

## ANTIBACTERIAL EFFECT OF ALOE VERA AND GLASS IONOMER MODIFIED BY ALOE VERA ON STREPTOCOCCUS MUTANS

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### **ABSTRACT**

**Objective:** To evaluate the antibacterial effect of Aloe Vera and glass ionomer modified by Aloe Vera on Streptococcus mutans in comparison to Chlorhexidine.

**Methods:** A total of fifty samples were used in the study and divided into 5 groups according to the materials used (n=10) Group 1: 2% Chlorhexidine gluconate (CHX); Group 2: Aloe Vera (AV); Group 3: Glass ionomer (GI); Group 4: 2% Chlorhexidine gluconate added to glass ionomer (CHX+ GI) and Group 5: Aloe Vera added to glass ionomer (AV+GI) and. The antibacterial effect of each group were assessed by disc diffusion methods after two-time intervals: 24 and 48 hours. Data were tabulated and statistically analyzed using Two-way ANOVA followed by Bonferroni's post-hoc test.

**Results:** There was a statistically significant difference between the materials used on inhibition zone diameter after 24 and 48 hours. CHX showed the statistically significantly highest mean inhibition zone diameter followed by AV > CHX + GI > AV + GI. GI group showed the lowest mean inhibition zone diameter with a statistically significantly.

**Conclusion:** Aloe Vera is beneficial in the inhibition of Streptococcus mutans growth up to 48 hours. The incorporation of Aloe Vera in glass ionomers seems to improve the antibacterial activity of glass ionomer restoration.

**KEY WORDS:** Aloe Vera, Chlorhexidine, Glass Ionomer, Streptococcus mutans, Antibacterial Activity.

### **INTRODUCTION**

Dental caries is one of the most common diseases affecting oral health. It is a multifactorial lesion related to the interaction between oral microorganisms, teeth and diet. <sup>(1)</sup> The incidence

of dental caries is depending on Streptococcus mutans which is the main factor that trigger dental caries.<sup>(2)</sup> These anaerobic microorganisms adhere to the tooth surface initiating plaque formation through their ability to synthesize and bind extracellular polysaccharides using glucosyltransferase enzyme.

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*Streptococcus mutans* also are able to metabolize sucrose into lactic acid which cause a significant drop in salivary pH below the critical pH (5.5).<sup>(3,4)</sup>

The continuous and repeated decrease in salivary pH can cause demineralization of the inorganic tooth structure and eventually tooth decay. Therefore, reducing these cariogenic microorganisms is beneficial in caries prevention.<sup>(5,6)</sup> For many years, scientific research continues to make progress in identifying the best practices for preventing dental caries. Minimal intervention dentistry focuses on the least invasive treatment options to preserve the natural tooth structure as much as possible.<sup>(7, 8)</sup>

Glass ionomer cement (GIC) is characterized by its antibacterial properties and fluoride release. The literature has proofed that the fluoride released from GIC is not sufficient to diminish the cariogenic bacteria. Furthermore, these bacteria are able to survive and remain viable under GIC restorations up to two years resulting in development of secondary caries.<sup>(9,10)</sup> Consequently, the use of cavity cleansers before the placement of the restorations are promising in the field of contemporary minimal intervention dentistry.<sup>(11)</sup>

Chlorhexidine (CHX) is a broad-spectrum antimicrobial effective against gram negative and gram positive bacteria. It is very effective mouthwash in reducing gingivitis and plaque accumulation.<sup>(12)</sup> Also Chlorhexidine and Chlorhexidine digluconate are widely used as cavity disinfectants due to their powerful effect in reducing the cariogenic microorganisms.<sup>(13)</sup> It exerts the antibacterial action by binding to the dentin amino acids.<sup>(14)</sup> However, the undesirable side effects of CHX such as brownish discoloration of teeth and restorations, mucosal soreness and desquamation and taste disturbances limit its clinical use.<sup>(15)</sup> Further researches were developed to find alternative sources of natural antibacterial agents with a focus on safety for humans and efficacy in the treatment and prevention of dental caries.<sup>(11, 13, 16)</sup>

Aloe Vera (AV) is an equatorial plant grown easily in hot and dry weather of Africa and Asia. It has

been used therapeutically for a long time due to its peculiar benefit.<sup>(17)</sup> Aloe Vera has various pharmacological actions such as anti-fungal, antiviral, anti-inflammatory, antibacterial, antioxidant, antitumor activities, detoxifier, hypoglycemic, antiseptic and tonic for the nervous system.<sup>(6,18)</sup>

Aloe Vera has been used in dentistry and showed good results in treatment of periodontitis, lichen planus, oral ulceration, oral candidiasis,<sup>(19,20)</sup> denture stomatitis, disinfectant in endodontic treatments, plaque control, healing of socket, denture adhesive, and anti-caries agent.<sup>(21,22)</sup> It has been successfully applied in toothpastes<sup>(6)</sup>, mouthwashes, and dental floss.<sup>(5,23)</sup> Although Aloe Vera have been used in dentistry for various purposes, till now no study has been conducted to investigate its effect after incorporation to dental restorative materials as glass ionomer restoration. Therefore, this in vitro study was designed to evaluate the antibacterial effect of Aloe Vera and glass ionomer modified by Aloe Vera on *Streptococcus mutans*.

## MATERIALS AND METHODS

### Materials used in the study:

In the current study three different materials were used; fresh mature Aloe Vera, 2% chlorhexidine gluconate (commercially available in syringe form, Consepsis, Ultradent) and Amalomer TM advanced glass ionomer esthetic anterior restoration (Advanced Health Care Ltd, Tonbridge, Kent, UK) water mix type.

### Sample grouping:

A total of fifty samples were used in the study and divided into 5 groups according to the materials used (n=10) Group1: 2% Chlorhexidine gluconate(CHX); Group 2: Aloe Vera (AV); Group 3: Glass ionomer (GI); Group 4: 2% Chlorhexidine gluconate added to glass ionomer (CHX+ GI) and Group 5: Aloe Vera added to glass ionomer (AV+GI). The antibacterial effect of each group were assessed after two-time intervals: 24 and 48 hours.

**Preparation of Aloe Vera:**

Fresh mature Aloe Vera leaves about 30-50 cm were selected and washed with distilled water then their spines were chopped. The thick epidermis was removed to collect the solid mucilaginous gel in a sterile container. The thick straw-colored gel crushed in a sterile mortar and pestle then the gel squeeze using a sterilized piece of gauze to collect Aloe Vera juice in a sterile syringe.<sup>(20, 24)</sup>

A filter paper discs of 5 mm diameter were prepared (Whatman filter paper No.1) and placed in a petri dish for sterilization in a hot air oven for two hours at 160°C. Regarding Group1 (CHX) and Group 2 (AV), the paper discs were immersed in 20 mL of CHX and 20 mL of AV respectively till saturation with the solutions. This has been done to avoid the spread of the solutions in a wider diameter in the plate to standardize the sample size (5 mm diameter) for all tested groups.

**Preparation of glass ionomer specimens:**

Glass ionomer specimens were made using a sterilized custom split Teflon mold (5mm in diameter x 2mm in height). For specimens' preparation, sterilized glass slide covered with a Mylar strip (Stripmat, POLYDENTIA, CH-6805 Mezzovico, Switzerland) was used. The glass ionomer powder and the distilled water were proportioned according to the manufacturer' instructions and mixed until a homogenous mix was obtained. The materials were packed into the mold as one increment then covered with another Mylar strip and a glass slide, then pressed for 10 seconds to expel the excess material and achieve a uniform smooth surface. Glass ionomer mix was left into the mold till complete setting. Regarding Group 4 (CHX+ GI) and Group 5 (AV+GI), the glass ionomer specimens were prepared with the same procedures as mentioned before except that the glass ionomer powder were mixed with CHX and AV respectively instead of distilled water.

**Testing of antibacterial activity:**

ATCC 25175 Type strain Streptococcus mutans (16S rRNA gene, Serotype c. carious dentin) were obtained from MIRCEN (Microbiological Resources Centre, Cairo, Egypt) and used in this in vitro study. Bacteria were cultured at 37°C overnight in Trypticase Soy agar (BD 236950, Difco™, USA) and used as inoculums. The McFarland 0.5 turbidity standard (Densimat, BioMerieux, France) was used to determine the turbidity of the suspension. On that absorbance, the concentration of bacteria is standardized to about 1x CFU/ml and used as a working microbial solution.

A total 100µl of the previously prepared working microbial solutions was spread evenly over blood agar (blood agar base (sheep) CM0854, Oxiod, UK) plate (TSA, Difco, USA). The plates were incubated at 37 °C a bacteriological incubator. The antibacterial effect for all groups were appraised by measuring the diameter of bacterial growth inhibition zones after 24 and 48 hours. The diameter of the bacterial growth inhibition zones was measured in millimeters using an electronic digital caliper (STECO, Germany) at three different points to obtain three measurements for each specimen.

**Statistical Analysis**

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed parametric distribution. Data were presented as mean, standard deviation (SD) and 95% Confidence Interval for the mean (95% CI) values. Two-way Analysis of Variance (ANOVA) was used to study the effect of material, time and their interaction on mean inhibition zone diameter. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

## RESULTS

Two-way ANOVA results showed that material, time as well as the interaction between the two variables had a statistically significant effect on mean inhibition zone diameter (table 1). Since the interaction between the variables is statistically significant, so the variables are dependent upon each other.

Descriptive statistics of inhibition zone diameter at the two follow up times regardless of material were represented by mean, standard deviation (SD), 95% Confidence Interval values in table (2). There was a statistically significant difference between the two follow up times ( $P$ -value  $<0.001$ , Effect size = 0.625). Pair-wise comparisons between the time periods revealed that 48 hours showed statistically significantly higher mean inhibition zone diameter than 24 hours.

All the five groups showed antibacterial activity. Figure (1) showed the zones of inhibition against

*Streptococcus mutans* around the tested material. Table (3) and figure (2) showed that there was a statistically significant difference between the materials used on inhibition zone diameter after 24 and 48 hours. Pair-wise comparisons between the materials revealed that CHX showed the statistically significantly highest mean inhibition zone diameter. AV showed statistically significantly lower mean inhibition zone diameter followed by CHX + GI then AV + GI group with a statistically significant difference between these groups. GI group showed the lowest mean inhibition zone diameter with a statistically significantly.

As regarding the effect of time, there was a statistically significant difference between the two follow up times. The mean inhibition zone diameter after 48 hours showed statistically significantly higher mean value than 24 hours in CHX group; AV group and CHX + GI group. While in AV + GI group and GI group, there was no statistically significant difference between the two follow up times.

TABLE (1): Two-way ANOVA results for the effect of different variables on mean inhibition zone diameter

Source of variation	Type III Sum of Squares	Df	Mean Square	$F$ -value	$P$ -value	Effect size ( <i>Partial eta squared</i> )
Material	987.312	4	246.828	2915.798	$<0.001^*$	0.997
Time	5.638	1	5.638	66.603	$<0.001^*$	0.625
Material x Time interaction	3.469	4	0.867	10.246	$<0.001^*$	0.506

*df: degrees of freedom = (n-1), \*: Significant at  $P \leq 0.05$*

TABLE (2): The mean, standard deviation (SD), 95% Confidence Interval values and the results of comparison between the inhibition zone diameters at the two time intervals regardless of material

24 hours		48 hours		$P$ -value	Effect size ( <i>Partial eta squared</i> )
Mean (SD)	95% CI	Mean (SD)	95% CI		
7.44 (4.35)	7.32 – 7.56	8.11 (4.74)	8 – 8.23	$<0.001^*$	0.625

*\*: Significant at  $P \leq 0.05$*



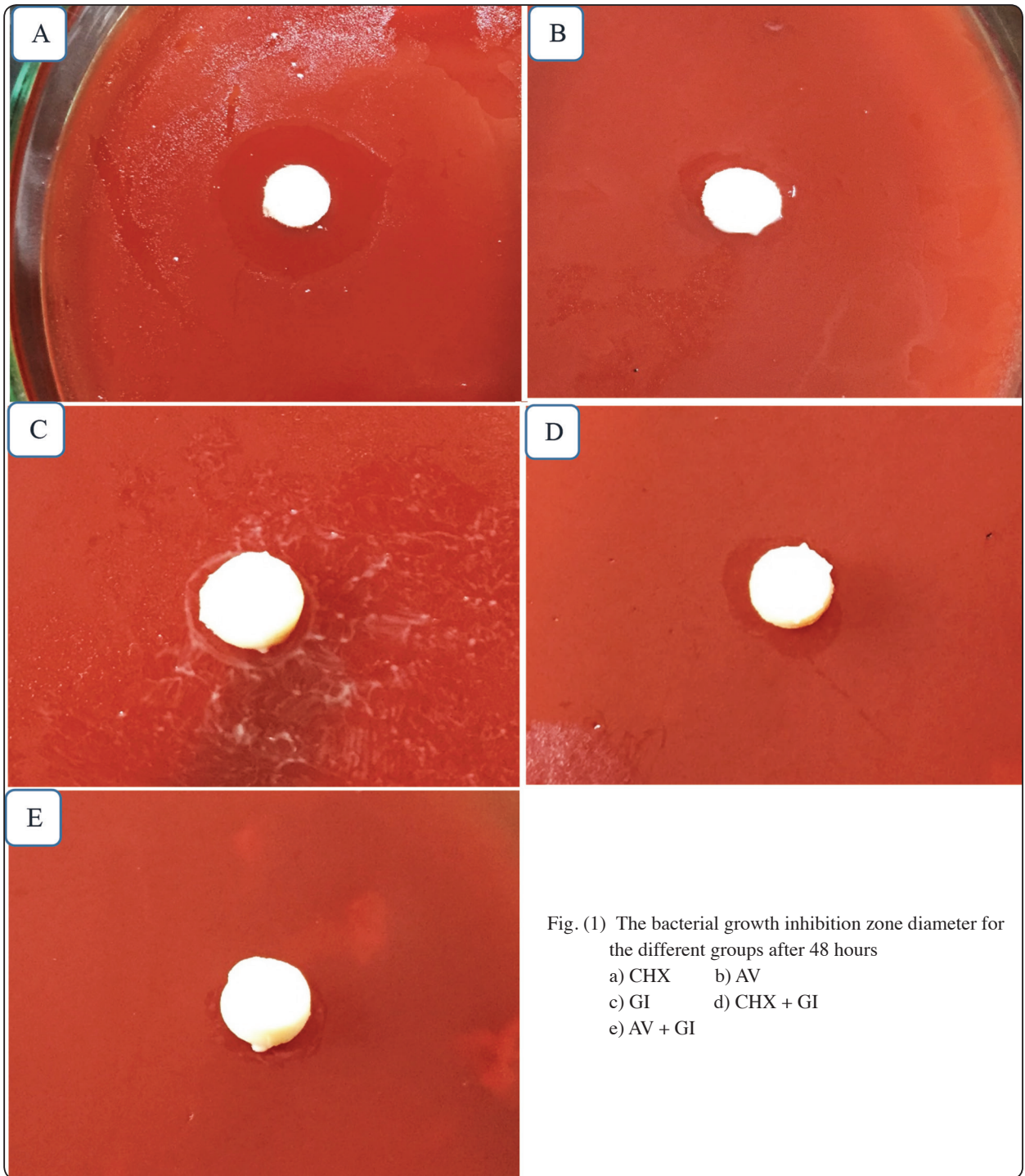


TABLE (3) The mean, standard deviation (SD), 95% Confidence Interval values and the results of comparison between the inhibition zone diameters for the different interactions

Material	24 hours		48 hours		P-value (Between times)	Effect size ( <i>Partial eta squared</i> )
	Mean (SD)	95% CI	Mean (SD)	95% CI		
CHX	15.31 (0.35) <sup>A</sup>	15.04 – 15.57	16.44 (0.12) <sup>A</sup>	16.18 – 16.7	<0.001*	0.487
AV	7.71 (0.24) <sup>B</sup>	7.44 – 7.97	8.73 (0.47) <sup>B</sup>	8.47 – 8.99	<0.001*	0.436
GI	2.71 (0.2) <sup>E</sup>	2.45 – 2.98	2.53 (0.14) <sup>E</sup>	2.27 – 2.79	0.323	0.024
CHX + GI	6.13 (0.53) <sup>C</sup>	5.87 – 6.4	7.22 (0.27) <sup>C</sup>	6.96 – 7.49	<0.001*	0.467
AV + GI	5.35 (0.13) <sup>D</sup>	5.09 – 5.61	5.64 (0.09) <sup>D</sup>	5.38 – 5.91	0.118	0.060
P-value (Between materials)	<0.001*		<0.001*			
Effect size ( <i>Partial eta squared</i> )	0.993		0.994			

\*: Significant at  $P \leq 0.05$ , Different superscripts in the same column are statistically significantly different

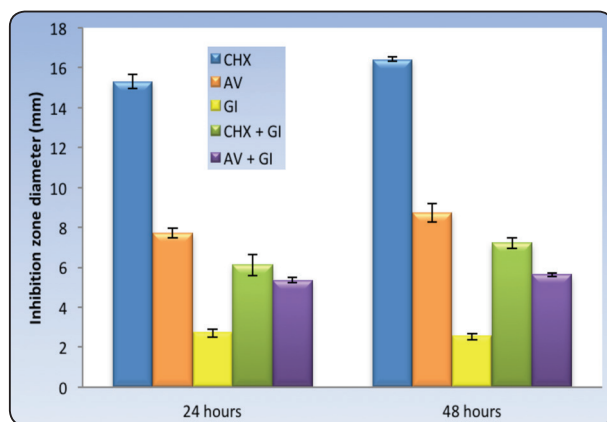


Fig. (2) Bar chart representing mean and standard deviation values for inhibition zone diameters with different interactions of variables

## DISCUSSION

The present study was conducted to evaluate the antibacterial effect of Aloe Vera and glass ionomer modified by Aloe Vera on *Streptococcus mutans*.

Dental caries development and progression are depending on the presence of acidic media. *Streptococcus mutans* is considered a main cause for acid production through metabolizing the dietary sucrose and subsequent demineralization of

tooth tissues. <sup>(5)</sup> Although this demineralized dentin contains bacteria, it can be left intact to maintain the pulp vitality. According to the minimally invasive dentistry, such treatment is acceptable providing the use of cavity disinfectant against these cariogenic bacteria. Also, it depends on the use of glass ionomer cements that characterized by anti-bacterial properties and fluoride release. <sup>(8,25)</sup>

The released fluoride contributes reduction in the number of residual bacteria and remineralization of soft dentin. However, viable bacteria have been found in the remaining affected dentin after different periods of evaluation, even after the removal of infected dentin and adequate sealing. <sup>(8,10)</sup> Therefore, the researchers enhance the antibacterial properties of GICs by adding antibacterial agents as antibiotics <sup>(9)</sup> and chlorhexidine. <sup>(26,27)</sup> Although chlorhexidine was able to reduce the residual bacteria but it has adverse effects on the microtensile and shear bond strengths. <sup>(11)</sup> Consequently, the use of natural antibacterial such as Miswak and proplis become a growing trend in modification of dental restorations to avoid the side effects of the chemical one. <sup>(28)</sup>

In the present study, all the tested materials showed statistically significant effect on mean inhibition zone diameter after 24 and 48 hours. The chlorhexidine gluconate 2% showed the statistically significantly highest mean inhibition zone diameter. Chlorhexidine has a proven antibacterial effect and it was used as cavity disinfectant at this concentration. <sup>(14, 15)</sup> Chlorhexidine enters bacterial cytoplasm destroying the cell membrane integrity. This leads to an increase in the bacterial cell wall permeability and osmotic imbalance. Chlorhexidine binds to phospholipids and dentin amino acids which cause damage to the cell molecular weight. Furthermore, it precipitates the phosphate groups in the cytoplasm causing coagulation and leakage of intracellular components which results in bacterial lysis and death. <sup>(5,14)</sup>

The results of this study were in accordance with **Sancakli et al., 2018** <sup>(14)</sup> who evaluated methods of antibacterial surface pretreatment against *S. mutans* within the infected dentin surface. They found that antibacterial dentin bonding and chlorhexidine have the highest antibacterial effects when compared with Er:YAG Laser and ozone.

In this in vitro study Aloe Vera showed statistically significantly effect on the mean inhibition zone diameter. This antimicrobial activity is attributed to the pharmacologically active component of Aloe Vera. The fresh Aloe Vera leaves excrete a mucilaginous colorless gel that contains high percent of water (98-99%) and about 1-2% active compounds. <sup>(21)</sup> It contains about 75 active ingredients as amino acids, salicylic acid, anthraquinones, anthracene, Aloe mannan, phenols, Aloe-emodin, polysaccharides, resistanol, aloetic acid, Aloin, vitamins, lignins, aloeride, sterols, antranol and saponin. Free Anthraquinones like emodin and aloin (a bitter-tasting yellow compound) are potent antimicrobial agents. <sup>(17, 29)</sup>

Aloe Vera compounds like anthraquinones and saponin have direct antibacterial activities as they can inhibit protein synthesis from bacterial cells.

This results in disruption of membrane permeability so the bacterial growth is inhibited. <sup>(30)</sup> Aloe Vera gel also can exert indirect bactericidal activity by some other components, such as acemannan through stimulation of leukocytes phagocytosis. Saponin has an antiseptic effect as it can dissolve the lipoprotein in the bacterial cell membrane. This is causing damage the cell membrane of bacteria results in bacterial lysis and death. <sup>(29, 31)</sup>

This result was in agreement with **Prabhakar et al., 2015** <sup>(11)</sup> who evaluated the efficacy of Aloe Vera and propolis as cavity disinfecting agents for minimally invasive hand excavation of dental caries. They found Aloe Vera extracts showed a significant reduction in the bacterial counts. **Fani and Kohanteb 2012** <sup>(20)</sup> found that the use of Aloe Vera gel at optimum concentrations in toothpastes or mouthwashes could be useful for prevention of dental caries and periodontal disease.

On the other hand, this result was in contradiction with **Sreekumar and Hegde 2018** <sup>(27)</sup> as they found that Aloe Vera failed to show any zone of inhibition against streptococcus mutans when compared with other herbal products. This may be attributed to that Aloe Vera extracts are highly technique sensitive and improper processing procedures lead to loss of its active ingredients.

In this in vitro study Aloe Vera showed statistically significantly lower mean inhibition zone diameter in comparison to CHX. This may attribute to that the mechanism of action of Aloe Vera that works by gradually damaging to the cell membrane of bacteria which is not progressive as chlorhexidine. <sup>(5)</sup>

These findings were in agreement with **Nonong et al., 2016** <sup>(5)</sup> who reported a significant difference between Chlorhexidine and Aloe Vera in decreasing the number of Streptococcus mutans colonies. Also, **Patri and Sahu 2017** <sup>(24)</sup> compared the efficacy of herbal antibacterial agents (Tea Tree Oil and Aloe Vera) with commercially available 2% chlorhexidine as cavity disinfectant used for minimally invasive

dentistry. They found that 2% chlorhexidine showed the highest reduction in Total Viable Count followed by 1% tea tree oil and aloe Vera gel.

**Gupta et al., 2014** <sup>(29)</sup> observed that the Aloe Vera mouth rinse has equal effect as 0.2% CHX. In the present study, 2% CHX showed better antibacterial effect than the Aloe Vera juice. This could be attributed to the difference in CHX concentration.

The present study showed that, there was a statistically significant difference between the two follow up times. The mean inhibition zone diameter after 48 hours showed statistically significantly higher mean value than 24 hours in CHX and AV groups. This might be attributed to the difference in rate of diffusion between CHX and AV. This finding was in agreement with **Nonong et al., 2016** <sup>(5)</sup> who reported that Aloe Vera and Chlorhexidine showed effective decreased in the *Streptococcus mutans* colonies after 2 days.

GI group showed the statistically significantly lowest mean inhibition zone diameter. The antibacterial activity of glass cements is one of the most appealing properties that has a direct correlation with the amount of fluoride release. Fluoride release may be influenced by many factors such as the released fluoride and other ions, the chemical composition of glass ionomer and low pH value during setting. It appears to be the most likely cause of the inhibitory effect on acid production and it also affecting the bacterial metabolism. <sup>(25,27)</sup>

This finding was in agreement with **Łuczaj-Cepowicz et al., 2014** <sup>(25)</sup> who determined the antibacterial activity of four glass ionomer cements against *Streptococcus* and *Lactobacillus*. They reported that there was different antibacterial activity of glass ionomer cements observed depending on the cement, time of evaluation and bacterial strain. Also, **Bariker and Mandroli 2016** <sup>(32)</sup> who evaluated the antibacterial activity of Fuji VII and Amalomer CR against streptococci mutans. They reported that Amalomer CR had the most antibacterial effect against *S. mutans*.

In GI group, there was no statistically significant difference between the two follow up times. This may be due to the initial low pH of the cements during setting which lasting from several minutes to 24 hours till reach the neutrality. <sup>(10)</sup>

In this study CHX + GI showed higher mean inhibition zone diameter GI with a statistically significant difference between these groups. This might be attributed to interaction between the cationic CHX molecule and the fluoride ion resulting in the precipitation of salts with lower solubility. <sup>(26)</sup> The mean inhibition zone diameter after 48 hours showed statistically significantly higher mean value than 24 hours in CHX + GI group. This may be due to the elution rate of the CHX from the GI, where synergism has been appeared to happen between fluoride and the cationic CHX. <sup>(28)</sup>

This results were in accordance **Yadiki et al., 2016** <sup>(26)</sup> compared the antimicrobial properties of two commercially available glass ionomer cements with and without chlorhexidine gluconate on strains of streptococci mutans. They confirmed that the addition of chlorhexidine gluconate to the tested cements produced a restorative material with increased antimicrobial properties. **Duque et al., 2018** <sup>(33)</sup> who found that the incorporation of chlorhexidine to glass ionomer cements GIC increased the in vitro antimicrobial activity, without changing chemical-mechanical properties of GIC and odontoblast-like cell viability.

In the present study, AV + GI showed statistically significantly higher mean inhibition zone diameter than GI. As the combined effect of Aloe Vera with the glass ionomer was not available in literatures, this may be due to the presence of anthraquinones that react with fluoride released from glass ionomer cement to form fluoroquinolones compound. These compounds are highly soluble in acidic pH and have greatly activity against many Gram positive bacteria. <sup>(34)</sup> Therefore, the acid base reaction of glass ionomer cement (initial acidic pH) enhance the solubility of fluoroquinolones. There was



no statistically significantly effect on the mean inhibition zone diameter in Aloe Vera+ GI group after 24 and 48 hours. This might be due to the low pH value of glass ionomer cements during the initial setting and the stability of fluoroquinolones compound in high pH.

To summarize, Aloe Vera as natural product has antibacterial effect against *Streptococcus mutans* that allow its use as a cavity disinfectant more affordable with no side effects than chlorhexidine. It has the ability to inhibit the growth of streptococci mutans. Also the incorporation of chlorhexidine gluconate and Aloe Vera to glass ionomer cements increased its antimicrobial properties.

## CONCLUSIONS

Despite the limitations of this study, the following could be concluded:

1. Aloe Vera has an inhibitory effect on *Streptococcus mutans* up to 48 hours.
2. The incorporation of Aloe Vera in glass ionomers seemed to improve the antibacterial activity of glass ionomer.

## RECOMMENDATIONS

1. Further long-term investigations assessing the antibacterial activity of Aloe Vera on *Streptococcus mutans* may be required.
2. Further investigations to evaluate the mechanical properties of glass ionomer modified by Aloe Vera.

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