

## Antibacterial Effect of Aloe Vera, Chlorhexidine and Fluoride Containing Tooth pastes. In-vitro study

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### Abstract:

**Background:** The dental bacteria have a role in the etiology of dental caries. One of the most important criteria of the toothpaste is the antibacterial activity. Aloe Vera has been known for its antibacterial effect.

**Aim or purpose:** This study aims to evaluate the antibacterial effect of Aloe Vera containing toothpaste versus fluoride containing and chlorhexidine containing tooth pastes.

**Abstract Materials and Methods:** Antibacterial effect of the materials tested were evaluated against *Streptococcus mutans* (SM) and *Lactobacillus Acidophilus* (LB) using Agar diffusion test and Direct contact test. In Agar diffusion test; 200 µl of bacterial suspension were spread on blood agar plates. Each tested material was inserted into uniform wells that were previously punched in the agar. After incubation at 37°C for 48 hours, the diameter of the inhibition zone formed was measured in millimeters in two perpendicular locations for each sample. In Direct contact test, measuring the optical density (OD) of bacterial growth was done using Spectrophotometer at 600 nm wavelength after incubation for 120hrs and measured after 5days. Two sets consisting of 96 well plates each were used. A 10 µl bacterial suspension was placed on the tested sample. The plates were then positioned horizontally and 245 ml of BHI broth were added to each well and incubated for 1 hour. Bacterial growth was estimated by following changes in OD in each well.

**Results:** in Agar diffusion test for SM and LB revealed a statistical significant difference between groups. Aloe Vera tooth paste group showed the highest mean value (mean± SD) of inhibition while fluoride toothpaste group showed the lowest mean value (mean± SD) of inhibition.

In Direct Contact test of the tested materials with SM and LB showed that there was a statistical significant difference between the groups. Pair wise comparisons between the groups revealed that fluoride toothpaste showed the highest statistical significant mean OD while Aloe Vera toothpaste showed the lowest statistical significant mean OD.

**Conclusion:** Aloe Vera has a potential antibacterial activity compared to chlorhexidine and fluoride toothpaste against SM and LB.

**Keyword:** Aloe Vera, antibacterial effect, herbal agent.

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### I. Introduction:

Dental plaque plays a major role in the etiology of dental caries<sup>1</sup>. It has been identified that a diverse microflora is found in dental plaque biofilms. Therefore, "The specific plaque hypothesis" was put forward as a major advance<sup>2</sup>. This hypothesis proposed that the disease is actively caused by only a few of the many species found in dental plaque biofilms. Thus, we can control caries disease by targeting preventative measures and interventions against these "specific" organisms<sup>3</sup>.

Over the years, various synthetic chemical agents have been evaluated for their antimicrobial effect in oral cavity. The chlorhexidine remains the benchmark control in the removal of plaque accumulated over enamel surface<sup>4</sup>. However, it cannot be used for a long period of time due to its reported side-effects like altered taste sensation and staining of tongue<sup>5</sup>.

Accordingly; patients are nowadays preferring to shift away from modern medicines, and preferring to use herbal preparations like Terminalia chebula, Aloe Vera, Azadirachta indica, piper betle, Ocimum sanctum<sup>6</sup>.

Aloe Vera extract has been considered as one of the natural oral hygiene aids to reduce plaque formation and accumulation. Aloe Vera has been used for many and different treatments like burns, hair loss,

skin infections, haemorrhoids, sinusitis, and gastrointestinal pain<sup>7</sup>. It has also been used as a wound healer for bruises, insect bites<sup>8</sup>.

In addition to having antihelminthics, anti-arthritis effect. It also aids in reducing radioderamtitis, psoriasis and genital herpes infection<sup>7</sup>. This is attributed to its various pharmacological actions which include anti-inflammatory, antibacterial, antioxidant, antiviral and antifungal actions<sup>9</sup>.

Literature is abundant on the health beneficial effects of Aloe Vera but to date, few studies have been conducted to test its antibacterial efficacy on enamel surface.<sup>9-13</sup> Hence, this study was conducted to evaluate the efficacy of Aloe Vera as antibacterial agent.

## **II. Materials and methods**

This in-vitro study was conducted at the Department of Microbiology, of Ain Shams University from December 2018 to December 2020.

**Study Design:** In-vitro study.

**Study Location:** This study was conducted at the Department of Microbiology, of Ain Shams University.

**Study Duration:** from December 2018 to December 2020.

To evaluate the antibacterial activity, three commercial toothpastes were used which are:

1. Aloe Vera containing toothpaste (Hello, Hello production LLC, United States)
2. Flouride containing toothpaste (Signal Kids, Unilever Mashreq ,Egypt)
3. Chlorhexidine containing toothpaste (Elgydium, Pierre Fabre Medicament ,France)

## **III. Methodology:**

Freshly collected Aloe Vera gel extract was obtained from cutting it into half and the inner *flesh (Mucin)* was extracted using a dental carver and inserted into sterilized vial until conduction of the test at room temperature 24 C.

Freeze dried stock culture of the reference strains *SM* (France, imported by El Magd company, derived from ATCC 35668TM, Lot no. 969-51-2, Ref.no. 0969P) and *LB* (France, imported by El Magd company, Lot no. 885-43-4, Ref.no. 0885P).

### **Tests:**

#### **Direct contact test:**

For testing the antibacterial effect of each material, two sets consisting of 96 well plates each were used (one plate for *SM* and second one for *LB*). The plates were held vertically, and the sidewalls of the wells were coated with thin layer of the tested materials. A 10 µl bacterial suspension (approximately 10<sup>6</sup> bacteria) was placed on the tested sample. Afterwards incubation of the wells vertically for 1 h in a humid atmosphere at 37°C was carried out where evaporation of the suspension's liquid was observed to ensure direct contact between all bacteria and surfaces of the tested materials. The plates were then positioned horizontally and 245 µl of BHI broth were added to each well containing the tested material and incubated for 1 hour.

The optical densities of bacterial growth using Spectrophotometer at 600 nm wavelength and measurements were taken on the fifth day.

#### **Zone inhibition test:**

200 µl of bacterial suspension (approximately 5x10<sup>7</sup> colony-forming units) were spread on blood agar plates. Each tested material (10 µl) was inserted into uniform wells that were previously punched (4 mm diameter and 4 mm depth) in the agar. After incubation at 37°C for 48 hours, the agar plates were then examined for bacterial inhibition zones. The diameter of the inhibition zone was measured in millimeters in two perpendicular locations for each sample<sup>14</sup>.

#### **Statistical analysis:**

Repeated measures ANOVA test was used to compare between the groups. Bonferroni's post-hoc test was used for pair-wise comparisons. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

## **IV. Results:**

#### **Results of direct contact test:**

After five days of both *SM* and *LB* incubation; there was a statistical significant difference between the groups. Pair-wise comparisons between the groups revealed that flouride toothpaste showed the highest statistical significant mean OD. While chlorhexidine toothpaste showed a lower statistical significant mean OD.

Aloe Vera toothpaste showed the lowest statistical significant mean OD as shown in table (1).

**Table (1):** Descriptive statistics and results of repeated measures ANOVA test for comparisons between Optical density of the different groups with SM and LB after five days incubation.

	Aloe Vera toothpaste		Chlorhexidine toothpaste		Fluoride toothpaste		P value	Effect size
	Mean	SD	Mean	SD	Mean	SD		
Streptococcus mutans	0.101 <sup>C</sup>	0.006	0.151 <sup>B</sup>	0.007	0.206 <sup>A</sup>	0.009	<0.001*	0.982
Lactobacillus	0.111 <sup>C</sup>	0.014	0.151 <sup>B</sup>	0.024	0.22 <sup>A</sup>	0.035	<0.001*	0.849

**Results of Agar diffusion test:**

Results of agar diffusion test are shown in table (2). As regards SM; there was a statistical significant difference between the groups ( $P$ -value <0.001, Effect size = 0.691). Pair-wise comparisons between the groups revealed that Aloe Vera toothpaste group showed the highest mean value of inhibition with no-statistical significant difference from fluoride control group. However, there was a statistical significant difference in respect to all other groups. Fluoride control group showed no-statistical significant difference from chlorhexidine toothpaste and chlorhexidine control groups. The lowest mean value was obtained with flouride toothpaste group with no-statistical significant difference from Aloe Vera pure gel group and a statistical significant lower mean value of inhibition compared to other groups.

As regards LB; there was a statistical significant difference between the groups ( $P$ -value <0.001, Effect size = 0.481). Pair-wise comparisons between the groups revealed that Aloe Vera toothpaste group showed the highest mean value of inhibition with no-statistical significant difference from flouride toothpaste group and a statistical significant difference in comparison to all other groups. Aloe Vera pure gel group showed the lowest mean value of inhibition with no-statistical significant difference to chlorhexidine toothpaste, chlorhexidine control and fluoride control groups.

**Table (2):** Descriptive statistics and results of one-way ANOVA test for comparisons between Agar diffusion results (cm) in the different groups

1.1.1 Group	1.1.2 Streptococcus mutans		1.1.4 Lactobacilli	
	1.1.3 Mean	M SD	1.1.4 Mean	M SD
Aloe Vera toothpaste	1.1.5 .49 <sup>A</sup>	1 0.34	1.98 <sup>A</sup>	0.15
Aloe Vera pure gel	1.1.6 .51 <sup>C</sup>	0 0.27	1.1.7 .49 <sup>B</sup>	1 0.26
Chlorhexidine toothpaste	1.1.8 .79 <sup>BC</sup>	0 0.21	1.7 <sup>B</sup>	0.21
Chlorhexidine control	1 <sup>B</sup>	0.18	1.59 <sup>B</sup>	0.21
Fluoride toothpaste	1.1.9 .5 <sup>C</sup>	0 0.24	1.1.10 .01 <sup>A</sup>	2 0.19
Fluoride control	1.1.11 .19 <sup>AB</sup>	1 0.24	1.69 <sup>B</sup>	0.23
<i>P</i> -value		<0.001*	<0.001*	
<i>Effect size (Eta Squared)</i>		0.691	0.481	

\*: Significant at  $P \leq 0.05$ , Different superscripts in the same column indicate statistically significant difference between groups

**V. Discussion:**

Dental plaque is an important risk factor for the formation of dental caries. Dental Plaque is the sticky, colorless film of bacteria that forms on teeth. Plaque develops when foods containing carbohydrates are frequently left on the teeth. Bacteria that live in the mouth grow well on these foods and as a result produce acids. Over a period of time, these acids destroy tooth enamel, resulting in dental caries<sup>1</sup>.

In this study, Aloe Vera was chosen as the intervening material for plaque control; owing mainly to its antimicrobial effect<sup>15</sup>. Aloe Vera extract have received exceptional attention because they are non-synthetic and organic in nature. Aloe Vera is added to toothpaste; due to its action against cariogenic bacteria.<sup>11</sup> Up till now, there is no adequate data assessing the effectiveness of a toothpaste containing Aloe Vera in the control of plaque and gingivitis<sup>12</sup>. Therefore, the purpose of the present study was to assess antimicrobial effect of this natural product compared to fluoride containing toothpaste and chlorhexidine toothpaste<sup>16</sup>.

An addition to the study, the active ingredients of the three toothpastes were evaluated to implement the results. The same percentage of Aloe Vera, chlorhexidine and fluoride in their toothpastes were used for standardization of the results.

Two types of bacteria were used in this study; SM and LB as it was found that the commencement of caries has a good relation with SM, while further advancement of caries has more relation with LB<sup>17</sup>. In addition, LB were the leading candidate in the causation of dental caries prior to the 1950s, when the SM started to dominate the literature<sup>18</sup>.

In this study, direct contact test (DCT) has been used to evaluate the antibacterial activity; due its reproducibility, immediate testing of more than 40 samples, and continuous measurement of bacterial outgrowth with multiple measurements per plate<sup>19</sup>.

Agar-diffusion test also has been used in this study as it offers many advantages over other methods as simplicity, low cost, the ability to test large number of microorganisms and antimicrobial agents<sup>19</sup>. But on the other hand, since the antimicrobial substance must diffuse through the aqueous agar medium, only water soluble agents can be tested<sup>20</sup>.

As per the direct contact test, Al. toothpaste showed significant antibacterial effect as it resulted in the least optical density for both SM and LB.

Besides, it showed the largest inhibitory zone in the agar diffusion test. This may be due to its 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 acid, salicylic acid, anthraquinone, anthracene, Aloemannan, phenols, Aloe-emodin, polysaccharides, resistanol, aloetic acid, Aloin, vitamins, lignins, aloeride, sterols, antranol and saponin. Free Anthroquinones like emodin and aloin (a bitter-tasting yellow compound) are powerful antimicrobial agents<sup>22</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>23</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 to the cell membrane of bacteria resulting in bacterial lysis and death<sup>8,24</sup>. All these factors would explain why the Aloe vera showed the lowest OD in direct contact test and the largest inhibitory zone in Agar diffusion test.

Our findings were in accordance with Sean et al. It was found that Aloe vera has antibacterial activity against SM using inhibitory zone test<sup>25</sup>.

As per agar diffusion test; Aloe vera pure showed less antibacterial activity than Al. toothpaste. This may be due to other agents in the toothpaste that can promote a moderate antiplaque effect. While fluoride pure showed higher percentage of antibacterial activity on SM and lesser percentage on LB than Fl.toothpaste. This may be attributed to additional action of one of the content of the toothpaste. Chlorhexidine pure material and chl. toothpaste, both showed equal antibacterial activity against SM and LB.

## VI. Conclusion:

Al. toothpaste has a potential antiplaque activity compared to chl. And fl. toothpaste in accordance to its antibacterial activity against SM and LB<sup>14,26</sup>.

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