

## Microbial Battlefront: Exploring the In-vitro Inhibitory Effects of Homeopathic Medicines on Streptococcus mutans

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### ABSTRACT

**Background:** Dental caries is known to be one of the commonest bacterial infectious disease in humans<sup>1</sup>. Streptococcus Mutans is the leading cause of dental caries worldwide. An ecological imbalance within the physiological processes amongst the minerals of tooth and oral microbial biofilms gives rise to dental caries.

### Materials and Methods

Samples of Streptococcus Mutans ATCC® 25175™\* procured from Nulife Consultants and Distributors Pvt. Ltd (An ISO 9001:2008 Certified Company). 30 pits of the organisms were incubated for growth in the Mitis Salivaris Agar culture media in 10 culture plates. 10 homeopathic medicines known to cure caries procured from Reckeweg company in 6, 30, and 200 potencies, 3 sets of each potencies were incubated for accuracy to observe the inhibition zone in the study samples.

### Results

10 homeopathic medicines namely Calc carb, Calc flour, Calc phos, Flour. ac., Hekla lava, Kreosote, Merc. sol., Mezerium, Plantago and Staphysagria; all medicines in 6, 30 and 200 potencies were applied to inhibit the colony growth by zone inhibition technique. Hekla lava has shown the best results by inhibiting completely in 6 and 200 potencies and Staphysagria remained most consistent by giving complete inhibition in 30 potency and 8 mm inhibition in 6 potency and 1 cm inhibition in 200 potency, Fluoric acid 200 also came up with complete inhibition. Almost nil/ no inhibition was found in Merc sol, Mezerium and Plantago in 6 potency and Calcarea flour in 30 potency.

**Conclusion(s)** – results are consistent with the fact that –

1. There is a definitive effect of homoeopathic potencies upon the cariogenic bacteria *Streptococcus mutans* in-vitro.
2. The medicines simply do not obey the concept that ‘more the material dose better the effect is’ since medicines found to act in higher potencies and not at all in lower potencies and vice-versa.
3. Whether the results are material or even there is a dynamic aspect operating in microbiological population in-vitro is now a valid question.

**Keywords** – Homoeopathic medicines, *Streptococcus mutans*, Mitis Salivaris Agar Media, In-vitro study.

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## Introduction

Dental caries is known to be one of the commonest bacterial infectious diseases in humans<sup>2</sup>, very much prevalent in industrialized countries; although the condition is not life-threatening, the cost involved to manage and treat the disease is substantial. Since *Streptococcus Mutans* is a cariogenic bacteria, understanding the bacteriology of oral microbiota is crucial to understand the disease and devising treatment strategies. *Streptococcus Mutans* is the major/leading cause of dental caries worldwide and understanding its pathogenesis and various related aspects is crucial to the understanding of the disease in greater extent. The research in this topic has been wide and still going on.

Caries is the result of complex interactions in between bacteria that produce acid, some chemicals that bacteria can metabolize and host factors like teeth and saliva.<sup>3</sup> Caries can develop due to result of imbalance of ecology of the physiological equilibrium between tooth minerals and oral microbial films.<sup>4,5</sup>. Bacteria live on teeth in micro-colonies that are

encapsulated in an organic matrix of polysaccharides, proteins, and DNA secreted by the cells, which provides protection from desiccation, host defences and predators and provides enhanced resistance to antimicrobial agents<sup>6, 7</sup>. Tooth provides non-shedding surfaces and facilitates piling up of huge no.s of micro-organisms and by-products within a biofilm matrix<sup>8, 9</sup>.

The pathogenesis of caries remain similar for all variants. The micro-organisms like *S. Mutans*, *S. Sobrinus* and *Lactobacilli* which usually habitat in buccal cavity biofilm generate organic acids weak in strength by fermenting carbohydrates. The acid produced by the endogenous bacteria effects fall of pH values to a level that can induce demineralization of teeth surfaces<sup>10, 11, 12</sup>. Given the continuation of demineralization of phosphate, carbonate and calcium the affected teeth shall develop cavity<sup>13, 14</sup>. The process of demineralization can be reverted in the early stages by intake of calcium, phosphate and fluoride. Fluoride facilitates calcium and phosphate diffusion into teeth that can remineralize the teeth. The newly built crystalline surface is now more resistant to attack. The enzymes from bacteria may help formation of caries<sup>15</sup>. The continuing process of demineralization and remineralization decides whether the lesion progresses, reverses or stalled. The demineralization and remineralization occurs on daily basis, several times in a day in most of the people. As time passes these processes decide whether the lesion shall get a repair and reversal/maintain a steady state<sup>16</sup>. When the pH of the biofilm is restored by saliva, remineralization occurs; the saliva acts like a buffer. New areas of remineralization are less porous and contain more fluoride as a result of calcium and phosphates mobilized from saliva.

When the dental biofilms goes on maturing and remain in place for long time, caries develop. Where a cavity forms, that helps the plaque organisms to adapt to a low pH gradually<sup>17</sup>. Cavity protects the biofilm and if the area in not cleansed the caries shall continue<sup>18</sup>. Initially the lesions start as white spot lesions, white spots are nothing but sub-surface demineralized area underlying the dental plaque. Caries over the root are similar to enamel caries, the difference in between these two is the root caries can cause softening of the teeth and allow bacteria to gain access to the tissue in early phases of the lesion<sup>19, 20</sup>.

Lots of studies have already been done on *Streptococcus mutans* since its discovery. In-vitro studies on *S mutans* are many and In-vitro studies in homoeopathy with *Streptococcus mutans* were very few. Although few in no. some very useful results that may substantiate the

efficacy of homoeopathic medicines in mother tinctures and potencies are there to show that it has some positive effect in inhibiting the growth of the bacteria.

In the year 1999 A G Jagtap and S G Karkera tested the aqueous extract of Terminalia Chebula to find out the ability of the solution to inhibit the growth and other physiological functions of Streptococcus Mutans<sup>21</sup>. The extract could inhibit growth, sucrose led adherence and glucan induced aggregation. They concluded with stating that the solution can inhibit glycolysis of salivary bacteria 90 minutes post-rinsing<sup>21</sup>.

In an article published in 2019 Yalgi and others took a study to find out antibacterial activity of four homoeopathic medicines namely Hypericum perforatum, Arnica montana, Echinacea angustifolia and Calendula officinalis against 02 strains of bacteria i.e., Streptococcus mutans and Enterococcus faecalis in mother tinctures. Hence it was concluded that all 04 medicines shown good antibiotic activity against both strains of bacteria<sup>22</sup>.

Basu Anushree and others did a study comparing antimicrobial efficacy of Triclosan-containing herbal against homoeopathic toothpaste in an in-vitro study. Their results showed formulation of kreosotum, plantago major and calendula were significantly effective against Streptococcus Mutans<sup>23</sup>.

Sajankumar et al assessed the efficacy of Azadirachta Indica (Neem) and Acacia Nilotica (Babool) upon Streptococcus Mutans in an in-vitro study with a chlorhexidine control. They studied 5%, 10% and 50% of Neem and Babool aqueous extract with a comparable solution of 0.2% chlorhexidine. The results elicited was 5% neem was significantly better than comparable strengths of Babool and chlorhexidine<sup>24</sup>. This is to mention here that Neem/Azadirachta Indica is used in homoeopathy as a medicine.

### **AIMS & OBJECTIVES:**

**AIMS:** To provide some biologic evidence of the effect of some homoeopathic drugs known to be effective for dental caries.

**OBJECTIVES:** To consolidate some evidence that may provide direct proof of effect of the commonest potentized homoeopathic drugs for dental caries on Streptococcus Mutans.

### **Results**

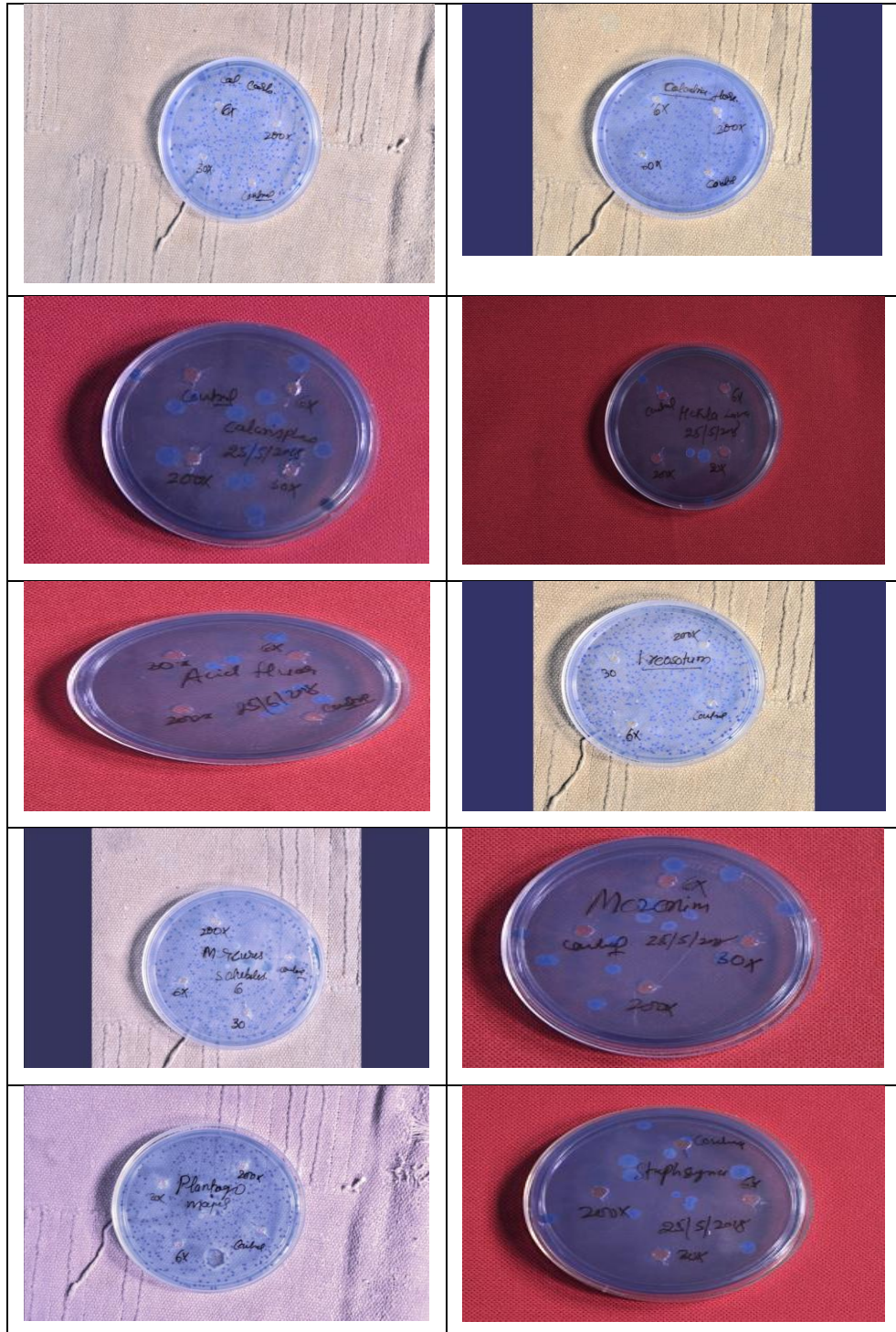
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This study was done with ten medicines (Calc carb., Calc flour., Calc phos., Flour. ac., Hekla lava., Kreosote, Merc. sol., Mezerium., Plantago, Staphysagria) mentioned in Garth Boericke's Repertory in three different potencies namely 6, 30, 200 besides a negative control and the results are summarized in Table 1. Very minimum (almost nil) i.e., 1 mm inhibition was observed in Calcarea fl 30 potency and Merc sol, Mezerium and Plantago in 6 potencies each and highest i.e., complete inhibition/ no growth of organism was observed in Fl ac 200, Hekla lava 6 and 200 potencies and Staphysagria 30 potency with three different ranges in between. (Refer Table 2). Inhibition in the range of 8 mm – 1 cm was observed in Fl. ac 30, Kreosote 6, 30; Mezerium 200 and Staphysagria 6 and 200. Inhibition in the range of 4 – 6 mm was observed in Calcarea ph 6, Fl. ac 6, Kreosote 200, Mezerium 30 and Plantago 30 potencies. Inhibition in the range of 2 – 3 mm was found in Calcarea c 30, 200; Calcarea fl. 6, 200; Calcarea ph 30, 200; Hekla lava 30; Merc sol 30 & 200 potencies and Plantago 200 potency. Hekla lava has shown the best results by inhibiting completely in 6 and 200 potencies and Staphysagria remained most consistent by giving complete inhibition in 30 potency and 8 mm inhibition in 6 potency and 1 cm inhibition in 200 potency. When complete inhibition and very high range of inhibition is considered 4 drugs namely Hekla lava, Staphysagria, Fluoric acid and Kreosote came up as the best to better performers.

Name of medicine	Control	6	30	200
Calcarea Carb	nil	2 mm	3 mm	2 mm
Calcarea flour	nil	3 mm	1 mm	2 mm
Calcarea phos	nil	4 mm	3 mm	2 mm
Flouric acid	nil	5 mm	<b>1 cm</b>	<b>No growth</b>
Hekla Lava	nil	<b>No growth</b>	3 mm	<b>No growth</b>
Kreosote	nil	<b>1 cm</b>	8 mm	4 mm
Merc Sol	nil	1 mm	2 mm	3 mm
Mezerium	nil	1 mm	6 mm	<b>1 cm</b>
Plantago	nil	1 mm	4 mm	2 mm
Staphysagria	nil	8 mm	<b>No growth</b>	<b>1 cm</b>





1 mm	Calcarea fl. 30 Mezerium 6 M sol. 6 Plantago 6
2-3 mm	Cal c. 30, 200 Cal fl. 6, 200 Cal ph. 30, 200 Hekla 1 30 M sol 30, 200 Plantago 200
4-6 mm	Cal ph 6 Fl ac 6 Kreosote 200 Mez 30 Plantago 30
8 mm – 1 cm	Fl ac 30 Kreosote 6, 30 Mezerium 200 Staphysagria 6, 200
Complete inhibition/no growth of organism	Fl. ac. 200 Hekla lava 6, 200 Staphysagria 30

## Discussion

Before the concept of nanoparticles came into being homoeopathic drugs used to be thought as not having any particle of the original drug substance of the drug beyond 12c or 24x potencies. Earlier the idea of any substance in homoeopathic medicines beyond 12 C and 24 X potencies were questioned of not having original molecule of the substance. But some studies have already been done in potencies beyond that showing clinical and laboratorial evidence of the potencies beyond the Avogadro's no. limit to be effective. But solid evidence

of the original substance in nano-form was only possible when the concept of nanoparticles came into effect and the study by various authoritative institutes/researchers with laboratory evidence of the higher potencies having nanoparticles of the original substance.<sup>25</sup> Enough evidence now available to produce for the presence of original molecule of the drug substance in diluted and potentized drugs<sup>26</sup>.

In the following analysis it has been found that in-vitro application of homoeopathic drugs in higher potencies have been able to inhibit the growth of *Streptococcus mutans* when grown in culture media.

This study aimed at inhibition of culture grown colonies of *Streptococcus mutans* in-vitro in different potencies of homoeopathic medicines that are known to cure/treat dental caries, the potencies being 6, 30 and 200. Two potencies used in the study were beyond the accepted levels of Avogadro's number to have any demonstrable molecules of the original substance. 10 medicines in three different potencies were studied to observe the in-vitro effects on inhibition of culture grown *Streptococcus mutans* colonies. The results were found to have variable effects of different medicines and across various potencies. There is a significant difference in inter-medicinal and inter-potency effects on colonies of bacteria; somewhere the lower potency had a better inhibitory effect when compared to higher and vice-versa. Some medicines had shown a better inhibitory effect across all potencies, one medicine in two out of three potencies. Some medicines had effects that can be compared to negative control and leveled as negligible/no effect. One important finding is that all potencies albeit of different medicines have shown complete inhibition i.e., 6, 30 and 200 potencies.

Very minimal or negligible inhibition were found in *Calcarea flour* 30, *Mezerium* 6, *Merc sol* 6 and *Plantago* 6. 2 – 3 mm inhibition were found in *Cal c* 30, 200; *Cal fl* 6, 200; *Cal ph* 30, 200 *Hekla lava* 30; *Merc sol* 30, 200 and *Plantago* 200. Colony inhibition in the range of 4 – 6 mm were observed in *Cal ph* 6, *Fl ac* 6, *Kreosote* 200, *Mezerium* 30 and *Plantago* 30. Inhibition in the range of 8 mm – 1 cm were found in the *Fl ac* 30, *Kreos* 6, 30; *Mezerium* 200; *Staphysagria* 6, 200. The highest ever inhibition/ complete inhibition were found in *Fl ac*, *Hekla l* 6, 200 and *Staphysagria* 30.

*Hekla l* 6 & 200 had a complete inhibitory effect upon the bacterial colonies, only medicine with 02 potencies having complete inhibition. One interesting fact here is that the 02



potencies are in the two extremes of the potency range used in this study and also 6 potency is the last mile potency to have molecules as regarded the Avogadro no. limit.

Staphysagria is the only medicine which has fared best when all three potencies taken into consideration. Staphysagria 30 had the complete inhibition and 6 and 200 potencies had 8 mm – 1 cm inhibitory range.

Fl. ac. had shown a unique pattern of inhibition like 6 potency had inhibition in the range of 4 – 6 mm; 30 potency had inhibition in the range of 8 mm – 1 cm inhibition and 200 potency had complete inhibition. There is a pattern that with rising potency the medicine had a clear empowered inhibitory effect. 6<sup>th</sup> potency had lowest and 200<sup>th</sup> potency had the complete inhibition and 30 potency had inhibition in-between.

Kreosote had given result again in 2<sup>nd</sup> and 3<sup>rd</sup> best inhibition range in all potencies in a reverse order of potencies, 200 giving inhibition in the range of 4 – 6 mm and 6, 30 potencies in the range of 8mm – 1 cm. All 3 potencies of Kreosote were in the the 2<sup>nd</sup> and 3<sup>rd</sup> higher range.

2 potencies of Mezerium had given 2<sup>nd</sup>, 3<sup>rd</sup> highest inhibition in the range of 4 – 6 mm and 8 mm – 1 cm; again in the increasing order of potency. Mezerium 6 had only negligible inhibition in the range of 1 mm only.

All potencies of Plantago have shown different levels of inhibition, in a series of 6, 200 and 30 potency in 1 mm, 2 - 3 mm and in 4 - 6 mm range.

One interesting finding is that all potencies of Merc sol and Calcarea group except only Calcarea ph 6 had shown very minimal inhibition in the lowest two ranges (1 mm and 2-3 mm ranges).

In the lowest range i.e., 1 mm inhibition out of 4 potencies of different medicines 3 were 6 potencies and 1 was 30 potency.

In the 2-3 mm range 200 potency had shown maximum presence i.e., 5 times, 30 potency 4 times and 6 potency once only.

In the 4 – 6 mm range 6 and 30 potencies has shown equal score i.e., 2 times and 200 potency only once.

When the two highest range of inhibition is considered together 200 potency has 4 times presence, 30 and 6 potencies has 3 times each presence.

When the highest range is considered alone 200 potency has shown result twice, while the 6 and 30 potencies once each.

When considered no. of times in the highest range and lowest range 200 potency has been present twice out of four in the highest range and 6 potency thrice out of four in lowest range. One of the similarities in highest and lowest range of inhibition is no. of potencies found to influence in each category are four. When four medicines were there in the minimum inhibition range, no. of medicines in the highest range are three. Clearly there is a trend that higher potencies have demonstrated more inhibitory effect on the culture-grown colonies.

**Conclusion(s)** – results are consistent with the fact that –

1. There is a definitive effect of homoeopathic potencies upon the cariogenic bacteria *Streptococcus Mutans* in-vitro.
2. The medicines simply do not obey the concept that ‘more the material dose better the effect is’ since medicines found to act in higher potencies and not at all in lower potencies and vice-versa.
3. Whether the results are material or even there is a dynamic aspect operating in microbiological population in-vitro is now a valid question.

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