# Project Completion Report

## "*Clinical Investigation to examine the effect of hygiene with tooth powder and toothpaste on dental plaque and gingivitis in conjunction with the analysis of oral bacteria*".

**Project No. CRA-2401**

**COLLABORATIVE RESEARCH AGREEMENT 2024**

Amongst

**School of Life Sciences, Mysuru, JSS Academy of Higher Education & Research, Mysuru**

&

**Chegus Ventures Pvt. Ltd., Bangalore**

&

**The Sadvaidyasala Pvt. Ltd., Nanjangud, Mysuru**


**Principle Investigator**

**Dr. D. Guru Kumar**

Assistant Professor

Division of Biochemistry

School of Life Sciences, Mysuru

JSS Academy of Higher Education & Research, Mysuru


**June 2024 - November 2024**

# JSS Academy of Higher Education & Research

**(Deemed to be University)**

## Sri Shivarathreeshwara Nagar, Mysuru - 570015

## CERTIFICATE FROM THE PRINCIPAL INVESTIGATOR'S

I declare that this Project report entitled **"Clinical Investigation to examine the effect of hygiene with tooth powder and toothpaste on dental plaque and gingivitis in conjunction with the analysis of oral bacteria"** has been submitted to JSS Academy of Higher Education & Research, Chegus Ventures Private Limited, Bangalore and The Sadvaidyasala Private Limited, Nanjangud, Mysuru during the period of June-November 2024 under our guidance and supervision.

**Date:**

**Signature of the Principal investigator**
**(Dr. D. Guru Kumar)**

**Signature of the Co-Principal investigator**
**(Dr. Nandlal B)**

**Signature of the Co-Principal investigator**
**(Dr. M.V.S.S.T. SubbaRao)**

# JSS Academy of Higher Education & Research

**(Deemed to be University)**

## Sri Shivarathreeshwara Nagar, Mysuru - 570 015

## CERTIFICATE FROM THE HEAD, SCHOOL OF LIFESCIENCES, MYSURU

This is to certify that the report titled **"Clinical Investigation to examine the effect of hygiene with tooth powder and toothpaste on dental plaque and gingivitis in conjunction with the analysis of oral bacteria"** has been successfully completed during the period of June-November 2024 at School of Life Sciences, Mysuru, JSS Academy of Higher Education & Research, Mysuru under the Supervision of Dr. D. Guru Kumar, Assistant Professor, Division of Biochemistry, School of Life Sciences, Mysuru, JSS AHER, Mysuru.

**Date:**

**Signature of the Head**

**(Dr. Raveesha K.A)**

# ACKNOWLEDGEMENT

The support and infrastructure facility provided by the School of Life Sciences, Mysuru, JSS Academy of Higher Education & Research (JSS AHER), Mysuru; JSS Dental College & Hospital, JSS AHER, Mysuru and Department of Biochemistry, JSS Medical College & Hospital, JSS AHER, Mysuru, India, are gratefully acknowledged. We also thank Chegus Ventures Pvt. Ltd., Bangalore, and The Sadvaidyasala Pvt. Ltd., Nanjangud, Mysuru, for their financial support through the Research Project under a Collaborative Research Agreement (2024). Additionally, the Principal Investigators (PIs) of the consultancy project express their gratitude to the authorities of JSS AHER for providing their constant support and infrastructure facilities that enabled the smooth completion of the project.

# Contents

## INTRODUCTION

Tooth powder is a type of pharmaceutical solid dosage form used to maintain cleanliness and polish teeth enamel, helping to prevent dental caries. Various dental care preparations can achieve this. Historically, the Romans used tooth powder to clean and whiten teeth, fix loose teeth, strengthen gums, and prevent toothaches. Their tooth powder was made from a range of substances, including bones, hooves, and horns of certain animals, as well as crab, oyster, and murex shells, and eggshells. These ingredients were ground into a fine powder, sometimes after being previously burnt. Some versions included honey, myrrh, salt, and hartshorn.

The use of powdered substances like charcoal, brick, and salt for cleaning teeth has been historically widespread in India, especially in rural areas. Mildly abrasive powders are used with a toothbrush to maintain oral hygiene. These abrasives, such as precipitated chalk, dicalcium phosphate dihydrate, calcium pyrophosphate, and alumina, are water-insoluble fine powders that mechanically scrape or abrade the enamel surface to remove plaque. Dental caries and periodontal diseases are among the most significant oral health challenges globally.

Epidemiological studies indicate that 60 to 90% of school children experience oral health issues, and periodontal disease is considered the 11th most prevalent disease worldwide. Despite the effectiveness of many commercially available chemical toothpaste formulations, there is a growing societal preference for natural compounds in oral health care. Therefore, it is essential to use tooth powder and evaluate its antimicrobial and antioxidant properties, as well as assess its effectiveness on the oral hygiene status of individuals.

### Review of Literature

### International status:

- Muhammad Khalil Khan et al in the year 2017 studied on Evaluating the Clinical Efficacy of Tooth Powder on Plaque-Induced Gingivitis: A Randomized Controlled Trial and concluded that tooth powder has been shown to be statistically superior to toothpastes in controlling dental plaque and gingivitis.

### National status:

- Sharath Asokan et al in the year 2019 studied the Effectiveness of a custom-made natural tooth powder on oral hygiene status of children and concluded that the newer custom-made natural tooth powder was found to have antimicrobial and antioxidant properties and found to be effective in oral hygiene maintenance. It could be an alternative to the commercially available natural tooth powder.
- Shilpa Santhosh et al in the year 2023 did a review on tooth powder and concluded that toothpowder has been shown to be statistically superior to toothpastes in controlling dental plaque and gingivitis. The impact of toothpowder in the healthcare system can’t be excluded.

- Kishor N. Shingnapure et al in the year 2024 studied on Herbal Tooth Powder Nature Secret For Oral Health and concluded that Maintaining oral hygiene through the use of herbal tooth powder is a reliable, safe, and cost-effective method.
- Rohit Sharma et al in the year 2023 studied on Herbal Tooth Powder and concluded that using herbal tooth powder is a dependable, safe, and affordable method to keep oral health. The study found that natural tooth powder is better and has fewer adverse effects than synthetic powder, which is worth highlighting in dentistry research.
- Chandrashekar Janakiram et al in the year 2018 studied on Comparison of Plaque Removal Efficacy of Tooth Powder and Toothpaste in Young Adults in India and concluded that There was no statistically significant difference in the plaque reducing potential of tooth powder and toothpaste in controlled conditions. It was also observed that the study participants preferred toothpaste over tooth powder due to its relative ease of use.

## AIMS & OBJECTIVES:

This pilot clinical study will compare the effect of twice daily oral hygiene with a randomly assigned test toothpowder and toothpaste on clinical parameters of dental plaque and gingivitis in conjunction with analysis of anaerobic dental plaque bacteria.

## MATERIALS AND METHODS

### MATERIALS:

1. Toothpaste 1: Commercially available fluoride paste
2. Toothpowder 2: Commercially available toothpowder [JSSTP00S1]
3. Toothpowder3: Commercially available toothpowder [JSSTP00S2]
4. Mouth mirror and periodontal probe, currete
5. 50 ml saliva collecting tube [Cat# 50041],
6. 15 ml tube [Cat# 50031]
7. 1.5ml Eppendorf tube to collect plaque samples [Cat# 500010]
8. Microbial culture plates [Cat# PW1132C]
9. Anaerobic jars [Cat# LEOO2A]
10. Sheep blood agar [Cat# MP1301]
11. Bacitracin [Cat# TC201]
12. Potassium Tellurite (1%) [Cat# FD052]
13. Mitis Salivarius agar base [Cat# M259]
14. Finnpipette F2 GLP kit 2.0 [Cat# 4700880]
15. Micropipette tips [Cat# 50041]
16. Micropipette tips 10µl [Cat# 21000]

17. Micropipette tips 200µl [Cat# 521014]
18. Micropipette tips 1000µl [Cat# 521016]

Plasticwares 5,6,7,15.16,17,18 was from Tarsons Products Pvt. Ltd, Reagents 8,9,10,11,12,13 were from HiMedia, India, Micropipette 14 was from Finnpipette.

**METHODS:**

The JSS Dental College & Hospital Institutional Ethics Committee after critically reviewing the research protocol during the review meeting held on date: 6th June 2024 hereby approves the research protocol number 44/2024 **(JSSDCH IEC Protocol No. 44/2024)**
Titled: "Clinical Investigation to examine the effects of hygiene with tooth powders and toothpaste on dental plaque and gingivitis in conjunction with analysis of oral bacteria - A pilot study."

**Data collection procedure**

**Type & duration of study**

**I. Study Design**

This is a double-blind, parallel design clinical study for 1 week duration.

**I. Study Population**

Study will enroll healthy adults with mild gingivitis of either gender who meet inclusion criteria.

**Source and Number of Subjects**

Study volunteers (age range 18-40 years) of either gender will be recruited from the local region. Sufficient subjects will be enrolled to ensure that nine (9) subjects will complete the study. Prospective adult subjects who indicate a willingness to participate in the study will be scheduled for a screening visit by the study investigator. During the screening visit, each volunteer will be provided the informed consent for completion. Subjects who can comply with study schedules and those who complete and sign the informed consent will be enrolled in the study.
The following forms will be completed for all enrolled subjects:

- Initial screening form
- Health questionnaire

All enrolled subjects will be examined by the dentist who will conduct an oral exam. During this initial exam, all subjects will undergo an oral soft and hard tissue assessment. The dentist will examine all subjects for dental plaque (Plaque Index; Silness-Loe Plaque Index), gingivitis (Gingival Index;Turesky Modification of the Loe-Silness gingival index),and conduct a full mouth periodontal examination using a periodontal probe. Subjects who meet all entry criteria listed below will be qualified for study participation. Study setting & source of data: dept of periodontia and pediatric dentistry, JSS Dental college and hospital and FLS, JSSAHER. Sample size and its estimation including sampling procedure: Pilot study hence 3 subjects in each group

**Sample selection criteria**

**Inclusion criteria:**

To be eligible for study participation, the subject must meet the following inclusion criteria:

1. Males and females in good general health aged 18 to 40 years.
2. A willingness to read, understand, and sign the Informed Consent Form after the nature of the study has been fully explained to them.
3. Subject should demonstrate a willingness to comply with all study procedures and sampling schedules.
4. A minimum of 20 natural teeth with facial and lingual scorable surfaces.
5. Adequate oral hygiene and no signs of oral neglect.
6. Selected subjects with mild gingivitis.
7. Subjects with gingival index (Loe-Silness gingival index) greater than 1 and plaque index (Quigley-Hein plaque index) greater than 2.0 will be enrolled.

**Exclusion Criteria**

Any of the following conditions will exclude subjects from eligibility for study participation:

1. History of significant adverse effects following use of oral hygiene products such as toothpastes and mouthrinses. Allergy to personal care/consumer products or their ingredients.
2. Teeth that are grossly carious, fully crowned or extensively restored on facial and/or lingual surfaces, orthodontically banded, abutments, or third molars
3. Significant oral soft tissue pathology, systemically related gingival enlargement, severe gingivitis
4. History of active severe periodontal disease with bleeding gums and loose teeth.
5. Gross dental caries, severe generalized cervical abrasion and/or enamel abrasion, large fractured or temporary restorations, will not be included in the tooth count.
6. Fixed or removable orthodontic appliance or removable partial dentures.
7. Participation in a dental plaque/gingivitis clinical study involving oral care products, within the past three months.
8. History of dental prophylaxis or treatments in the past three months.
9. Self-reported pregnancy or lactation.
10. Subjects known to be an alcoholic/Smokers, or a recovering alcoholic or smoker.

11. History of diabetes or hepatic or renal disease, or other serious medical conditions or transmittable diseases, e.g., heart disease or AIDS. History of rheumatic fever, heart murmur, mitral valve prolapses or other conditions requiring prophylactic antibiotic coverage prior to invasive dental procedures.
12. Subjects on antibiotic, anti-inflammatory or anticoagulant therapy during the month preceding the baseline exam.

**Withdrawal criteria:**

If the subject reports taking medication, a history of allergy, and/or a chronic disease which in the opinion of the dental examiner will not affect the clinical parameter(s) being assessed or the safety of the subject, the subject may be enrolled in the study and it will be noted on the Subject Screening Form.

**Prohibited/Allowable Medications or Precautions:**

The use of any mouthrinse, dentifrice, or any oral hygiene devices other than the test materials during the study period is prohibited.

**Facilities available for the proposed research project/ short study**

OPD facilities at Dept of Periodontia and SIG Dental Cariology, Pediatric dentistry, Microbiological lab, SLS Mysuru, JSS AHER.

# JSS DENTAL COLLEGE AND HOSPITAL

A Constituent college of JSS Academy of Higher Education & Research,
Sri Shivarathreeshwara Nagar, Mysuru-570015

JSS ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED TO BE UNIVERSITY) MYSURU

**JSS Dental College & Hospital Institutional Ethics Committee**
NECRBHR: DHR Reg No: EC/NEW/INST/2022/2764
CDSCO Reg No. ECR/1170/Inst/KA/2019/RR-22

+91-821-2548349 / 350 jssdchiec@gmail.com https://jssuni.edu.in/jssaher/dental-college/dch-iec-home.html




## JSS DENTAL COLLEGE & HOSPITAL
## INSTITUTIONAL ETHICS COMMITTEE APPROVAL LETTER

JSSDCH IEC Research Protocol No: 44/2024 Date: 12-06-2024

To,
Dr. D Guru Kumar, Assistant Professor,
Division of Biochemistry,
School of Life Sciences,
Mysuru, JSS AHER Mysuru
Under the guidance of
Dr B. Nandlal, Professor,
Department of Pediatric & Preventive Dentistry,
JSS Dental College & Hospital, Mysuru

The JSS Dental College & Hospital Institutional Ethics Committee after critically reviewing the research protocol during the review meeting held on date: 6th June 2024 hereby approves the research protocol number 44/2024,

Titled:

"Clinical Investigation to examine the effects of hygiene with tooth powders and toothpaste on dental plaque and gingivitis in conjunction with analysis of oral bacteria – A pilot study.".

12/6/2024

Signature of the Chairman
**Dr R.N. Suresha**
**Chairman**
JSS Dental College & Hospital
Institutional Ethics Committee
JSS DCH, Mysuru

Shivanandi

Signature of the Member Secretary
**Dr Shivananda S.**
**Member Secretary**
JSS Dental College & Hospital
Institutional Ethics Committee
JSS DCH, Mysuru

## RESULTS AND DISCUSSION

1. **Demographics table age and gender of the subjects.**

| Subjects Sl. No. | Age | Sex |
|---|---|---|
| PO1 | 20 | Male |
| PO2 | 19 | Male |
| PO3 | 20 | Male |
| PO4 | 20 | Female |
| PO5 | 25 | Female |
| PO6 | 35 | Female |
| PO7 | 49 | Male |
| PO8 | 18 | Male |
| PO9 | 20 | Male |

**2. Percentage of the Product utilization:**

| Groups | subjects | Day 1 W(g) | Day 8 W(g) | Quantity Product utilised W(g) |
|---|---|---|---|---|
| Toothpowder S1 | PO2 | 92 | 67 | 25 |
| | PO4 | 93 | 77 | 16 |
| | PO8 | 93 | 79 | 14 |
| Toothpowder S2 | PO1 | 94 | 63 | 31 |
| | PO3 | 99 | 77 | 22 |
| | PO6 | 93 | 68 | 25 |
| Toothpaste C | PO5 | 120 | 101 | 19 |
| | PO7 | 120 | 90 | 30 |
| | PO9 | 120 | 92 | 28 |

**3. Plaque samples by weight:**

| Groups | Subjects | | Day 1 | Day 8 |
|---|---|---|---|---|
| | | Plaque sample | W(mg)/V(µl) | W(mg)/V(µl) |
| Toothpowder S1 | PO2 | Before | 6/600 | 10/1000 |
| | | After | 7/700 | 9/900 |
| | PO4 | Before | 6/600 | 3/300 |
| | | After | 2/200 | 4/400 |
| | PO8 | Before | 8/800 | 3/300 |
| | | After | 3/300 | 4/400 |
| Toothpowder S2 | PO1 | Before | 8/800 | 20/2000 |
| | | After | 3/300 | 6/600 |
| | PO3 | Before | 8/800 | 10/1000 |
| | | After | 15/1500 | 5/500 |
| | PO6 | Before | 5/500 | 3/300 |
| | | After | 3/300 | 3/300 |
| Toothpaste C | PO5 | Before | 6/600 | 2/200 |
| | | After | 3/300 | 3/300 |
| | PO7 | Before | 7/700 | 6/600 |
| | | After | 4/400 | 2/200 |
| | PO9 | Before | 8/800 | 2/200 |
| | | After | 6/600 | 3/300 |

**Unstimulated Saliva samples approximate volume of 5ml collected in a Sterile 50ml Falcon tube.**

**Mean Plaque Score:**

| Groups | Subjects | Day 1 | Day 8 |
|---|---|---|---|
| Toothpowder S1 | PO2 | 1.2 | 1.0 |
| | PO4 | 1.1 | 1.1 |
| | PO8 | 3.2 | 1.0 |
| Toothpowder S2 | PO1 | 2.3 | 1.1 |
| | PO3 | 1.3 | 1.1 |
| | PO6 | 1.9 | 1.0 |
| Toothpaste C | PO5 | 1.8 | 1.0 |
| | PO7 | 3.1 | 1.0 |
| | PO9 | 3.1 | 1.1 |

**Mean Gingivitis Score:**

| Groups | Subjects | Day 1 | Day 8 |
|---|---|---|---|
| Toothpowder S1 | PO2 | 1.6 | 1.1 |
| | PO4 | 0.9 | 0.9 |
| | PO8 | 2.0 | 0.7 |
| Toothpowder S2 | PO1 | 1.9 | 1.1 |
| | PO3 | 1.1 | 1.0 |
| | PO6 | 2.3 | 1.2 |
| Toothpaste C | PO5 | 1.9 | 1.0 |
| | PO7 | 1.1 | 0.0 |
| | PO9 | 1.3 | 1.0 |

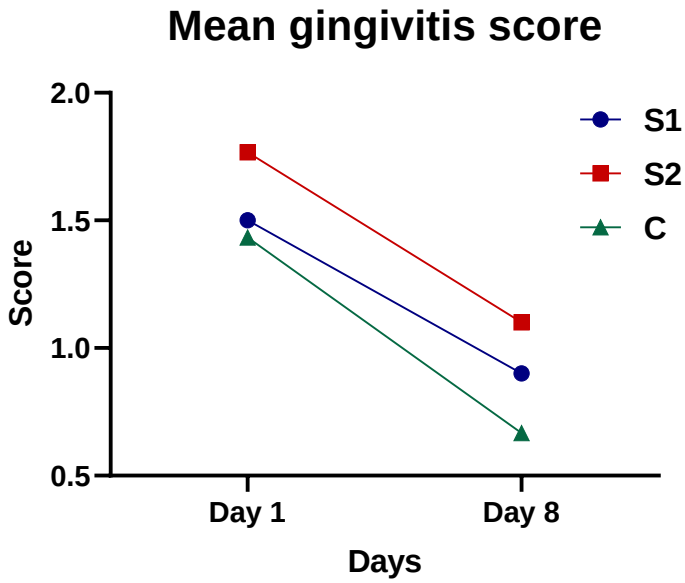



Figure 1: The average whole-mouth dental plaque score of the entire population and among subjects are shown

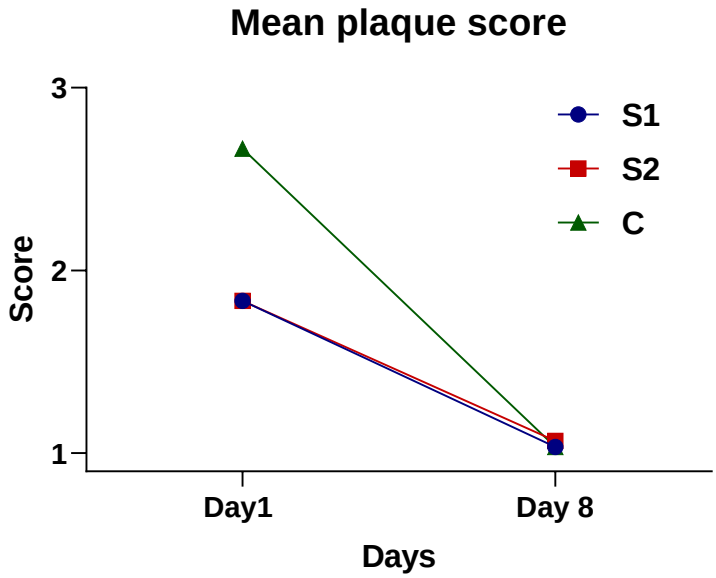



**Figure 2:** The average whole-mouth dental Gingivitis score of the entire population and among subjects are shown

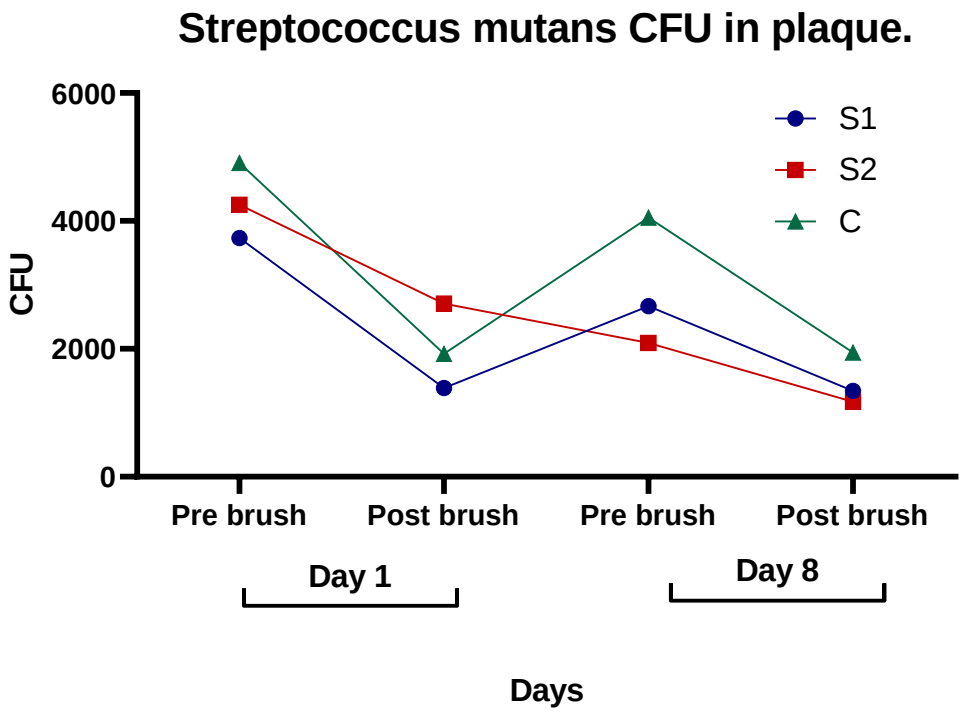



**Figure 4:** Strptococcus mutans CFU obtained from plaque sample on the first day and after 7 days brushing

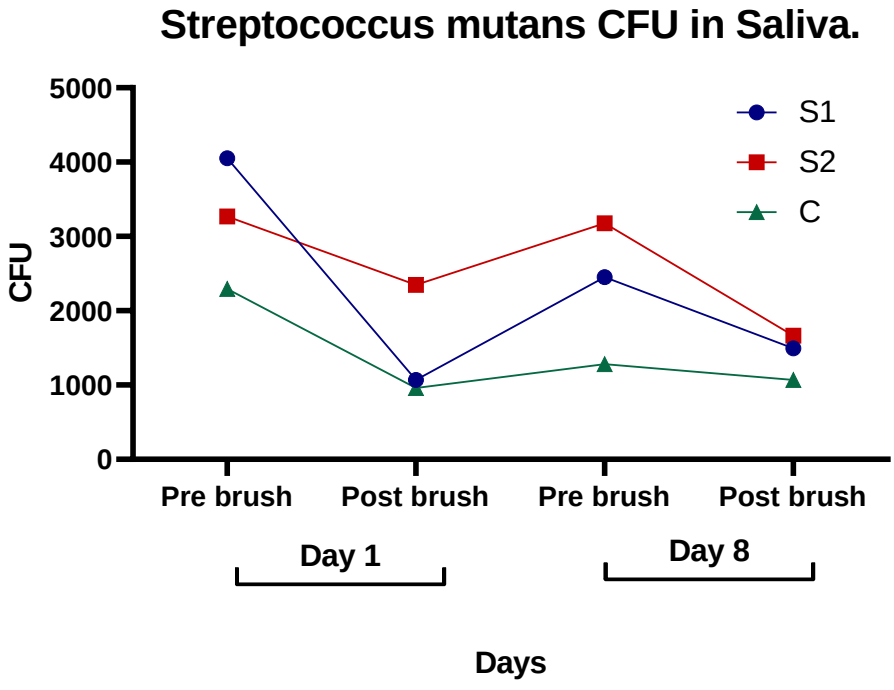



**Figure 5:** Strptococcus mutans CFU obtained from Saliva sample on the first day and after 7 days brushing

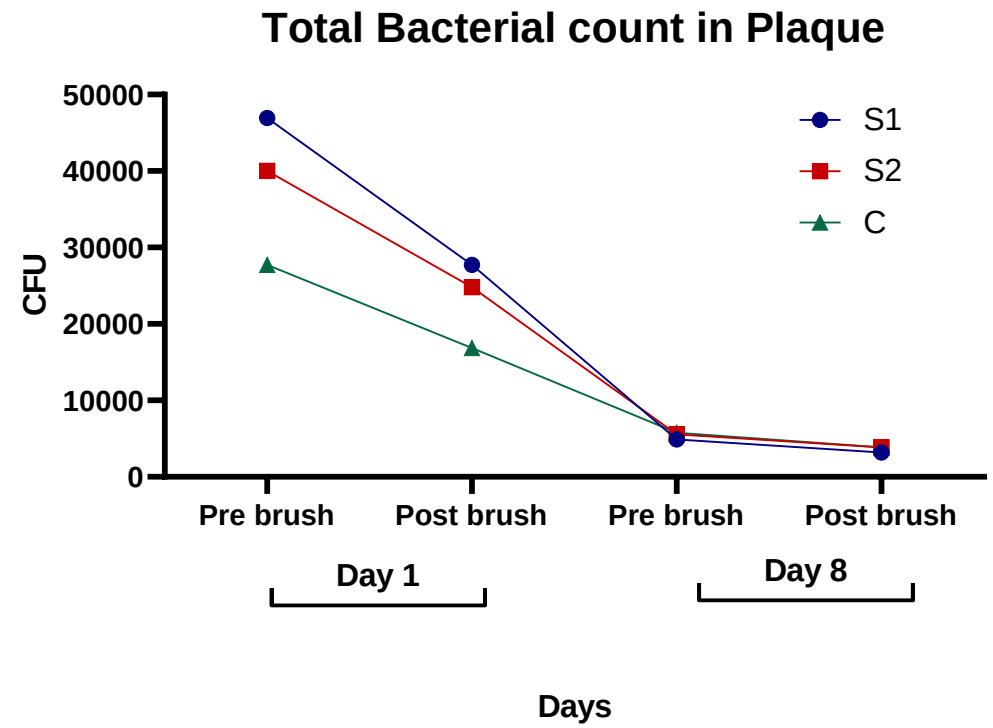



**Figure 6:** Total bacteria CFU obtained from plaque sample on the first day and after 7 days brushing

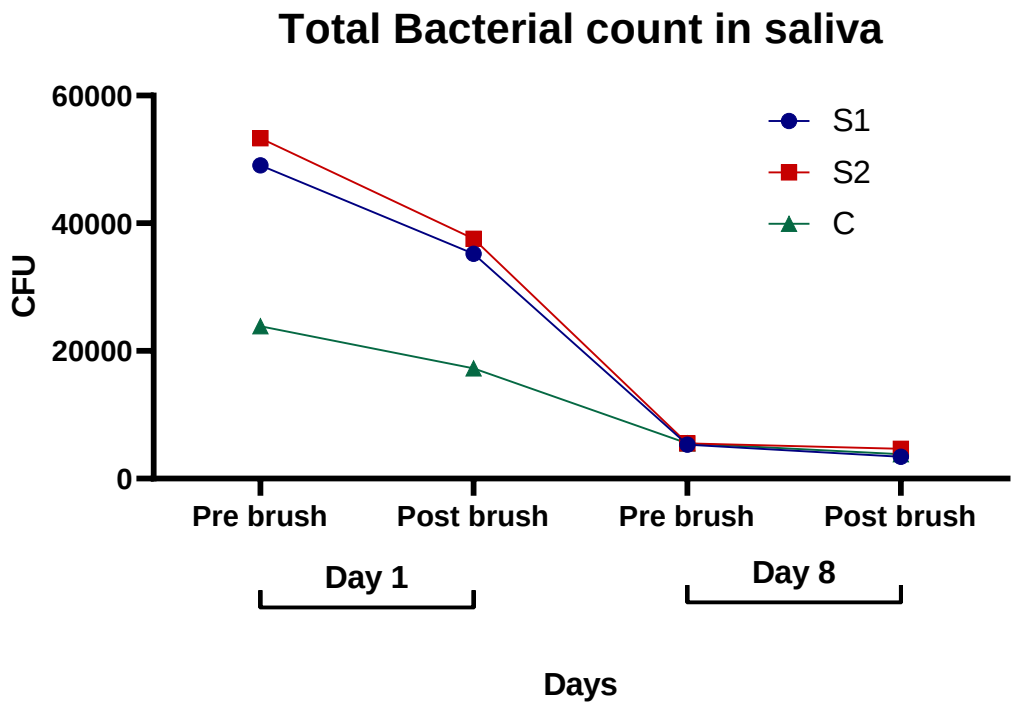
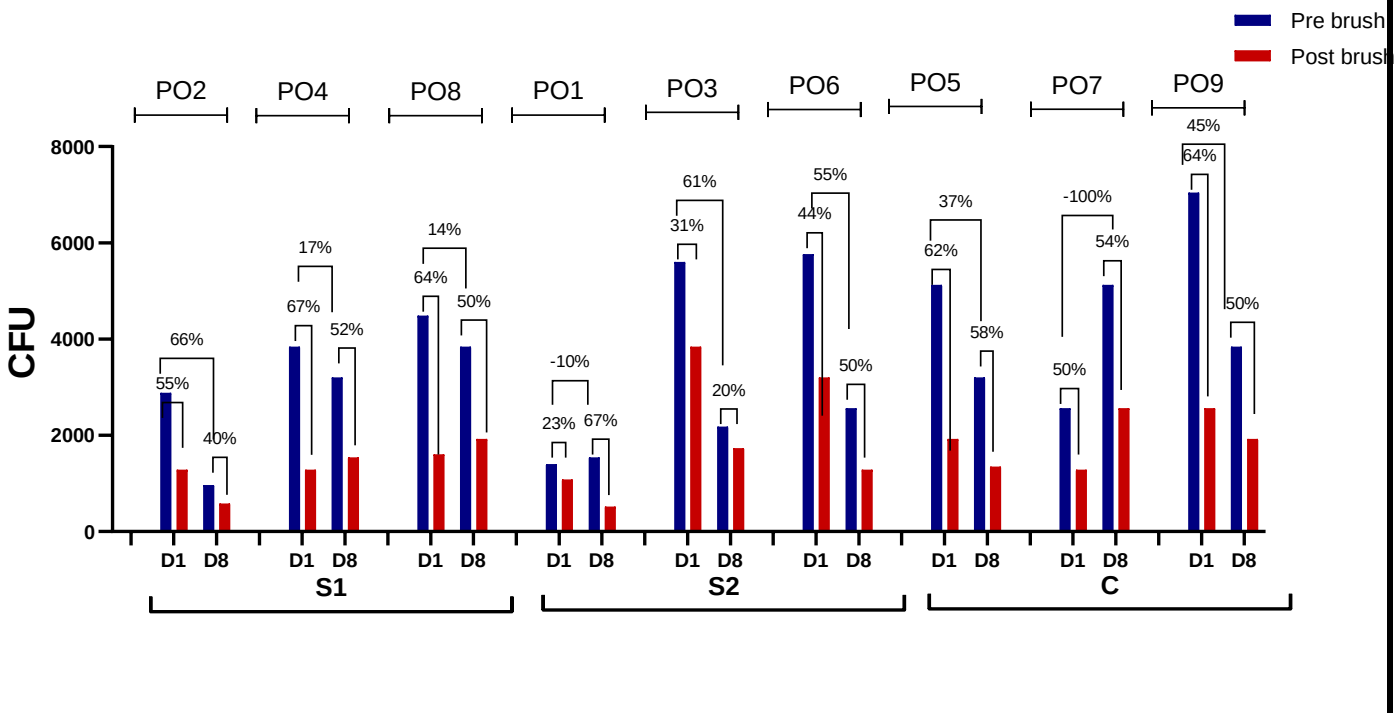



**Figure 7:** Total bacteria CFU obtained from Saliva sample on the first day and after 7 days brushing

## Percentage reduction in Streptococcus mutans CFU in plaque on Post Brushing.




## Days

**Figure 8: Strptococcus mutans CFU obtained from plaque sample on the first day and after 7 days brushing representing in % reduction**

- **% Reduction of SM in Plaque with S1 ranged from 55 %- 67 % on day 1 and40 %- 52% on day 8 while reduction was 14 %- 66% from day1 to Day8.**
- **% Reduction with S2 ranged from 31 %- 62% on day 1 and 20 %- 58 % on day 8 while reduction was -10 %- 61 % from day1 to Day8.**
- **% Reduction with C ranged from50 %-64% on day 1 and 50%- 58 % on day 8 while reduction was -100 %- 45 % from day1 to Day8**

## Percentage reduction in Streptococcus mutans in Saliva on Post Brushing

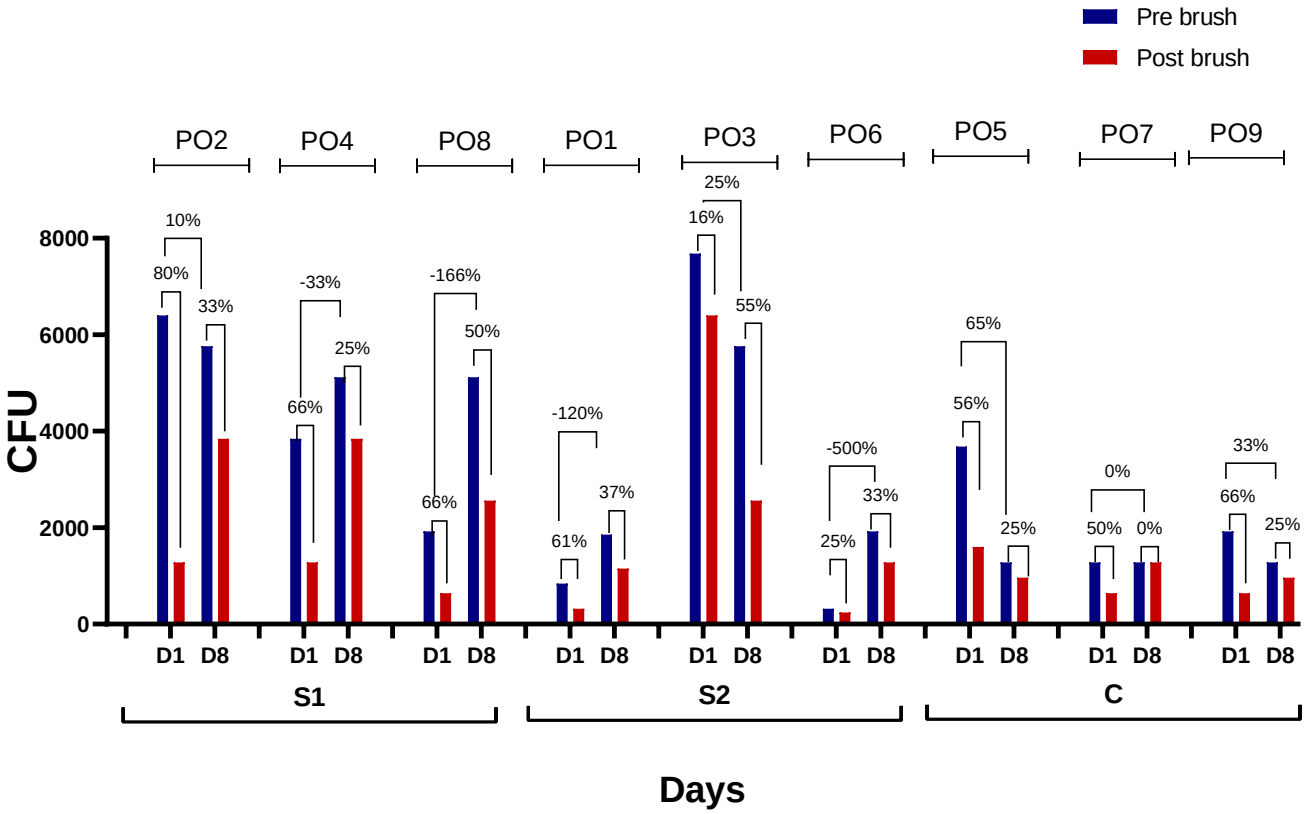



**Figure 9: Strptococcus mutans CFU obtained from Saliva sample on the first day and after 7 days brushing representing in % reduction**

- **% Reduction of SM in Saliva with S1 ranged from 66%- 80 % on day 1 and 25 %-50% on day 8 while reduction was -166 -10 % from day1 to Day8.**
- **% Reduction with S2 ranged from16%- 61% on day 1 and 33%-55 % on day 8 while reduction was -500%-25% from day1 to Day8.**
- **% Reduction with C ranged from 50 %- 66% on day 1 and 0%- 25 % on day 8 while reduction was 0%-65 % from day1 to Day8.**

## Percentage reduction in Total Bacterial count in Plaque on Post Brushing

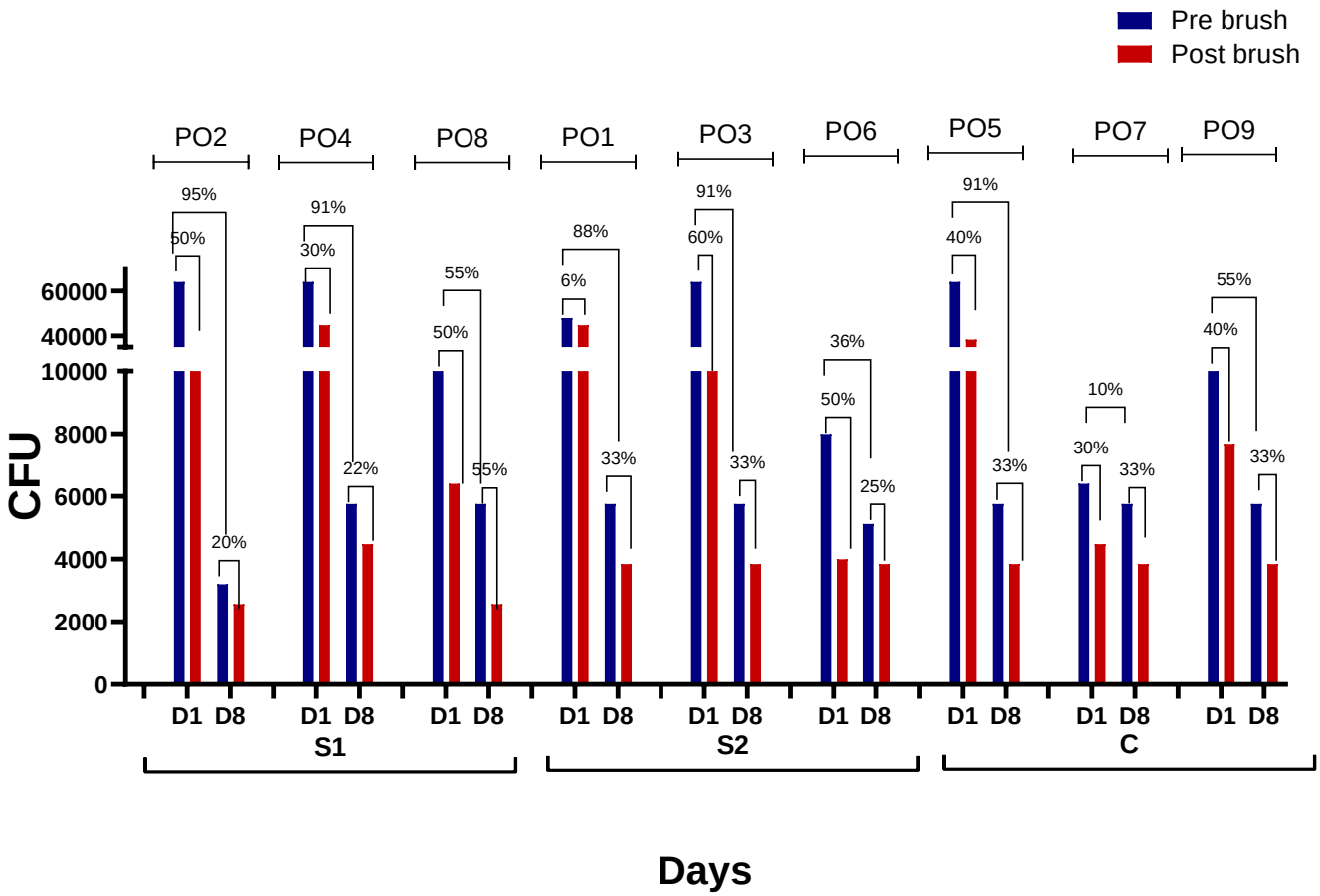



**Figure 10: Total bacterial CFU obtained from plaque sample on the first day and after 7 days brushing representing in % reduction**

- **% Reduction of TBC in Plaque with S1 ranged from 30%-50% on day 1 and 20%-55% on day 8 while reduction was 55%-95% from day1 to Day8.**
- **% Reduction with S2 ranged from 6 %- 60 % on day 1 and 25 %-33% on day 8 while reduction was36%- 91 % from day1 to Day8.**
- **% Reduction with C ranged from 30 %- 40 % on day 1 and 33% on day 8 while reduction was 10%- 91 % from day1 to day8**

# Percentage reduction in Total Bacterial count in Saliva on Post Brushing

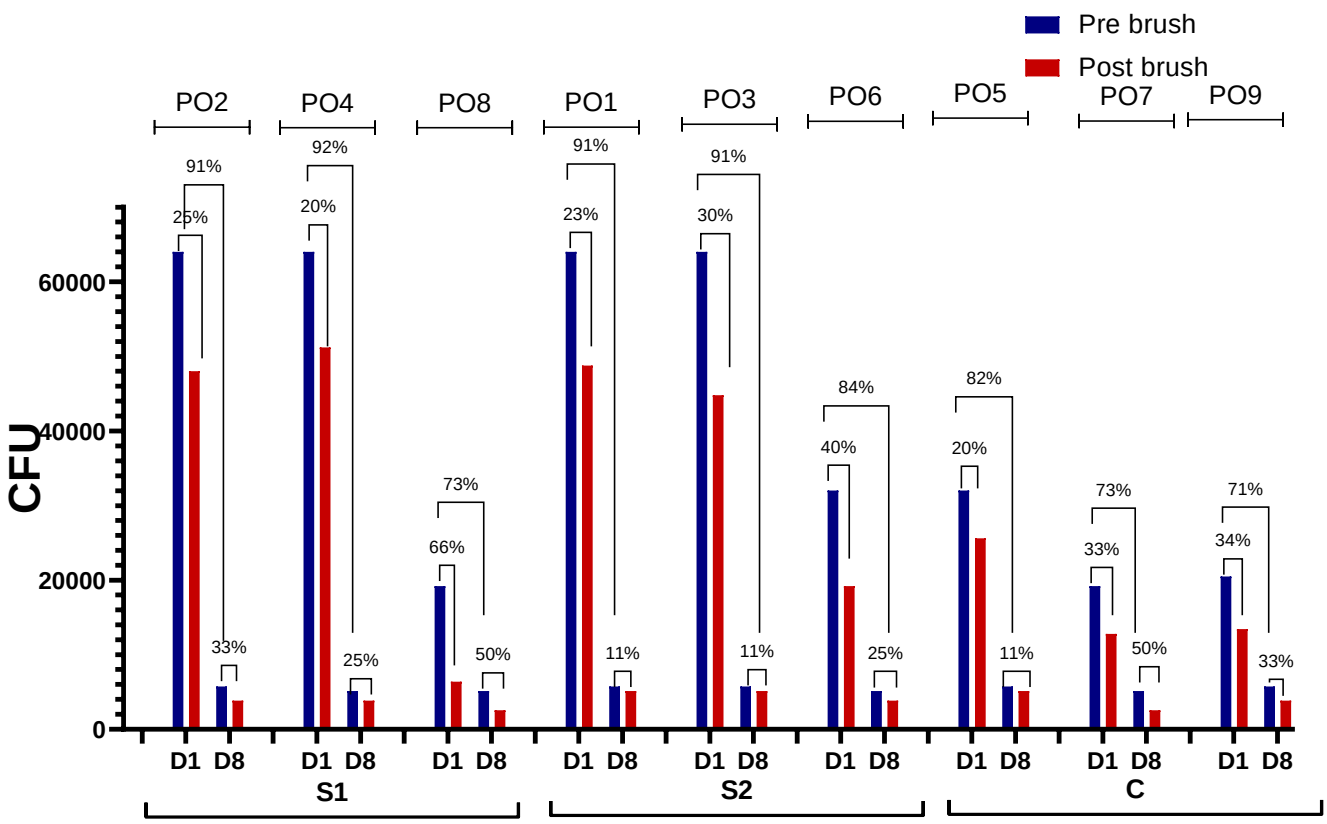



**Figure 11: Total bacterial CFU obtained from plaque sample on the first day and after 7 days brushing representing in % reduction**

- **% Reduction of TBC in saliva with S1 ranged from 20 %-66 % on day 1 and 25%-50 % on day 8 while reduction was 73%-92% from day1 to Day8.**
- **% Reduction with S2 ranged from 23%-40 % on day 1 and 11 %- 25 % on day 8 while reduction was 84 %- 91 % from day1 to Day8.**
- **% Reduction with C ranged from 20 %- 34 % on day 1 and 11%- 50 % on day 8 while reduction was 71 %- 82 % from day1 to Day8.**

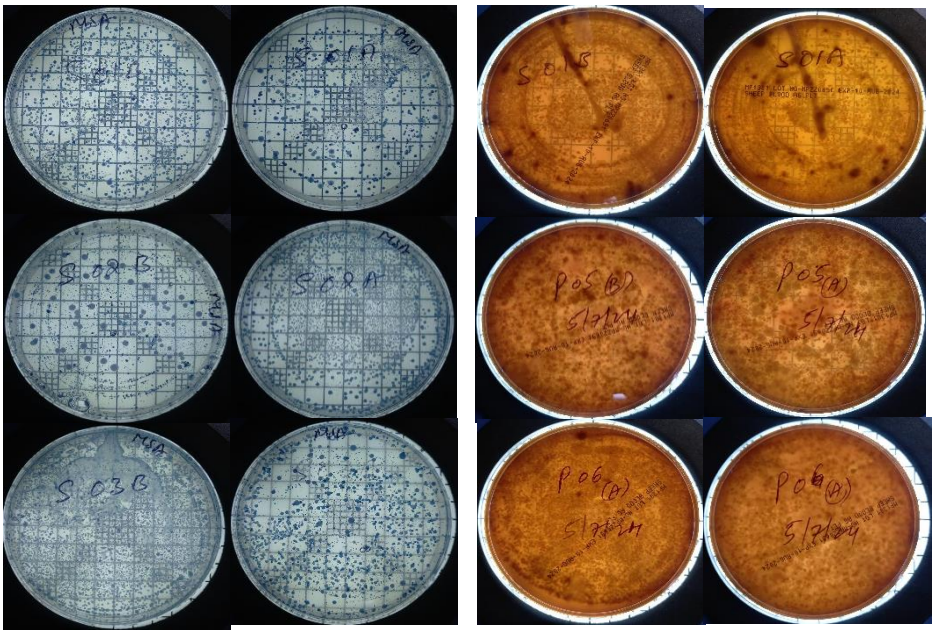


**Figure 12:** CFU obtained from plaque and saliva samples in both MSB agar & Sheep blood agar plates.

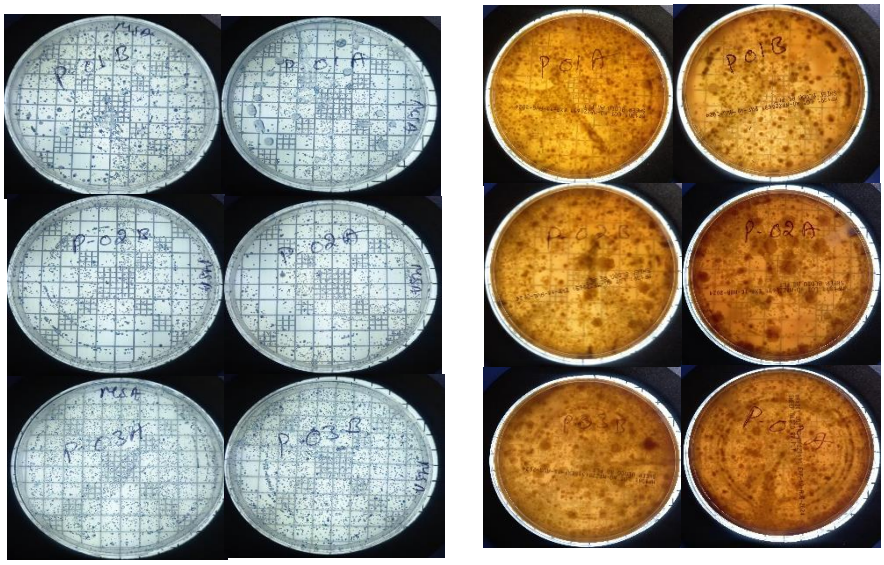


**Figure 13:** CFU obtained from plaque samples in both MSB agar & Sheep blood agar plates.

## Final Conclusion

Percentage reduction of TBC in Plaque

- S1 ranged from 30% to 50% on day 1 and 20% to 55% on day 8 while reduction was 55% to 95% from day1 to Day 8.
- S2 ranged from 6% to 60% on day 1 and 25% to 33% on day 8 while reduction was 36% to 91% from day1 to Day8.
- C ranged from 30% to 40 % on day 1 and 33% on day 8 while reduction was 10% to 91% from day1 to Day8

Percentage reduction of TBC in saliva

- S1 ranged from 20% to 66% on day 1 and 25% to 50% on day 8 while reduction was 73% to 92% from day1 to Day8.
- S2 ranged from23% to 40% on day 1 and 11% to 25 % on day 8 while reduction was 84% to 91% from day1 to Day8.
- C ranged from 20% to 34% on day 1 and 11% to 50 % on day 8 while reduction was 71% to 82% from day1 to Day8.

Percentage reduction of SM in Plaque with

- S1 showed upto 67% on day 1 and 52% on day 8, and reduction was 66% from day1 to Day8.
- S2 showed upto 62% on day 1 and 58 % on day 8 and reduction was 61 % from day1 to Day8.
- C showed upto 64% on day 1 and 58 % on day 8 while reduction was 45 % from day1 to Day8

Percentage reduction of SM in Saliva

- S1 showed upto 80% on day 1 and 50% on day 8 while reduction was 10% from day1 to Day8.
- S2 showed upto 61% on day 1 and 55 % on day 8 while reduction was 25% from day1 to Day8.
- C showed upto 66% on day 1 and 25 % on day 8 while reduction was 65% from day1 to Day8.

## Limitations

- Small Sample size.
- Baseline is not balanced based on the dietary habits - veg or mixed.
- Short term study shows less changes seen on the lag phase in microbial count.
- Salivary proteins levels as per age and conditions might affect the microbial counts.

## Suggestions

- Increase sample size upto 15 subjects in each group for different age groups and stages of health and diseases - Gingivitis and periodontitis.
- Increase the study duration to 3 weeks for a better reduction to evaluate cumulative effects.

## References: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## TIME LINE

| Sl. No | Month | Details of Experiment carried out |
|---|---|---|
| 1 | June 2024 | Ethical Committee work & Methodology standardization |
| 2 | July 2024 | Study progress |
| 3 | August 2024 | Statistical analysis |
| 4 | September 2024 | Final CRA Meeting |

**CRA meeting at JSS Medical College, JSS AHER Mysuru.**


**CRA final presentation meeting at JSS AHER, Mysuru.**