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COMPARATIVE *IN VITRO* ANTIBACTERIAL EVALUATION OF SOME COMMERCIALY AVAILABLE BRANDS OF MOUTH WASHES AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT: Colonization of bacteria causes various diseases of oral cavity such as plaque and gingivitis. *Staphylococcus* and *Streptococcus* species are the most important pathogens causing dental caries. The present study was designed to screen the anti bacterial action of four different marketed mouth washes namely Listerine zero, Colgate plax, Signal expert protection and Aloe dent against selected human pathogenic strains *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Escherichia coli*. All the brands of mouth wash demonstrated an excellent spectrum of activity against *Staphylococcus aureus*. In this study the antibacterial action of Listerine zero was significant when compared to Colgate plax against *Staphylococcus aureus* at $P < 0.05$ level. However, the spectrum of action of Colgate plax was extremely significant against *Bacillus subtilis* at $P < 0.001$ level when compared to rest of mouthwashes screened. The spectrum of activity varied with brands of mouth washes screened against organisms. This study demonstrated that all the brands of mouth washes revealed good anti bacterial effect.


INTRODUCTION: Gargling with mouthwash is good to follow in order to maintain the oral health and thus get free from bad breath. It is very effective after brushing because during gargle it can reach the areas where the toothbrush can't reach like in between the teeth, back of throat, and on the sides of inside of the cheeks. The oral cavity may be affected by plaque and periodontal disease due to bacterial colonization¹. *Streptococcus pyogenes*, *Streptococcus mutans*, *Staphylococcus aureus* are the major organisms of dental carries, plaque and periodontitis². The purpose of this study was to find the efficacy of various commercially available brands of mouthwash in the Kingdom.

In this work, the mouth washes have been selected on random basis, among these Listerine zero and Aloe dent contains thymol and *Citrus grandis* seed extract respectively, the other two contains specified anti bacterial chemicals. However, all the brands of mouth wash are free from alcohol.

MATERIALS AND METHODS:

Collection of Materials and Properties of mouthwashes: Commercially available 4 different brands were purchased from local Pharmacy, Jazan, KSA and their colour, homogeneity and pH were noted on preliminary basis. All Chemicals used in this study was analytical grade from Sigma Aldrich, Saudi Arabia.

Strains used: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Escherichia coli* were used in this study. The cultures were isolated from clinical samples obtained from Jazan Hospital, Jazan.

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24h cultures were prepared by sub culturing from the stock culture and the working culture was determined as 10^{-6} CFU/mL.

Antibacterial screening: All the mouth washes were diluted in sterile distilled water and predetermined concentrations of 0.5, 1, 1.5, 2, 2.5 and 3% v/v of respective mouthwashes were prepared and used in this study. The concentration of mouth washes was fixed by means of determining the minimum inhibitory concentration (MIC) using nutrient broth dilution method as described by Kamal Rai *et al.*, 2010³. Based on MIC value, the concentration of mouthwashes was fixed to evaluate the spectrum of antibacterial property. The agar well diffusion technique was performed for antibacterial susceptibility test¹. Muller Hinton (MH) agar was prepared and plated in aseptic condition. 0.1 ml of standardized bacterial culture was poured on the MH agar plate individually and spread with L shaped glass spreader. After spreading, the wells were made by using sterile cork borer and 0.1 ml of 1% v/v mouth washes were placed in the respective wells and kept in a bacteriological incubator for 24 h at 37⁰C. The

zone of inhibition was measured, tabulated and statistical analysis was performed.

Statistical Analysis: All the experiments were performed six times (n = 6) throughout the experimental studies. The data were subjected to one way analysis of variance (ANOVA), the level of significance is $P < 0.05$ and $P < 0.001$ using Graph pad InStat software system, USA.

RESULTS: The study demonstrated the spectrum of antibacterial activity of four different commercially available mouthwashes Listerine zero, Colgate plax, Signal expert protection and Aloe dent. Based on MIC studies, 1% v/v concentration was fixed for all the mouthwashes in order to screen against selected human pathogenic bacteria. **Table 1** depict that the spectrum of activity of all mouth washes is in mixed fashion. However, in general all the mouth washes were exhibiting predominant action against *Staphylococcus aureus* except Colgate plax. On comparative study among mouth washes, Listerine zero exhibited significant activity against *Streptococcus pyogenes* when compared to Colgate plax at $P < 0.05$ level.

TABLE 1: ANTI BACTERIAL EFFECT OF DIFFERENT BRANDS OF MOUTHWASHES AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

Organisms	Zone of inhibition (mm)			
	Listerine Zero	Signal Expert Protection	Colgate Plax	Aloe Dent
<i>Streptococcus pyogenes</i>	22.1 ± 1.1*	21.5 ± 2*	16.5 ± 4.4	20.1 ± 2.8
<i>Staphylococcus aureus</i>	24 ± 2	22.5 ± 2.4	21.1 ± 2.7	20.8 ± 2.1
<i>Bacillus subtilis</i>	17 ± 2.2	17.7 ± 3.8	25 ± 2.2***	15.5 ± 2
<i>Escherichia coli</i>	16.5 ± 3	17.3 ± 3	20 ± 1.6*	15 ± 3.5

#Each value is the mean of 6 batches with standard deviation, $P < 0.05$ significant level by performing Tukey Kramer test (post test). * Significant when compared to Colgate plax. *** Extremely significant at $P < 0.001$ when compared to rest. * Significant when compared to Aloe dent

It is interesting to note that Colgate plax showed extremely significant activity against *Bacillus subtilis* at $P < 0.001$ when compared to rest. As shown in **Fig. 1**, the Colgate plax exhibited lesser effect against *Streptococcus pyogenes* when compared with other mouth washes that were screened in this work. Overall all the mouthwashes were exhibiting predominant activity against coccus bacteria when compared to bacilli.

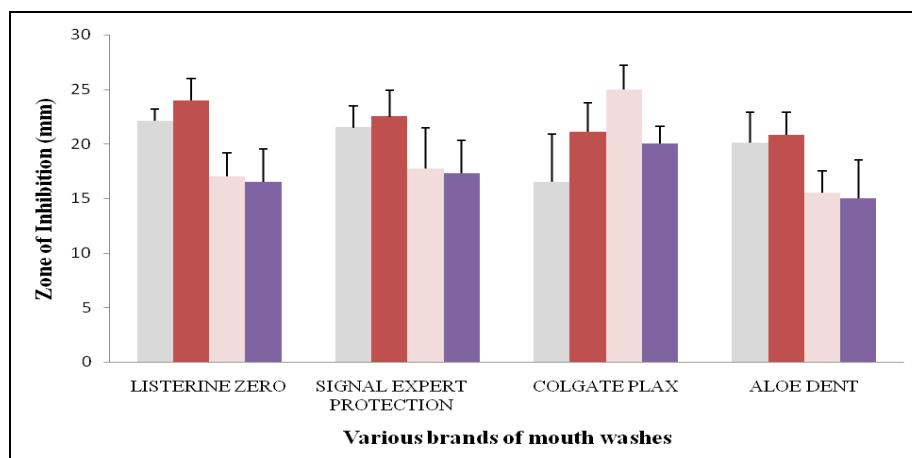
DISCUSSION: Mouth washes are used very commonly to maintain oral hygiene. Generally, mouthwash solutions include antibacterial substances that ensure the bacterial free solutions which aids in preventing future dental carries,

gingivitis and periodontitis⁵. Results of this study show that mouthwash solution possesses variable antibacterial activity due to their chemical composition. Majed *et al.*, 2013⁶ reported that mouth washes containing cetylpyridinium chloride, a quaternary ammonium compound exhibited antibacterial activity against all the organisms that screened except *P. aeruginosa* and *K. pneumoniae*. However, many other researchers also proved that cetylpyridinium chloride exhibited good spectrum of activity against selected bacterial pathogens⁷⁻⁹.

In this study, the Colgate plax contains cetylpyridinium chloride that showed maximum activity against *Bacillus subtilis* followed by

Staphylococcus aureus. However, Aloe dent mouth wash composed of *Citrus grandis* seed extract showed good anti microbial action. In contrast to earlier report, Naiana et al., 2012¹⁰ Listerine zero exhibited the predominant activity against *Staphylococcus aureus* and *Streptococcus pyogenes* when compared to rest (Fig.1). Among natural extract mouth washes, thymol and eucalyptol based

Listerine zero showed little more activity when compared to Aloe dent, which has *Citrus grandis* seed extract as active constituent. In this study the results demonstrate the anti bacterial activity of various brands of commercially available mouth washes proving that all the four brands of mouth washes are good in protecting against selected bacterial human pathogenic bacteria.



Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis and Escherichia coli

FIG. 1: THE SPECTRUM OF ANTI BACTERIAL VARIOUS BRANDED MOUTH WASHES AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

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CONFLICT OF INTEREST: The author declares no conflict of interest relevant to this article.

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