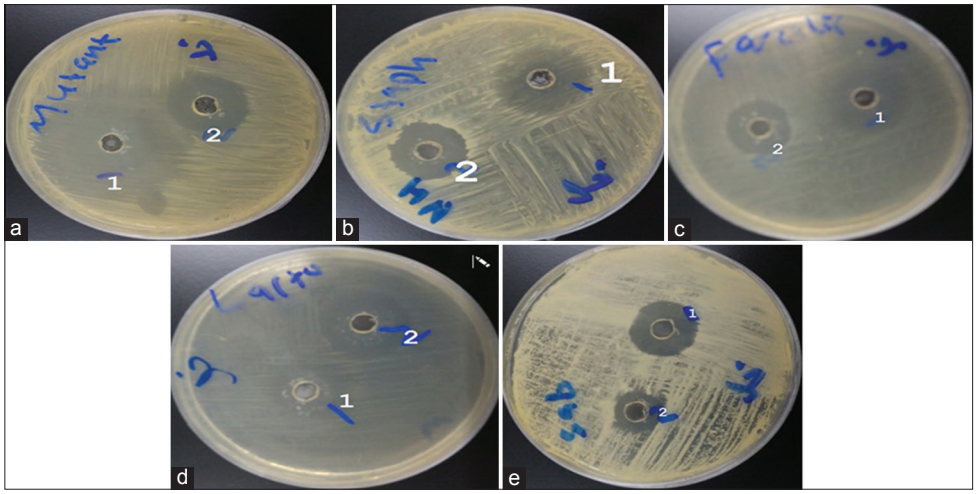


Figure 1: Zones of inhibition produced against five tested microorganisms in disk diffusion test. (a) *Streptococcus mutans*; (b) *Staphylococcus aureus*; (c) *Enterococcus faecalis,* (d) *Lactobacillus salivarius* and (e) *Candida albicans.* (1) Vinegar, (2) chlorhexidine.

plaque inhibitory potential.[15,16] Giuliana *et al.*[18] have mentioned that active agents in oral mouthwashes have special effects in reducing dental plaque. Chlorhexidine has fast bactericidal action on plaque bacteria and plaque fungi and one of the most effective active agents to reduce and inhibit plaque accumulation.[18,19] It is able to kill both Gram-positive and Gram-negative microbes. This may be due to the mechanism of action of chlorhexidine on bacteria, which includes the disruption of bacterial cell membrane.

Due to low toxicity and low cost, and pointing to a viable home-use application vinegar, was evaluated in this study. Vinegar has been suggested as an alternative disinfectant in other areas. It has been reported in the literature,[20] the use of an acetic acid-based solution for the disinfection, for the treatment of oral inflammation (as mouthwash) and as an antiseptic for sores. Few studies have reported the use of vinegar in dentistry. Silva *et al.*[21] (2008) reported a good antimicrobial effectiveness against *S. aureus* and *C. albicans* in the disinfection of acrylic resin. El-Shamy and Ammar[23] (2015) studied the effect of vinegar on transverse strength of heat-polymerized acrylic resin, and they concluded that vinegar did not demonstrate any significant change in flexural strength. Chibebe and Pallos[24] (2001) observed the decrease in the number of *Streptococcus pyogenes* inoculated in toothbrushes after disinfection with vinegar. Azuma *et al.*[25] (2006) examined different concentrations of several trademarks of vinegar and detected that low concentrations (3-6%) were effective *in vitro* against *C. albicans.*

In this study, the vinegar tested exhibited antimicrobial and antifungal effects against the five microorganisms tested, as showed by agar-disk diffusion methods. It was probably owing to the formulations of vinegar active constituents. According to Malicki *et al.*,[26] organic acids are considered weak acids and have an antimicrobial effect caused by its undissociated forms. They passively diffuse through the bacterial cell wall during neutral pH and dissociate into anions and protons. Discharge of the protons causes the internal pH to be reduced that applies repressive effects on the bacteria.[26] Various researchers have verified the antibacterial effect of organic acids on completely different types of infective microorganism.

Zasshi *et al.*[27] who revealed that the bacteriostatic and bactericidal activities of vinegar products against *E. coli*; the bactericidal activities of vinegars were not related to microorganism inoculums' sizes but was dependent of growth phase. Bacteria of logarithmic growth phase were a lot of sensitive than those of stationary phase. Medina *et al.*[8] found that the vinegar and liquid extracts of virgin olive oil have strong bactericidal activity against *S. enteritidis.*

Wood vinegar, in Thailand was not just used in farming, it was likewise used to treat skin diseases and dandruff, as the vinegar has antibacterial and antifungal activities against skin and gastrointestinal tract microbes. Moreover, they demonstrated antioxidant activity that may act as anti-inflammatory against skin or wound diseases. In this way, the development of *S. faecalis, S. aureus, E. coli, S. epidermidis,* and *Propionibacterium*

acnes were restrained by vinegars.[28] In spite of the fact that, vinegar contained a high measure of natural acids, particularly acidic, corrosive which their antimicrobial impact was greatly frail. All these things contribute to the bacteria cannot grow in the presence of vinegar.

Conclusion

This scientific study reported the antibacterial activity of vinegar against four out of five microorganisms was found to be superior to the chlorhexidine, which is actually considered as one of the most effective antimicrobial agents. In addition, vinegar appears to satisfy all of the criteria for antibacterial agents, being cheap and safe.

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