

## INVESTIGATION OF ALOE-VERA TOOTH GEL CONTAINING ACTIVE SALT AND ALUM BY TIME KILL TEST

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### ABSTRACT

The aim of the present study was to investigate efficacy and performance of Aloevera tooth gel against selected bacteria and fungi. This test method measures the changes of a population of Aerobic microorganism within a specified time period, when tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* which were selected as a test micro-organism against which Aloe-vera tooth gel was tested. It included the evaluation of dentifrices by antimicrobial testing.

This investigation showed that newly formulated tooth gel has shown 99.99% reduction of test bacteria viz *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* in 30 sec, 60 sec and 2 minutes, Bright Tooth Paste has shown >99.99% reduction of test bacteria *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* in 30 sec, 60 sec and 2 minutes, Bright Tooth Paste has shown no reduction of *Pseudomonas aeruginosa* in 30 sec, 60 sec and 2 minutes, when analysed as per ASTM E 2315 – 03 (Re. 2008) Method.

**Key words :** Aloe gel dentifrice, *Staphylococcus aureus*, *Streptococcus mutans*, *Candida albicans*, *Escherichia Coli*, *Pseudomonas aeruginosa*.

### Introduction :-

Dental Caries, Cavities and other oral diseases are the most common diseases worldwide including both developed and developing countries affecting peoples of all ages and sex. These diseases are caused by a mixture of micro-organism and food debris. Specific types of acid producing bacteria like *Streptococcus mutans* colonize the dental surface structure in the presence of fermentable carbohydrates, for example sucrose, fructose and glucose. Also other microflora are also associated with it. Teeth provide a unique environment for bacterial colonization, since unlike other parts of the mouth they are non-shedding inorganic structure retentive areas.

Aloe-vera is a stemless or very short succulent, cactus-like plant that actually is part of the lily family, growing to 60-100 cm (24-39 inches) tall, spreading by off sets. There are more than 300 varieties of the Aloe plant but the *Aloe-barbadensis* variety exhibits the best medicinal properties. It has been suggested for a wide variety of ailments but its use in dentistry is limited.

Literature survey showed that though Aloe-vera gel were studied and can be used for minor burns, skin abrasions and moisturizing properties but comparatively less work has been reported on its use in dentistry. In the view of this, the present study the Aloe tooth

gel was prepared by incorporating Aloe-vera along with active salt and Alum in it and then the formulation was screened for time kill test.

Time Kill Test is a basic microbiology method of assessment of Antimicrobial activity of an Anti-Microbial test material or Disinfectant. The kill time test is carried out to evaluate the microbial reduction by a disinfectant against selected bacteria or fungi. Various organism are studied depending upon the type of analysis and test materials. However most common organism tested include : Staphylococcus aureus, Pseudomonas aeruginosa, E-coli etc.

The test product or a dilution of the test product is brought into contact with a known population on of micro-organisms approximately  $10^6$  CFU/ml specified period of time at a specified temperature. At selected time points including zero time aliquots are removed and placed into a neutralizer blank. Dilutions of the neutralizer are made and selected dilutions plated onto agar. Colonies are enumerated.

The percentage or log reduction or both form either on initial microbial population, or test blank is calculated. The amount of sample required is 100 ml per lot per organism.

### **Materials and Methods :**

For this purpose following materials were requested to different companies for samples are ;

1. Aloe-vera extract – kingvish Company, Mumbai
2. Precipitated silica (ABSIL – Madhu silica Pvt. Ltd.Gujrat)
3. Hydrated Silica (M-Fil – Madhu Silica Pvt. Ltd Gujrat)
4. Carraeingn gum (VTP-Sarin industries FMC Biopolymer Navi-Mumbai. )
5. Clolours – Neelikon food Dyes and chemicals Ltd. Mumbai.

All the reagents used were of analytical grade. After collecting all the Samples, Aloe-vera tooth gel were formulated by incorporating Aloe-vera, active salt and alum as are active ingredients, to checkout the effectiveness against different micro-organisms with in a specified period of time.

### **EXPERIMENTAL WORK :**

Finalized base formulation for gel tooth paste with aloe – vera extract , salt and alum as an active ingredients with other common ingredients of gel dentifrice.

**Table 1: Formulation of Aloe-Vera Gel containing dentifrice**

S. N.	Ingredients	% (w/w)
1	Sorbitol	65.00
2	VTP Gum ( Carrageenan gum)	0.55
3	F - Sil 100 ( Abrasive Silica )	13.5
4	M - Fil 100 ( Hydrated Silica )	6.5
5	Poly ethylene glycol 400	0.5
6	Sodium Saccharin	0.3
7	Sodium fluoride	0.22
8	Sodium lauryl sulphate	2.5
9	Methyl Paraben	0.1
10	Propyl Paraben	0.01
11	Colour	0.1
12	Flavour	0.5
13	Water	0.92
14	Aloe – vera gel	9.0
15	Sodium salt	0.2
16	Alum	0.1

### Preparation of Aloe Tooth gel :

In order to optimize the concentration of gelling agent to achieve proper consistency of the gel formulations were prepared with different gel formers, Carboxy methylcellulose sodium, Carbomer 934, HPMC and different concentration of viscosity enhancer vis. 1.0, 2.0, 3.0 and 4.0 % were tried and finally gel that showed good spreadability and consistency was selected.

Weigh all the ingredients. Take sufficient quantity of water . Add sodium saccharin, Sodium fluoride , preservatives , colour, Sodium salt, alum in it . Mix it properly. Then warm the water up to 45 – 50<sup>o</sup>c . Then at this temperature add VTP gum. Then add sorbitol, Poly-ethylene glycol. Then add both silica powder i.e. abrasive silica & hydrated silica to it. Mix it 15 minutes. The add Aloe – vera gel & flavour to it and mix it 10 -15 minutes properly. Lastly add sodium lauryl sulphate to the formulation by making a slurry. Slight amount of foam will generate. Remove air by vacuum.

Finalized base formulation for gel tooth paste with aloe – vera extract , salt and alum as an active ingredients with other common ingredients of gel dentifrice.

**Antimicrobial Analysis of Aloe Tooth Gel by Time Kill Test :****Name of Test :** Time Kill Test**Test Standard ;** ASTM E 2315 – 03 (Re 2008)**Test Inoculum :**

1. Staphylococcus aureus ATCC 6538
2. Streptococcus mutans ATCC 25175
3. Escherichia coli ATCC 10535
4. Pseudomonas aeruginosa ATCC 9027
5. Candida albicans ATCC 10231

**Test Conditions :**

Test Product	: 1:2 dilution
Diluent / Neutraliser	: DE broth
Contact Time	: 30 seconds, 60 seconds and 2 minutes
Contact Temperature	: Room Temperature
Media and Reagent	: Soyabean-casein digest agar, plates incubated at 37°C; Sabourauds Dextrose agar, incubated at 28°C

**Procedure :**

1:2 dilution product was inoculated with test organisms bacteria / fungi individually (approximately 10<sup>6</sup> CFU /ml). After the specified exposure time of 30 seconds, 60 seconds and 2 minutes, surviving microorganisms were recovered by drawing an aliquot, neutralizing it and performing Standard Pour Plate Technique, Culture count was ascertained by dilution Blank. Adequate Validation of Neutralizing agent was also carried. Test was carried out in duplicate and average count was taken as CFU/ml.



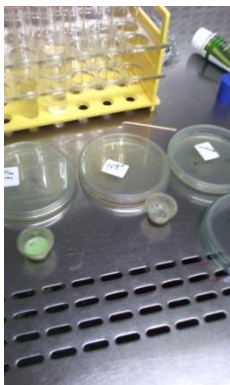
Sample Measurement  
Tube Vortex Shaker



Remove Sample with Micropipette



Mixing Sample in



Preparation of Plates



Inoculation of Microbial Plate



Spiral Plater

#### Neutralizer Validation :

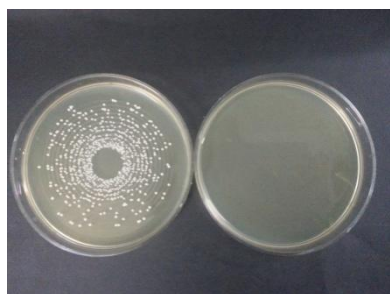
Validation Test		
Test Organism	Viable count in Saline (CFU/ml)	Viable Count in Neutraliser
Staphylococcus aureus	78	80
Streptococcus mutans	45	46
Escherichia	53	52
Pseudomonas aeruginosa	80	78
Candida albicans	32	30

**Results :**

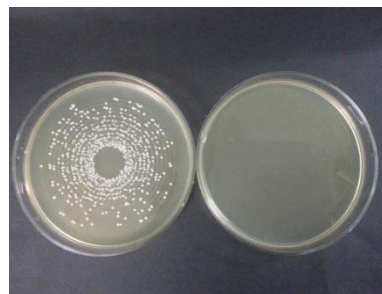
Sample Identification	Test Organism	Exposure Time	Count of Test Organism			Antimicrobial Activity	
			Initial Count	After Exposure		Log Reduction	Percentage Reduction
				CFU/ml	Log		
Aloevera Gel Tooth Paste	Staph. Aureus	30 Sec	<b>1.45x10<sup>5</sup></b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ms	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.16</b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
Bright Tooth Paste		30 Sec	<b>1.41x10<sup>5</sup></b>	<10	<1	<b>&gt;4.14</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ms	<10	<1	<b>&gt;4.14</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.14</b>	<10	<1	<b>&gt;4.14</b>	<b>&gt;99.99</b>
Aloevera Gel Tooth Paste	Streptococcus Mutans	30 Sec	<b>1.35x10<sup>5</sup></b>	9.50x10 <sup>4</sup>	4.97	<b>0.16</b>	<b>29.62</b>
		60 Sec	CFU/ml	1.00x10 <sup>4</sup>	4.0	<b>1.13</b>	<b>92.59</b>
		2 Min	<b>L = 5.13</b>	<10	<1	<b>&gt;4.13</b>	<b>&gt;99.99</b>
Bright Tooth Paste		30 Sec	<b>3.50x10<sup>5</sup></b>	<10	<1	<b>&gt;4.54</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.54</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.54</b>	<10	<1	<b>&gt;4.54</b>	<b>&gt;99.99</b>
Aloevera Gel Tooth Paste	Escherichia Coli	30 Sec	<b>1.08x10<sup>5</sup></b>	<10	<1	<b>&gt;4.16</b>	<b>29.62</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.16</b>	<b>92.59</b>
		2 Min	<b>L = 5.03</b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
Bright Tooth Paste		30 Sec	<b>1.10x10<sup>5</sup></b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.04</b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
Aloevera Gel Tooth Paste	Pseudomonas aeruginosa	30 Sec	<b>1.09x10<sup>5</sup></b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.03</b>	<10	<1	<b>&gt;4.16</b>	<b>&gt;99.99</b>
Bright Tooth Paste		30 Sec	<b>1.15x10<sup>5</sup></b>	>1.00x10 <sup>6</sup>	>6.0	<b>&lt; 1</b>	<b>0.00</b>
		60 Sec	CFU/ml	>1.00x10 <sup>6</sup>	>6.0	<b>&lt; 1</b>	<b>0.00</b>
		2 Min	<b>L = 5.06</b>	>1.00x10 <sup>6</sup>	>6.0	<b>&lt; 1</b>	<b>0.00</b>
Aloevera Gel Tooth Paste	Candida albicans	30 Sec	<b>4.60x10<sup>5</sup></b>	<10	<1	<b>&gt;4.66</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.66</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.66</b>	<10	<1	<b>&gt;4.66</b>	<b>&gt;99.99</b>
Bright Tooth Paste		30 Sec	<b>2.60x10<sup>5</sup></b>	<10	<1	<b>&gt;4.41</b>	<b>&gt;99.99</b>
		60 Sec	CFU/ml	<10	<1	<b>&gt;4.41</b>	<b>&gt;99.99</b>
		2 Min	<b>L = 5.41</b>	<10	<1	<b>&gt;4.41</b>	<b>&gt;99.99</b>

Percentage Reduction of Microorganism = 100 (Initial – After Exposure)/ Initial

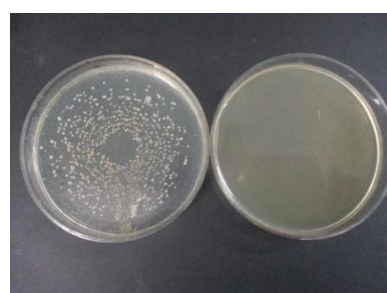
Log Reduction = Log Initial – Log after Exposure



Candida\_Aloe\_2\_mins



Candida\_Bright\_L\_control and\_right\_2\_min

S\_mutans\_Aloe\_L\_control\_  
and\_right\_2\_mi

S\_mutans\_Bright\_L\_control\_ and\_right\_2\_min

### Conclusion :

Test Product named as Alovera Tooth Gel has shown >99.99% reduction of test bacteria viz Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Pseudomonas aeruginosa and Candida albicans in 30 sec, 60 sec and 2 minutes, Bright Tooth Paste has shown >99.99% reduction of test bacteria viz Staphylococcus aureus, Streptococcus mutans, Escherichia coli in 30 sec, 60 sec and 2 minutes, and Bright Tooth Paste has shown no reduction of Pseudomonas aeruginosa in 30 sec, 60 sec and 2 minutes when analysed as per ASTM E 2315 – 03 (Re. 2008) Method.

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