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Comparison of the Inhibitory Effect of Different Concentrations of Theobromine Paste on the Growth of *Streptococcus mutans* as Measured in the Zone of Inhibition

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Abstract: **Dental caries remains as a public health concern as it is one of the most common diseases that affect children and adult worldwide. *Streptococcus mutans*, which thrives in the dental plaque biofilm, is considered as the primary contributor to the progression of dental caries. Theobromine is used as a bioactive component since it is known to contain antioxidant, antiviral, and/or antimicrobial property. Theobromine has gained attention as a material for development in dentistry because of its potential role in enamel strengthening and caries prevention. This study aimed to evaluate the effects of different concentrations (0.02%, 0.04%, 0.06%, 0.08%, and 1.00%) of theobromine on the growth of *S. mutans*. All the different concentrations (0.02%, 0.04%, 0.06%, 0.08%, and 1.00%) of ThP reflected a very active inhibitory inference against *S. mutans* comparing to the control (0.00%), which only showed an active inhibitory effect. It showed that theobromine can be utilized as a bioactive component to the development of alternative or adjunctive materials that maybe used oral health improvement. Further studies may still be done to find out the best concentration for the formulation of theobromine paste. It is crucial to characterize theobromine's interactions with various dental materials to fully understand its compatibility, stability, and efficacy in different formulations.**

Keywords: **theobromine, dental caries, *Streptococcus mutans*, theobromine paste.**

I. INTRODUCTION

Dental caries is one of the most common diseases that affect billions of children and adults worldwide. [1] Tooth decay occurs over time as a result of a complex interaction between acid-producing bacteria, fermentable carbohydrates, and host factors like teeth and saliva. [2] The formation of dental plaque, a critical factor in the development of dental caries and periodontal disease, can be initiated by various strains of oral streptococci. Among the primary etiological agents, the α-hemolytic streptococci *Streptococcus mutans* and *Streptococcus sobrinus* are considered highly cariogenic. [3] The primary virulence factors contributing to dental caries include the synthesis of water-insoluble glucan from sucrose, acidogenicity, and acid tolerance. Among these factors, bacterial activity is suggested to have the most significant influence on the prevalence and incidence of dental caries. [4]

S. mutans is a Gram-positive, facultatively anaerobic bacterium and a key contributor to dental caries. As a member of the mutans streptococci group, it thrives in dental plaque biofilms, metabolizing sucrose into organic acids through fermentation. Its acidogenicity and acid tolerance enable survival in low pH conditions, leading to enamel demineralization and caries progression. Since its identification by Clarke in 1924 [5], research has focused on its cariogenic mechanisms, aiding the development of preventive strategies such as antimicrobials, vaccines, probiotics, and biofilm disruptors to control dental caries. [6]

Various sectors, including healthcare, government, pharmaceutical, and commercial industries, have been actively developing strategies to reduce the incidence of dental caries. Preventive measures such as fluoride supplementation, oral hygiene education, and the development of caries vaccines are among the approaches utilized to mitigate the disease. Given that dental caries remains a major public health concern, researchers worldwide continue to explore alternative methods to prevent its occurrence and progression. [7]

Theobromine (3,7-dimethylxanthine) is one of the main component of cacao beans. [8] It is used widely used for its antioxidant, antiviral, and/or antimicrobial property. [9] It has been studied for its pharmacological benefits, including vasodilation, diuretic effects, and antioxidant properties. In dentistry, theobromine has gained attention for its potential role in enamel strengthening and caries prevention. [10]

This study aimed to evaluate the effects of different concentrations of theobromine on the growth dynamics of *S. mutans*. The findings may contribute to the development of alternative or adjunctive therapeutic strategies for oral health management.

II. MATERIALS AND METHODS

A. Material Preparation

Six different concentrations of paste containing theobromine (0.0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%) were prepared and tested against *Streptococcus mutans* using the agar cup/ agar-well diffusion method. Twenty eight wells for each concentration of theobromine paste (ThP).

B. ThP Preparation

ThP was made by mixing 1 cup of sifted sodium bicarbonate ($NaHCO_3$), 16 tablespoons of glycerine and 1/8 cup of distilled water until homogenous with a consistency of toothpaste. Theobromine was ground and weighed before addition to the mixture. Different amounts of theobromine were added to the paste to make the different concentrations of 0.2%, 0.4%, 0.6%, 0.8%, 1.0%. The control did not contain any theobromine material.

C. Agar Well Preparation

S. mutans ATCC 25175 in lyophilized form were propagated in brain heart infusion broth following the manufacturer's instructions. This was incubated for 48 hours at 37°C in anaerobic condition. Bacterial cell suspensions were diluted to obtain cell samples containing 1-2 × 10^8 CFU/ml (0.5 McFarland standard). [11] Streaking method using a sterile triangular rod was used to inoculate the sample. The streaked agar was then allowed to stand for a minimum of 5 minutes before placing the agar wells. Seven agar plates were allotted for the control and each concentration of ThP. To make the agar wells, the plate was divided into quadrants. Four small circles 12 mm in diameter, properly spaced at the center of each quandrant, were made in each agar plate. Properly sized and fabricated sterile aluminum tubes (diameter = 12mm) were used to punch each agar well in the agar. A total of 28 wells for each concentration of ThP were made. After the agars have been streaked and the wells made, the ThP was then loaded using sterile wire loops. Each agar well was filled up to the brim. All the plates were incubated at 37°C for 70 hours.

E. Measurement of the Zone of Inhibition (ZOI)

After incubation, for 70 hours, the zones of inhibition were measured using a Vernier caliper. The ZOI were measured through the center of the disc from top to bottom and were recorded. A reference table by Guevara (2005) was used to interpret the ZOI. [12]

III. RESULTS AND DISCUSSION

TABLE 1: Inhibitory Activity Concentration based on Guevara (2005)

ZOI Measurement (mm)	Inference
<10	Inactive
10-13	Partially Active
14-19	Active
>19	Very Active

Shown on Table 1 are the ZOI measurements in millimetres and the interpretation of the inferences based on Guevara (2005). A score of >19 would mean a very active inhibitory activity, 14-19 as very active, and 10-13 as partially active. A

measurement of <10 would render the inhibitory effect inactive. This interpretation was used to analyze the inhibitory effect of the prepared ThP on *S. mutans*.

TABLE 2: Inferred Inhibitory Activity on *S. mutans* per ThP Concentration based on Guevara (2005)

ThP Concentration	Mean ZOI (mm)	Inference (Inhibitory Activity)
0.00%	18.25	Active
0.02%	19.86	Very Active
0.04%	20.93	Very Active
0.06%	19.75	Very Active
0.08%	21.04	Very Active
1.00%	20.05	Very Active

Table 2 shows the inhibitory activity of the different concentrations of ThP against *S. mutans*. All the different concentrations (0.02%, 0.04%, 0.06%, 0.08%, and 1.00%) of ThP reflected a very active inhibitory inference against *S. mutans* comparing to the control (0.00%), which only showed an active inhibitory effect, without the theobromine. The most active concentration was reflected on the mean score of 0.08% ThP concentration. It was followed by 0.04% (20.93), 1.00% (20.05), 0.02% (19.86), and 0.06% (19.75). And the least ZOI was observed for the control sample (18.25) containing 0.00%.

In a study by Lakshmi et al. [8], they have also found that theobromine showed greater ZOI results against *S. mutans* compared to other bioactive ingredients in toothpaste. Similarly, in a study by Cevallos Gonzales et al. [13], they found that biofilm formation of *S. mutans* was decreased in their glass ionomer samples infused with theobromine. Additionally, Demir et al. [14], investigated the antimicrobial effects of different natural ingredients in toothpaste and found the theobromine presented the best antimicrobial effect against *S. mutans*.

IV. CONCLUSION

As observed in the data of the study, the addition of theobromine in the paste created an antimicrobial inhibitory effect against *S. mutans* when compared to the control. When theobromine was added to the mixture, all the different concentrations' inhibitory effect became very active. It showed that theobromine can be utilized as a bioactive component to the development of alternative or adjunctive materials that maybe used oral health improvement. Among the different concentrations of ThP, 0.08% showed a promising antimicrobial effect against *S. mutans* but further studies may still be done to find out the best concentration for the formulation of ThP. It is crucial to characterize theobromine's interactions with various dental materials to fully understand its compatibility, stability, and efficacy in different formulations. Investigating its mechanism of action and long-term effects will contribute to the development of safe and effective dental products. Future studies should also explore potential synergies between theobromine and other antimicrobial agents to enhance its efficacy in preventing oral infections.

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