COMPARATIVE EVALUATION OF ANTI-BACTERIAL EFFICACY OF CHLORHEXIDINE MOUTHWASH AND *MOMORDICA CHARANTIA, SPINACIA OLERACEA* MOUTHWASH AGAINST *STREPTOCOCCUS MUTANS, LACTOBACILLUS SPP.* AND *PORPHYROMONAS GINGIVALIS* - AN IN VITRO STUDY

By
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Dissertation

Submitted to KLE Academy of Higher Education and Research (KAHER), Belagavi
In Partial Fulfillment of the Requirements for the Degree Of

MASTER OF DENTAL SURGERY

In

PEDIATRIC AND PREVENTIVE DENTISTRY

(BRANCH - VIII)

DEPARTMENT OF PEDIATRIC AND PREVENTIVE DENTISTRY
KAHER'S KLE VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES,
NEHRU NAGAR, BELAGAVI -10, KARNATAKA.

2020-2023

KLE Academy of Higher Education and Research, Belagavi

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ABSTRACT

Background: Various advances have been made in pharmacology and synthetic organic chemistry, the dependency on natural products, particularly on herbs, remains relatively unchanged. Although many studies have tested the antibacterial effect of the *Momordica charantia* extract and *Spinacia oleracea* extract on various organisms but there is a dearth of literature pertaining to their effect against organisms causing dental caries i.e *Streptococcus mutans* and *Lactobacillus acidophilus*. Among, various herbal products that are being used in dentistry, efficacy of mouthwash prepared using *Momordica charantia* extract and *Spinacia oleracea* extract on prevention of caries in children has not been researched yet. Hence, in this study the antibacterial efficacy of the *Momordica charantia, Spinacia oleracea* mouthwash were evaluated and compared with Chlorhexidine mouthwash against *Streptococcus mutans* and *Lactobacillus acidophilus, Porphyromonas gingivalis.*

Aim: To evaluate and compare the antimicrobial efficacy of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis*

Method: Ethanolic extract of *Momordica charantia and Spinacia oleracea* were prepared. MIC and MBC of these extracts were then determined against *Streptococcus mutans, Lactobacillus acidophilus and Porphyromonas gingivalis* using resazurin method and agar plate streaking method. Herbal mouthwashes were then prepared from the extracts using MIC and MBC values and its cytotoxicity was determined using MTT assay. Antibacterial susceptibility was then determined using Agar Well Diffusion method and Time Kill Assay.

Results: There is no statistically significant difference in the effectiveness of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash

against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

Conclusion: *Momordica charantia* extract mouthwash and *Spinacia oleracea* extract mouthwash can be used as an herbal alternative as it has equal antibacterial efficacy against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* as compared to 0.2% Chlorhexidine mouthwash.

KEYWORDS: Dental Caries, Herbal extract, *Momordica charantia*, mouthwash, *Spinacia oleracea*.

LIST OF ABBREVIATIONS

1.	AgNPs	Silver Nanoparticles
2.	BHI	Brain Heart Infusion
3.	CCT	Controlled Clinical Trials
4.	Carbon Dioxide	CO_2
5.	CFU	Colony forming unit
6.	CHX	Chlorhexidine
7.	^oC	Degree Celsius
8.	DMEM	Dulbecco Modified Eagle Medium
9.	DMFT	Decayed, Missing and Filled Teeth
10.	DMFS	Decayed, Missing and Filled Surface
11.	Dmfs	Decayed missing filled surface
12.	DMSO	Dimethyl Sulfoxide
13.	ECC	Early Childhood Caries
14.	et al	et alia
15.	etc.	et cetera
16.	F^-	Fluoride
17.	GI	Gingival Index
18.	HA	Hydroxyapetite
19.	i.e.	Id est
20.	ICMR	Indian Council of Medical Research
21.	L. acidophilus	Lactobacillus acidophilus
22.	LD	Lesion Depth

23.	MBC	Minimum Bactericidal Concentration
24.	MCEE	Momordica charantia Ethanolic Extract,
25.	mg/ml	milligram/ milliliter.
26.	MIC	Minimum Inhibitory Concentration
27.	µg/ml	microgram/milliliter
28.	µl	Microliter
29.	min	Minute
30.	ml	Milliliters
31.	M. charantia	Momordica charantia
32.	MS	Mutans streptococci.
33.	MTCC	Microbial type culture collection
34.	MTT	3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
35.	MW	Molecular weight
36.	n	Sample Size
37.	NaF	Sodium Fluoride
38.	Nm	Nanometer
39.	NCD	Non-Communicable Disease
40.	No.	Number
41.	OD	Optical Density
42.	%	Percentage
43.	pH	Pouvoir Hydrogene
44.	PI	Plaque Index
45.	P. gingivalis	Porphyromonas gingivalis
46.	Ppm	Parts per million

47.	R	Resistance
48.	RCT	Randomised Controlled Trial
49.	S	Sensitive
50.	SD	Standard Deviation
51.	SE	Standard Error
52.	sic	Significant caries index
53.	Sl.	Serial
54.	SOEE	Spinacia oleracea Ethanolic Extract.
55.	spp.	Species
56.	SPSS	Statistical package for the Social Sciences
57.	UV	Ultraviolet
58.	w/v	Weight/volume
59.	w/w	Weight/weight
60.	WHO	World Health Organization
61.	WMD	Weighted mean difference

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INTRODUCTION

"Study nature, love nature, stay close to nature,
It will never fail you."

-Frank Lloyd Wright

Dental caries is the most common non-communicable disease (NCD) and a major public health problem worldwide. With time, there is demineralization of the tooth structure due to interaction of cariogenic microbes and fermentable dietary carbohydrates. Due to complex interaction of social, cultural, nutritional, behavioral and biological risk factors that are allied with its commencement and progression it is a disease that will be difficult to be eradicated from the world.[1]

Incidence of dental caries varies from 49% to 83% across different countries. According to 2015 Global Burden of Disease Study, dental caries is prevalent among (2.3 billion people) where it ranks first for decay of permanent teeth and 12th for deciduous teeth prevailing among 560 million children and affecting humans of all ages throughout the world. Moreover, it remains the major dental health problem among school children globally.[2] Prevalence of dental caries in India as per 2018 report was found to be 68.4%. Occurrence of dental caries was found to be high and varied across India irrespective of age of affected population. Various factors like socioeconomic status, lack of preventive measures, and dietary changes have contributed to increased occurrence of dental caries in children. The direct medical treatment costs of dental diseases accounted for US$298 billion in 2010, corresponding to an average of 4.6% of total global health care expenditure.[3]

Preservation of health and integrity of primary teeth till they naturally exfoliate is one of the preliminary objectives of Pediatric dentistry. Prevention of a

disease is often more affordable option than the treatment of an established condition.[2] In the areas where the embattled disease burden or risk factors associated with it are higher, it is highly necessary to highlight that health preventive interventions will generally be more cost-effective, therefore the intervention will be able to avert more cases.[4]

The most prevalent organism in pit and fissure and buccal surfaces of tooth is *Streptococcus mutans*, which is a facultative, Gram-positive anaerobic organism and is the pioneer organism for the commencement of dental caries. The bacteria of the genus *Lactobacillus acidophilus* is a Gram-positive, facultative anaerobe associated with progression of carious lesions, especially those in the coronal areas and it is important in further caries development, especially in the dentin.[5] Dental caries can be thus prevented by controlling the activities of the microbes which play a vital role in its development. *Lactobacillus acidophilus* is a presiding part of the flora inhabiting the widespread cavities, and its number relates with the amount of carbohydrates. *Porphyromonas gingivalis* is mainly associated for gingival inflammation in children. It has been observed that about 60% of healthy children of 2-18 yrs children have detectable *Porphyromonas gingivalis* in their plaque.[6]

Prevention treatment modalities like, oral hygiene measures, different forms of fluoride application, pit-and-fissure sealants, xylitol, development of a dental caries vaccine and the role of the primary caregiver for infants were found to be essential. Amongst the different antimicrobial delivery systems, especially for young children, mouthwash is one of safest and effective vehicles as they have ability to deliver therapeutic ingredients to all accessible surfaces and interproximal surfaces. It has

been found that medicinal plants can be used to treat ailments and preserve the normal harmony of the human body.[5]

The traditional used medicinal or herbal plants were replaced by the chemical form of medicine i.e allopathic form of drugs. Though these allopathic drugs do have rapid onset of action and are able to treat various diseases in effective manner, they are considered to be "double-edged sword" as they can cause serious side effects and discomfort to the individual who is taking the drugs for any specific disease.[4]Hence, in the past few years there has been a paradigm shift in the concept of treating a particular disease by use of herbal plants for treating a particular disease.[3]

Chlorhexidine mouthwash is widely used for the inhibition of commencement of dental caries.[6] Even though, Chlorhexidine is considered to be a "gold standard" to prevent the development of dental caries, due to risk of various adverse effects such as brown staining of the tooth, taste alteration and mucosal erosions etc., thus there is a need for an herbal substitute to avoid the side effects.[7]

There has been a change in global thinking in past few decades, with a growing tendency to go natural. Although various advances have been made in pharmacology and synthetic organic chemistry, the dependency on natural products, particularly on herbs, remains relatively unchanged. There is 80% reliance of the world population on Medicinal plants for their basic health care needs.[8] *Momordica charantia* (Bitter gourd), *Spinacia oleracea* (Spinach), *Salvadora persica* (Miswak), *Newbouldia laevis* (blood root plant), *Syzgium aromaticum* (clove oil), *Azadirichta indica*(neem), *Allium sativum* (Garlic), *Nidus vespae* (Honey Comb) have proved to pose significant antibacterial effect against cariogenic bacteria.[6]

Momordica charantia (Bitter gourd) has been used for centuries in various cuisine of India, China and Latin America. It is a good source of vitamin C, Vitamin A, phosphorus and iron and has been extensively used in folk medicine.[5] The leaves of *Momordica charantia* has been used for thousands of years for its medicinal properties. Although many studies have tested the antibacterial effect of the bitter gourd extract using aqueous, petroleum ether, ethanol, chloroform and ethyl acetate on various organisms but there is a dearth of literature pertaining to their effect against organisms causing dental caries and gingivitis i.e. *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis.*[6] Aqueous and ethanolic extract of *Spinacia oleracea* (Spinach) has an antibacterial effect against *Streptococcus mutans* and *Lactobacillus acidophilus.* The antibacterial effect can be attributed to its silver nanoparticles (AgNPs). Therefore, silver nanoparticles (AgNPs), phenolics, and flavonoids exhibit great potential as novel antimicrobial agents.[9]

When literature search was carried out it was seen that among, various herbal products that are being used in dentistry, efficacy of mouthwash prepared using *Momordica charantia* extract and *Spinacia oleracea* extract on prevention of caries and gingivitis in children has not been researched yet.[10]

Hence, in this study the antibacterial efficacy of the *Momordica charantia, Spinacia oleracea* mouthwash were evaluated and compared with chlorhexidine mouthwash against *Streptococcus mutans* and *Lactobacillus acidophilus, Porphyromonas gingivalis.*

AIM AND OBJECTIVES

AIM OF THE STUDY:

To evaluate and compare the antimicrobial efficacy of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

OBJECTIVES OF THE STUDY:

1. To evaluate the antibacterial efficacy of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*
2. To compare the antibacterial efficacy of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

RESEARCH HYPOTHESIS

NULL HYPOTHESIS:

There is no statistically significant difference in the effectiveness of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

ALTERNATIVE HYPOTHESIS:

There is statistically significant difference in the effectiveness of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

REVIEW OF LITERATURE

Literature regarding prevalence of dental caries in children:

1. A systematic review carried out to find the prevalence of dental caries in children in India where total 1468 titles were screened after which 191 articles were shortlisted for further analysis as per inclusion and exclusion criteria. Final analysis was carried on 69 studies and it was found that at interval of 5 yrs, the collective caries prevalence was between 50.84% and 62.41%. It was also seen that there was reduction in caries prevalence in 2-5 and 11-15 years of age group where it was found that Significant caries index (SiC) of more than 3 was found in all the age groups. The systematic review concluded that prevalence of dental caries was seen in more than half of Indian children Population.[11]
2. Systematic review that aimed to determine the prevalence of dental caries in children who were 5–15 years of age in South-East Asia Region (SEAR) countries of World Health Organization (WHO) and to find out different indices used for measuring severity of caries used in these population-based studies. It was seen that the most commonly used index for measurement of dental caries was the dentition status as per 1997 WHO criteria. The systematic review concluded that dental caries is still a major oral health problem among children in the SEAR countries with a wide variability in the prevalence across different ages and countries.[12]
3. Systematic review carried out to correlate the influence of early childhood caries on the development and incidence of caries in preschool children which included studies published in English language with children who were less than six years with greater than 1000 sample size. This study found that use of diagnostic criteria describe dental caries in young children were not consistent as there was

lack of reporting standards for assurance and prevalence for early disease process in children. As per the data from different countries, the dental caries is still considered to be a global concern with dental caries in maxillary anterior teeth of young children still being a major concern.[13]

4. A study assessed the caries prevalence in 1257 children aged 5, 6, 11 & 15 years where there were total 628 boys and 629 girls in all the four age groups. It was observed that, the point prevalence was found to be 57.9%, 73.1%, 66.2% and 62.2 % at age of 5, 6, 11 and 15 years respectively while the overall prevalence of dental caries was about 64.3%. The study concluded that, 63.6% children required dental treatment due to various oral conditions however, there was difference in prevalence of dental caries in different age groups.[14]

5. In a review, studies have proved the evidence that the level of *Streptococcus mutans* and to a lesser level of proportion of *Lactobacillus acidophilus* in plaque is associated statistically to the presence of dental caries or the onset of development of dental caries. The data provided in the review confirms that *Streptococcus mutans,* perhaps *Streptococcus sobrinus, Lactobacillus acidophilus,* species are human odontopathogens and are associated with oral diseases. The review also explains that dental caries is a diagnosable and treatable condition and moreover, *Streptococcus mutans* being acidogenic in nature is responsible for demineralization of teeth by lowering pH and causing dental caries.[15]

6. A clinical study studied the role of *Streptococcus mutans* and *Streptococcus sanguis* in development of dental caries. It was seen that *Streptococcus mutans* accounted for about 60% of the total cultivable flora of dental plaque from caries lesions. It was seen that proportions of *Streptococcus sanguis,* from the plaque

of upper anterior teeth and from posterior teeth showed very low count in contrast to those of *Streptococcus mutans*, which supports for the role of *Streptococcus mutans* in the initiation and development of dental caries.[16]

Literature regarding preventive measure of dental caries:

7. The study aimed to determine the effects on dental caries by using pit and fissure sealant mixed with Silver nanoparticles with help of monthly measurement of fluorescence with DIAGNODENT over a period of six months. This study was divided in two experimental phase and clinical phase. In the experimental phase, evaluation of the adhesion and microleakage of the pit and fissure sealant was done. Conventional (Group A) and Silver nanoparticles added to the pit and fissure sealant (Group B) were applied to two groups of 10 teeth with no carious lesions. For the clinical phase, a split-mouth study was carried on 40 children who were aged 6-10 years on caries free, erupted permanent first molars. Conventional pit and fissure sealant or a Silver nanoparticle-mixed sealant was randomly applied on teeth which was then subjected to repeated measures analysis. The study concluded that the Silver nanoparticle-mixed sealant reduced tooth demineralization and slightly increased remineralization as compared to the conventional pit and fissure sealant.[17]
8. An in vitro study compared the efficacy of two toothpastes containing hydroxyapatite or 500 ppm fluoride that promoted remineralization and inhibited caries development. Two enamel blocks (human primary teeth), one which was sound and one which artificially produced caries lesion, were applied with toothpaste either, 10% hydroxyapatite or 500 ppm F^- (amine fluoride). Microradiography was used to determine the baseline and post-test mineral loss and lesion depth (LD). It was found that 10% hydroxyapatite achieved

comparable efficacy with 500 ppm F^- in remineralizing initial caries and preventing demineralization.[18]

9. A longitudinal, cluster randomized, non-inferiority trial was conducted in lower socioeconomical class children with high-caries risk. The primary objective is to compare the non-inferiority of Silver diamine fluoride varnish versus Glass ionomer therapeutic sealants in the prevention of carious lesion. The comparative effectiveness of alternate caries inhibiting modes is considered to be one of the highest research significances in the developed nations. Silver diamine fluoride being simple and affordable could be considered as a feasible alternative for the arrest of dental caries in high-risk children.[19]

10. A randomized controlled trial of caries free children aged 2 to 3 years was conducted where 1,096 (549 intervention, 547 control) were assessed at baseline and after 3 years. The clinical trial failed to keep children caries free, but once children developed caries, the intervention slowed down the progression of caries.[20]

Literature regarding prevalence of gingivitis in children:

11. A cross-sectional survey of including 200 children who were aged 2–5 years was performed from March 2015 to February 2016. In the survey the investigators measured the Gingival Index (GI) and pocket depth of fully erupted teeth. It was observed that the t-test comparison of mean pocket depth was least in children who were aged 3 and 4-year-old (0.89 mm) and was highest in 2 and 4 year old children (3.09 mm). It was also observed that the mean GI among boys and girls also differ suggestively which was highly statistically significant. It was observed that the boys had a higher GI and pocket depth than girls moreover, the school going children had less gingival index score and pocket depth than non-

school going children. The review concluded that the socioeconomical condition significantly influences that gingival health overall as there was significant difference in mean GI and pocket depth of children of upper and lower socioeconomical class.[21]

12. In a cross-sectional study that aimed to estimate the prevalence of gingivitis and calculus among 12 yr old children where a probability-based sample of children from 113 schools were selected proportional to enrolment and graded by health region, school type, and gender. After evaluation of 1586 children it was observed that gingivitis was found in 80.41% of children. The results also showed that children from urban-public school had a slightly greater prevalence (83.24%) as compared to private (79.15%) while it was seen that those in rural-public (77.59%) and private schools had similar prevalence. It was seen that extensive gingivitis was present in 60.81% of all children. Rural and urban public-school children had significantly higher bleeding on probing as compared to children from private schools while when gender comparison was done, dental calculus was seen in 61.59% of the children with boys presenting significantly higher supragingival calculus. It was observed that prevalence of subgingival calculus and gingivitis was higher in rural-public school than private school children which was statistically significant.[22]

Literature about Chlorhexidine:

13. A randomized controlled trial was carried which aimed to discuss scope and demerits of commercially available chemical oral hygiene agents like Chlorhexidine against pathogenic microbes and caries incidence. Chlorhexidine was further evaluated in different forms like gel, varnish and mouth wash. The

study concluded that maximum reduction in *Streptococcus*. *mutans* count was seen with Chlorhexidine varnishes, followed by gels and mouthwashes.[23]

14. A study which aimed to compare the anti-bacterial efficacy in reducing colony counts of *Streptococcus mutans* using Probiotic mouthrinse, 0.12% Chlorhexidine (CHX) and 0.05% Sodium fluoride (NaF) mouthrinse in children. A triple-blinded crossover randomized trial was carried between interventional groups where fifty-one children between 8 to 12 years of age were randomly allocated into three groups (I, II, and III) and were made to use three mouthrinses (A, B, and C) for two weeks with an inter-phase washout period of four weeks. The baseline *Streptococcus mutans* count were assessed and compared with post-interventional *Streptococcus mutans* counts (CFU/mL) which were then analyzed statistically, it was seen that there was no statistically significant differences with $p > 0.05$. The study concluded that the Probiotic mouthrinse was equally effective in reducing *Streptococcus mutans* count as compared to Chlorhexidine and Sodium fluoride mouthrinses in old children 8 to 12 year old.[24]

15. A double blinded randomized control clinical study was carried for comparative evaluation of efficacy of magnetized water and 0.2% Chlorhexidine as a mouth rinse in inhibition of plaque and gingivitis in children aged 12-15 years. 20 children aged 12-15 years were randomly categorized into two groups, magnetized water and 0.2% Chlorhexidine. Plaque index (PI) scores and Gingival (GI) scores were evaluated at baseline. 10 children from each group were then made to rinse with respective mouthwashes and Plaque index (PI) scores and Gingival (GI) scores were

compared after 2 weeks and at 3 weeks. It was observed that there was no statistically significant difference in the scores. It was thus concluded that magnetized water can be used daily as a mouthwash and could be a safe alternative to Chlorhexidine in reducing plaque and gingivitis, which could also provide benefit to children along with daily toothbrushing with minimum side effects.[25]

Literature about herbal products:

16. A study aimed to evaluate and compare effect on plaque and gingival indices using Neem and Mango chewing stick mouthwashes. 105 children aged 12-15 years were randomly categorized into three groups, namely Neem, Mango, and Chlorhexidine mouthwash groups. Baseline Gingival and Plaque indices were recorded of the subjects and later they were made to use ten millilitres of herbal and Chlorhexidine mouthwashes (0.2%) twice daily for 21 days. The subjects were then made to discontinue the mouthwashes and later Gingival and Plaque Indices were reassessed at 21 days and at 1 month, 2 months, and 3 months. It was found that there was statistically significant reduction ($P < 0.001$) in Gingival index in all the three mouthwash groups at 21 days and at 1 and 2 months after discontinuation of mouthwash. It was thus concluded that, all the three mouthwashes were equally effective as antiplaque and as antigingivitic agents.[26]

17. A study that evaluated and compared the effectiveness of mouthwash prepared with Triphala and commercially available Chlorhexidine mouthwash on dental plaque scores, gingival inflammation, and *Streptococcus mutans* count. The students were randomly divided into three groups namely, Group I (n = 457)

using Triphala mouthwash (0.6%), Group II (n = 440) using Chlorhexidine mouthwash 0.1% (positive control), and Group III (n = 412) using distilled water (negative control). The variable chosen for assessment were plaque scores, gingival scores, and the microbiological analysis where Streptococcus and lactobacilli counts were estimated. Both the Group I and Group II showed considerable reduction in plaque scores from baseline to the end of 9 months however, for Group III where distilled water was used by the subjects, increase in plaque scores from the baseline to the end of 9 months was observed. It was further seen that Group I and Group II showed comparable effect on gingival status. There was considerable inhibition on microbial counts in Group I and Group II however, in those subjects where Triphala mouthwash was used, reduction in *Lactobacillus* count was more as compared to Chlorhexidine. The study concluded that there was no statistically significant difference between the Triphala and the Chlorhexidine mouthwash.[27]

18. A study was conducted to evaluate effectiveness of two herbal mouthwashes containing *Aloe vera* and Tea tree oil in school children aged 8–14 years. The participants of the study were allocated into four groups based upon the mouthwash used: (Group 1) *Aloe vera*, (Group 2) Chlorhexidine, (Group 3) Tea tree oil and (Group 4) Placebo. Plaque index, gingival index and salivary *Streptococcus mutans* counts, were recorded at baseline, four weeks after usage of mouthwash and after two weeks of stopping the mouthwash. A statistically significant reduction in all variable scores was noted after the use of both the herbal preparations at the end of four weeks which was further maintained after the two week washout time where mouthwash was not used by subjects. It was seen that the use of *Aloe vera* and tea tree oil mouthwashes can significantly

decrease plaque, gingivitis and *Streptococcus mutans* count in the oral cavity of children. Thus, it was concluded that the effectiveness of both herbal mouthwashes are comparable to Chlorhexidine.[28]

19. A study was conducted to compare effectiveness of a commercially available herbal mouthwash with Chlorhexidine on status of plaque, gingiva, and salivary *Streptococcus mutans* count. 55 children, aged 8-14 years, were allocated into two random groups by random allocation method. 10 mL of test mouthwash "Freshol" and Chlorhexidine was given to Group A (35) and Group B (20) respectively during phases 1 and 3 of the clinical trial which was of 10 days each. In Phase between phase 1 and phase 2 was the washout time during which there was no mouthwash given. It was seen that Freshol was found to have better anti-bacterial efficacy in reducing salivary *Streptococcus mutans* count than Chlorhexidine and was found the be equivalent than Chlorhexidine in reducing the Plaque and Gingival scores. Thus, it was concluded that Herbal products can prove to be more effective and safer alternate to commercially available products used for treatment.[29]

20. An in vitro study was carried for assessment of antibacterial efficacy of various concentrations of Coffee extract with 0.2% Chlorhexidine mouthwash against different gram-negative periodontal pathogens mainly, *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans*. The anti-bacterial susceptibility test was carried for comparing the antimicrobial efficacy of coffee extract and 0.2% Chlorhexidine by disc diffusion method. It was seen that 0.2% Chlorhexidine mouthwash showcased larger zone of inhibition against all periodontal pathogens. While, Coffee when used in concentration of 20% and 15% showed

significant antimicrobial activity against *Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans. However, Fusobacterium nucleatum* was resistant to all concentrations of coffee extract. Moreover, commercially available Coffee extract does indeed possess antimicrobial activity against the periodontal pathogens, *Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans.*[30]

21. Randomized Controlled Trial aimed to compare and evaluate the effectiveness of the herbal rinses - Tulsi and *Black myrobalans* and Sodium fluoride mouth rinse was done to determine change in the pH of saliva and *Streptococcus mutans* count. Based on standard sample size calculating formula, 105 children were screened orally and 60 children were randomly selected. The selected children were randomly allotted into two groups. In Control group children were given Sodium fluoride rinse 0.05% (Kidodent mouth rinse), Group B - Tulsi mouth rinse 4% (Experimental group), and Group C - *Black myrobalans* 2.5% (Experimental group) by random lottery method. Determination of baseline values of saliva was done of the three groups after which, each group was given their respective mouthwash. Comparison of salivary pH was done on 8th day and it was found that both the experimental groups and the control group were statistically significant with $P \leq 0.023$. It was seen that the salivary pH was found to be high in the control group (Sodium fluoride mouth rinse), followed by Tulsi leaf extract mouth rinse however, *Black myrobalans* mouth rinse was found to have least salivary pH. It was also found that Tulsi showed its effect immediately, whereas, *Black myrobalans* mouth rinse showcased a prolonged effect on *Streptococcus mutans* count.[31]

22. A study carried out to summarize various herbal products that can be used as a mouthwash for maintaining oral health. Various herbal mouthwashes formulated from extracts of Guava, Pomegranate, Neem, Propolis, Tulsi, Green Tea, Cranberry, Alum, Grapefruit can be used as an effective caries control measure and thus concluded that the use of these herbal products as mouthwash was effective as well as economical without causing any side effects to the oral health.[32]

23. In a study that assessed the existing evidence on the antimicrobial efficacy of 10 herbal extracts on dental caries and plaque microbes in various internet search engines, it was found that the herbal extracts of *Azadirachta Indica*, *Ocimum sanctum*, *Murraya koenigii*, *Acacia nilotica*, *Eucalyptus camaldulensis*, *Hibiscus sabdariffa*, *Mangifera indica*, *Psidium guajava*, *Rosa indica*, and *Aloe barbadensis* all have the antibacterial property against oral pathogenic micro-organisms and hence various herbal formulations can be used in dentistry.[33]

24. In a study that aimed to determine the scientific origin of few indigenously used medicinal plants in India which have known anti-microbial potential against *Streptococcus mutans*. The studies included in the systematic review have studied the potential active component, efficacy against inhibiting *Streptococcus mutans* using solvents like water, chloroform, ethanol. It is seen that chloroform extract of *Streptococcus acmella* shows antimicrobial activity against Streptococcus mutans with MIC of 256 μg/ml. Studies have shown that *Prosopis spicigera* leaf extract contain active ingredient piperidine alkaloid spicigerin which shows prominent anti-microbial activity against *Streptococcus mutans* and *Streptococcus bovis* with the least MIC being 4.88 μg/ml. The active phytochemical Curcumin isolated from the roots of turmeric plant does have

potential to inhibit *Streptococcus mutans* with Minimum Inhibitory Concentration (MIC) of 175 μmol/L. Similarly, Kuwanon G isolated from root bark has shown potential action against *Streptococcus mutans* with MIC of 8.0 μg/ml it was also seen that Kuwanon G completely inactivated *Streptococcus* mutans at the concentration 20 μg/ml in 1 min. *Acacia nilotica* stem bark extracts contain alkaloids, saponins, cardiac glycosides, tannins, flavonoids and anthraquinones which have high inhibitory activity against *Streptococcus mutans* with a MIC in the range of 9.75-313μg/ml. This study explains various phytochemicals which have active potential in inhibition of caries by inactivating *Streptococcus mutans.* These studies can prove beneficial for commercialization of products derived from herbal plants that can compete with various pharmacological products available in market.[34]

25. An in vitro comparison was done to study antimicrobial properties of *Aloe vera,* Stevia mouth rinse against *Streptococcus mutans*. Anti-bacterial susceptibility test was done using agar well diffusion method to evaluate the antimicrobial property by comparing zone of inhibition of the three extracts after 24 h incubation. Mean zone of inhibition values for Stevia, Thulasi, and *Aloe vera* mouthrinse were found to be 22.33 mm, 11 mm, and 0 mm respectively while it was about 13.6mm for positive control Chlorhexidine. The study thus concluded that medicines derived from herbal ingredients could be used as a potential alternative as a cariostatic agent.[35]

26. The systematic review analyzed efficacy of *Aloe vera* mouthwash on plaque and gingival problems. A comprehensive search of PubMed, Embase, Scopus, and Web of Science was done to find all relevant studies done as per the inclusion and exclusion criteria. The eligibility criteria were randomized clinical trials that

carried out comparative assessment of anti-microbial efficiency on plaque and gingivitis of *Aloe vera* mouthrinse and Chlorhexidine respectively. Six randomized clinical trials comprising 1,358 subjects were included in this systematic review. All studies that were included in the systematic review showcased that aloe vera had proved beneficial in reducing plaque and gingival inflammation. Four studies compared the plaque scores after using Aloe vera and Chlorhexidine mouthrinse and found that *Aloe vera* mouthwash was as effective as Chlorhexidine in reducing plaque scores, while two studies found Chlorhexidine to be more effective than *Aloe vera.* Three studies also found that *Aloe vera* mouthrinse was equally effective to Chlorhexidine in reducing gingival inflammation, while one study found Chlorhexidine to be a better choice in reducing gingival inflammation. When comparative assessment was done with respect to patient acceptability *Aloe vera* mouthrinse was better choice than Chlorhexidine since it didn't produce and side effects like bitter taste and staining to teeth as that of Chlorhexidine.[36]

Literature about antibacterial efficacy of *Momordica charantia*:

27. An experimental, in vitro study was carried to determine antibacterial action of *Momordica charantia* extract against *Streptococcus mutans* and *Lactobacillus acidophilus. Momordica charantia* extract was prepared using different solvents like petroleum ether, ethyl alcohol, and chloroform which then was diluted serially to obtain 1 mg/ml, 2.5 mg/ml, 5 mg/ml, 7.5% mg/ml, 10 mg/ml solutions in sterile test tubes using distilled water. Agar well diffusion method was used to further analyze the antimicrobial activity of the extract. It was further concluded that *Momordica charantia* has an active compound named tras-nerolidol which had potential quality to be antibacterial against both *Streptococcus mutans* and

Lactobacillus acidophilus when used chloroform, ethanol and petroleum ether as solvent.[6]

28. Comparative evaluation and assessment of antimicrobial potential of different commonly used herbs namely Neem (*Azadirachta indica*), Bitter gourd (*Momordica charantia*) was carried out to determine whether their extracts could be used as an endodontic irrigants against *Enterococcus faecalis*. Anti-bacterial susceptibility test mainly Agar well diffusion test was performed to determine zone of inhibition where inoculums of *Enterococcus faecalis* and *Candida albicans* were streaked on the blood agar plate, and wells were made using cork borers. It was found that the zone of inhibition of *Momordica charantia* extract was maximum and thus it was concluded that *Momordica charantia* has a potential anti-bacterial activity against wide range of bacteria.[37]

29. In a study that aimed to introspect in vitro antimicrobial and antioxidant activity of *Momordica charantia* extracts in aqueous and methanol form. Phytochemical analysis and thin layer chromatography was carried out to assess different phytochemicals in the extract and it was found that *Momordica charantia* extract contains glycosides, alkaloids, phytosterols, saponins, phenolic compounds, proteins, fats and fixed oils and flavonoids. Further, anti-microbial activity test and anti-bacterial susceptibility test was carried out to evaluate anti-microbial potential of ethanolic and aqueous extract of *Momordica charantia*. It was found that aqueous extract showed milder antimicrobial activity compared to ethanolic extract, which clearly depicts that ethanolic extract harbors greater concentration of active antimicrobial agents such as alkaloids, glycosides, volatile oils. Leaf extracts of *Momordica charantia* was assessed for its anti-microbial activity against *Enterococcus faecalis* using three types of solvents namely water,

ethanol, methanol where broad spectrum activity of *Momordica charantia* was determined. The study thus determined that the *Momordica charantia* extract has rich phytochemicals which have free radicals scavenging activity and thus in future more studies can be carried out to determine chemical nature of different other active ingredients and anti-oxidants present in the plant.[5]

30. The study depicts complete botanical explanation of the parts of plant *Momordica charantia* mainly its fruits, leaves, stem, etc. The study showcases the active chemical component in plant which has potent antimicrobial and antifungal action with respective to values of anti-microbial activity tests using Minimum Inhibitory Concentration (MIC) in accordance to method used in each study. The studies included in the review have analyzed that this plant contains wide diversity of chemically active ingredients with beneficial potential like charantin, α-momorcharin and MAP30, and thus highlighting its properties. The review seeks acquaint us with phytochemical and pharmacological properties of *Momordica charantia*, which would be useful for future research for determining new chemical compounds of the plant, studies on its safety and efficiency, as well as the comparative assessment of its possible synergistic act in combination with other antimicrobials, in order to develop new therapeutic substitutes against bacterial resistance.[38]

31. In an in vitro study screened for potential antimicrobial and antioxidant qualities unripe/ripe seed and fruit ethanol extracts of *Momordica charantia*. Disc diffusion and broth microdilution methods were used to determine the antimicrobial activities of the extract against four-gram positive bacteria, seven-gram negative bacteria, and one yeast. The unripe fruit extract has maximum antibacterial activity against tested microbes in the study with higher zone of inhibition and

lesser minimum bactericidal or fungicidal activities than the other extracts. Total antioxidant activity, free radical scavenging activity (DPPH assay), iron (III) and cupric ion reduction assay was used to determine the antioxidant qualities of the extract where it was found that ripe fruit extract has the strongest antioxidant capacity compared to unripe extracts.[39]

Literature about antibacterial efficacy of *Spinacia oleracea*:

32. Study conducted to inspect the in vitro antimicrobial and antioxidant activity of aqueous and ethanolic extracts of *Spinacia oleracea* leaves. It was concluded that the *Spinacia oleracea* leaves are rich in phytochemicals which have free radicals scavenging activity and in future attempts can be made to identify and isolate the organic nature of the antioxidant and anti-microbial qualities present in the plant.[9]

33. Study which aimed to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and ethanolic extracts of *Spinacia oleracea* against *Streptococcus mutans* and *Lactobacillus acidophilus*. The results of the study showed that the aqueous and ethanolic extract of *Spinacia oleracea* has a good antibacterial efficacy against *Streptococcus mutans* and *Lactobacillus acidophilus* where its antibacterial property can be attributed to its Silver nanoparticles as Silver is a natural antimicrobial agent. Along with their antimicrobial properties, Silver nanoparticles have also been reported to show antiangiogenesis, anti-inflammatory, and antiplatelet activities. This study thus concluded that there is evidence for the antimicrobial activity of *Spinacia oleracea* extracts against *Streptococcus mutans* and *Lactobacillus acidophilus* and this raises the possibility that it could be used to prevent dental caries and other oral infections such as gingival and periodontal conditions.[40]

34. An in vitro study evaluated the antimicrobial and antifungal activities of ethanolic extract of *Spinacia oleracea* leaves by carrying antibacterial activity test using Minimum Inhibitory Concentrations (MIC) and anti-bacterial susceptibility testing using well diffusion method. It was found that the ethanolic extract when used in concentration 25 mg/ml to 100mg/ml showed significant zone of inhibition ranging from 6mm to 22mm with ethanolic leaf extract having maximum zone of inhibition. It was thus found that the leaf extract has higher antibacterial and antifungal activity against bacterial and fungal species while root extract has low antibacterial and antifungal activity. It was also found that the activity of plant extract was increased by the increasing concentration of extracts where very low zone of inhibition was found at concentration 25 mg/1 ml DMSO which ranges from 6 mm to 14 mm while very high zone of inhibition was found at concentration 100 mg/ml which ranged from 6 mm to 22 mm. So, it was concluded that the ethanolic extracts of *Spinacia oleracea* has good efficacy against bacterial and fungal species.[10]

35. An in vitro study was carried out to screen the phytochemicals and to determine the anti-bacterial activity of *Spinacia oleracea*. The extract showed presence of phytochemicals including phytocobalamin, saponins, phenol, tannins, glycosides, flavonoids, steroids, terpenes and cardenolides. The extract with their phytoconstituents were reported for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities. The antibacterial activities of aqueous extract, ethanol and ethyl acetate crude extracts of the leaves were tested for studying the antibacterial activity where it was found that ethyl acetate crude extract showed maximum antibacterial effect among the three solvents. It was found that ethyl acetate crude extract being an organic solvent would dissolve

organic compounds better, hence, could release the active component required for antimicrobial activity. The study concluded that the constituents of *Spinacia oleracea* extract are rich source for pharmacological activities.[41]

36. An in vitro study evaluated the antimicrobial activities of ethanolic extract of *Spinacia oleracea* leaves by carrying antibacterial activity test using minimum inhibitory concentrations (MICs) and anti-bacterial susceptibility testing using well diffusion method. It was found that the ethanolic extract when used in concentration 25 mg/ml to 50mg/ml showed significant zone of inhibition ranging from 7mm to 23mm with ethanolic leaf extract having maximum zone of inhibition. It was thus found that the leaf extract has higher antibacterial. It was also found that very high zone of inhibition was found at concentration 100 mg/ml which ranged from 7 mm to 23 mm. So, it was concluded that the ethanolic extracts of *Spinacia oleracea* has good efficacy against bacterial species.[10]

MATERIALS AND METHOD

The present in-vitro study was designed to evaluate and compare the antibacterial efficacy of *Momordica charantia, Spinacia oleracea* mouthwash and Chlorhexidine mouthwash against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

The study was conducted in the Department of Pediatric and Preventive Dentistry at KAHER's KLE VK Institute of Dental Sciences, Belagavi in association with Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research, Belagavi

The herbs *Momordica charantia, Spinacia oleracea* were procured from market and authenticated by the Indian Council of Medical Research, Belagavi (ICMR).

The following armamentarium was used in the study. The armamentarium used was divided in the following steps as follows:

[i] Armamentarium for extract preparation [Figure No. 1]**:**

- Dried *Momordica charantia* (Bittergourd)
- *Spinacia oleracea* (Spinach)
- Grinder
- Pulverizer (Mill Power Industries, Ahemdabad, Gujarat)

- Weighing scale (Citizen CY220, Citizen Scale(I) Pvt. Ltd., Malad, Mumbai)
- Waterbath (Labline, Labline Equipments Private Limited, Vadodara, Gujarat)
- Beaker (Borosil Glass Works Limited, Mumbai)
- Ethanol (Antares Chem Pvt Ltd, Mumbai)
- Conical flask (Borosil Glass Works Limited, Mumbai)
- Whatman Qualitative filter paper (Sigma- Aldrich Co., USA)
- Parafilm (Bemis Company, Inc.)

Figure No. 1: Photograph showing armamentarium for extract preparation of *Momordica charantia, Spinacia oleracea*.

[ii] Armamentarium for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) [Figure No. 2 and 3(a) and (b)]**:**

- Ethanolic extract of *Momordica charantia*
- Ethanolic extract of *Spinacia oleracea*
- MTCC Strains of *Streptococcus mutans* & *Lactobacillus acidophilus, Porphyromonas gingivalis*
- Weighing scale (Citizen CY220, Citizen Scale (I) Pvt. Ltd., Malad, Mumbai)
- Dimethyl Sulfoxide (DMSO) [MERCK, Specialty Pvt. Ltd.]
- Eppendorf tubes (Tarsons Products Pvt. Ltd., West Bengal)
- Vortex Mixer (IKA Industries, India)
- Pipette with micro pipette tips (Tarsons Products Pvt. Ltd., West Bengal)
- Brain Heart Infusion (BHI) Broth (HiMedia laboratories Pvt. Ltd.,Mumbai)
- Incubator (Yorco, York Scientific Industries, India)
- Platinum Inoculum loops (Sigma- Aldrich Co., USA)
- Electric loop sterilizer (HiMedia laboratories Pvt. Ltd., Mumbai)
- Resazurin (Sigma- Aldrich Co., USA)
- Agar Plates (Borosil Glass Works Limited, Mumbai)

Figure No. 2: Photograph showing armamentarium for determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

a **b**

Figure No. 3: Photograph showing (a) Vortex Mixer (b) Incubator

[iii] Armamentarium for Herbal mouthwash Preparation [Figure No. 4]:

- Ethanolic extract of *Momordica charantia*
- Ethanolic extract of *Spinacia oleracea*
- Glycerine (Molychem, Maharashtra, India)
- Xylitol (HiMedia Laboratories Pvt. Ltd., Mumbai)
- Sodium Benzoate (SDFCL Sd Fine Chemicals Ltd, Chennai, Tamil Nadu)
- Methyl Paraben (SDFCL Sd Fine Chemicals Ltd, Chennai, Tamil Nadu)
- Propyl Paraben (HiMedia Laboratories Pvt. Ltd., Mumbai)
- Magnetic Stirrer (Remi Laboratory Instruments, Mumbai, Maharashtra)
- Beaker (Borosil Glass Works Limited, Mumbai)
- Conical Flask (Borosil Glass Works Limited, Mumbai)
- Distilled Water

Figure No. 4: Photograph showing magnetic stirrer used for Herbal mouthwash preparation.

[iv] Armamentarium for cytotoxicity [Figure No. 5(a) and 5(b)]**:**

- L929 fibroblast (National Centre for Cell Science, Pune, Maharashtra)
- *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash
- Chlorhexidine mouthwash
- Buffering Solution: 0.5M Phosphate Buffer (pH 7)
- Dulbecco's Modified Eagle Medium(DMEM) (Genetix biotech Asia Pvt. Ltd.)
- Dimethyl Sulfoxide (DMSO) [MERCK, Specialty Pvt. Ltd.]
- Pipette with micro pipette tips (Tarsons Products Pvt. Ltd., West Bengal)
- 96 well titer plate (SPL Life Sciences Co., Ltd., Korea)
- 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent) [HiMedia laboratories Pvt Limited, Mumbai; CAS no.: 298-93-1]
- Compound Microscope (Olympus CH20iBIMF, Olympus Pvt. Ltd.,Noida)
- Laminar Air Flow (Yorco, York Scientific Industries, India)
- CO_2 Incubator (New Brunswick, Eppendorf, Canada)
- Microplate Absorbance Reader (Bio-Rad Laboratories India Pvt. Ltd., Haryana, India)

(a)

Figure No. 5(a): Photograph showing armamentarium for cytotoxicity evaluation.

(b)

Figure No. 5(b): Photograph showing microplate absorbance reader used for cytotoxicity evaluation.

[v] Armamentarium for Agar Well Diffusion Method [Figure No. 6]**:**

- *Momordica charantia* mouthwash
- *Spinacia oleracea* mouthwash
- Chlorhexidine mouthwash
- MTCC strains of *Streptococcus mutans* & *Lactobacillus acidophilus.*
- Agar Plates (Borosil Glass Works Limited, Mumbai)
- Brain Heart Infusion Agar (HiMedia laboratories Pvt. Ltd.,Mumbai)
- Incubator (Yorco, York Scientific Industries, India)
- Pipette and micro pipette tips (Tarsons Products Pvt. Ltd., West Bengal)
- Zone of Inhibition Interpretation Scale (HiMedia laboratories Pvt. Ltd.,Mumbai)
- Cotton Swab (HiMedia laboratories Pvt. Ltd.,Mumbai)
- Weighing Scale (Citizen CY220, Citizen Scale (I) Pvt. Ltd., Malad, Mumbai)
- Eppendorf tubes (Tarsons Products Pvt. Ltd., West Bengal)
- Parafilm (Bemis Company, Inc.)
- Conical Flask (Borosil Glass Works Limited, Mumbai)

Figure No. 6: Photograph showing armamentarium for Agar Well Diffusion Method.

[v] Armamentarium for Time Kill Assay [Figure No. 7(a) and 7(b)]**:**

- *Momordica charantia* mouthwash
- *Spinacia oleracea* mouthwash
- Chlorhexidine mouthwash
- MTCC strains of *Streptococcus mutans* & *Lactobacillus acidophilus.*
- Brain Heart Infusion (BHI) Broth (HiMedia laboratories Pvt. Ltd.,Mumbai)
- Eppendorf tubes (Tarsons Products Pvt. Ltd., West Bengal)
- Test Tubes (Borosil Glass Works Limited, Mumbai)
- Pipette with micro pipette tips (Tarsons Products Pvt. Ltd., West Bengal)
- Equilots
- Absorbance Reader (Shimadzu Corporation, Kyoto, Japan)

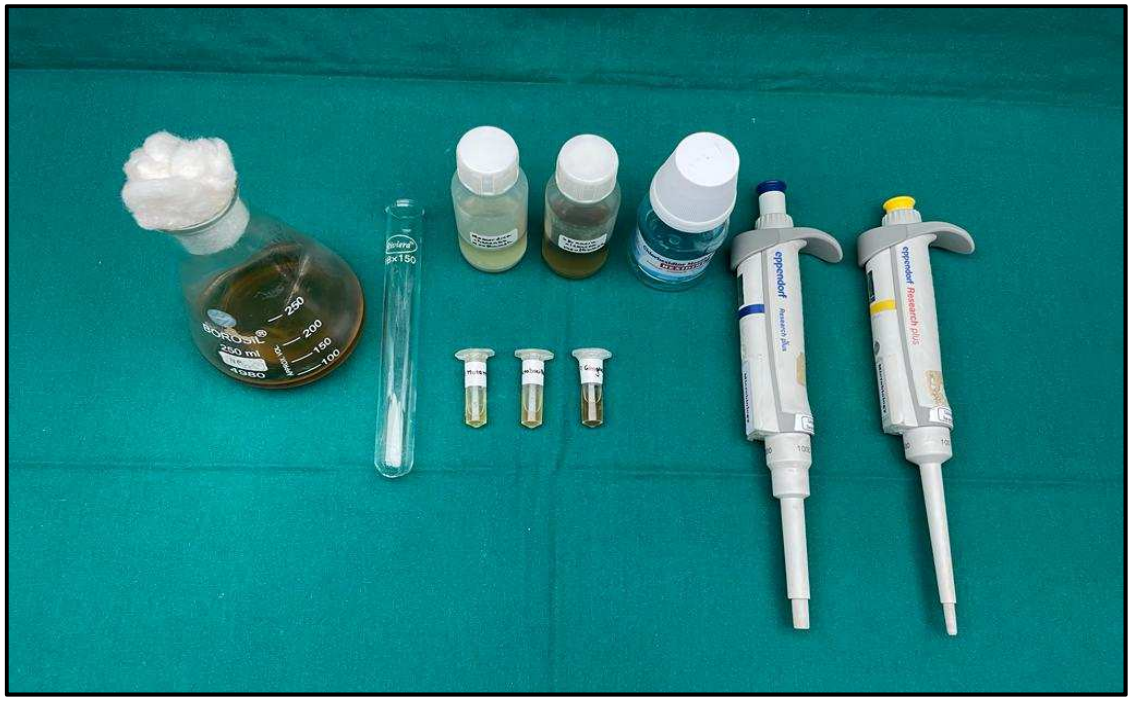


Figure No. 7(a): Photograph showing armamentarium for Time Kill Assay.

Figure No. 7(b): Photograph showing absorbance reader used for Time Kill Assay.

SOURCE OF DATA:

The study was conducted at Department of Pediatric and Preventive Dentistry, KLE V.K. Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi.

SAMPLE SIZE:[5]

1. Evaluation of Antibacterial effectiveness

Sample size given was n=5,

where n= number of times the antibacterial test is repeated for each organism.

STUDY GROUP:

The study groups were equally divided into two groups namely:

- **Group I (Control Group)**: Chlorhexidine mouthwash
- **Group II (Experimental Group)**: *Momordica charantia* mouthwash
- **Group III (Experimental Group)**: *Spinacia oleracea* mouthwash

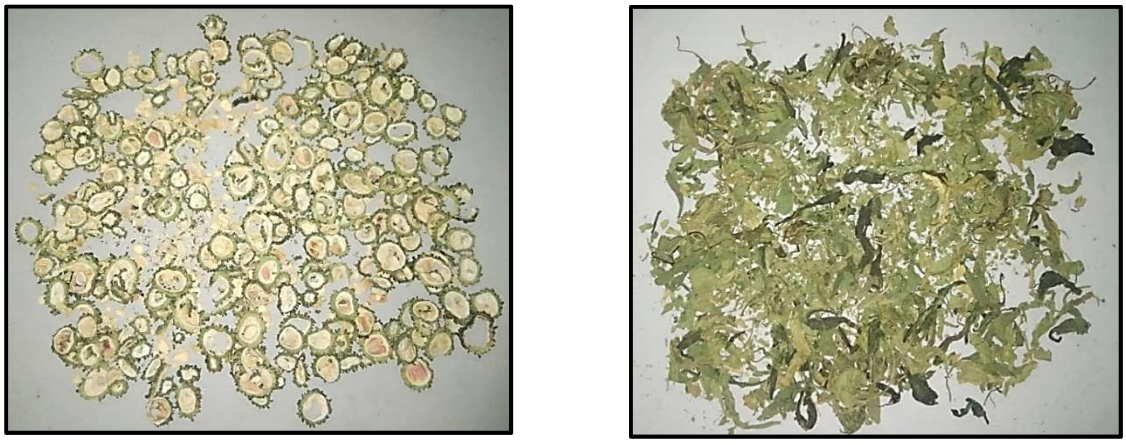
METHOD OF COLLECTION OF DATA

A) PREPARATION OF EXTRACT:

The extract was prepared in the KLE Academy of Higher Education and Research's Dr. Prabhakar Kore Basic Science Research Center, Belagavi.

Preparation of ethanolic extract of *Momordica charantia and Spinacia oleracea*:

500 grams of bitter gourd and spinach obtained from local market which were thoroughly washed and cut into small pieces, shed dried at room temperature for 2-3 days under standard aseptic condition and then collected in a jar [Figure No. 8(a) and 8(b)]. The dried pieces were powdered in mixer grinder [Figure No. 9(a) and 9(b)]. 20-gram Powder was poured in 100 ml of ethanol [Figure No. 10(a) and 10(b)]. The solution was kept for 48 hours with continuous mixing on rotary shaker [Figure No. 11]. The extract was then filtered through a sterilized Whatman No.1 filter paper (Sigma Labsys) [Figure No. 12(a) and 12(b)] to obtain solution containing ethyl alcohol with dissolved active ingredients. The solvent was then evaporated using electric water bath to obtain concentrated extract [Figure No. 13(a) and 13(b)]

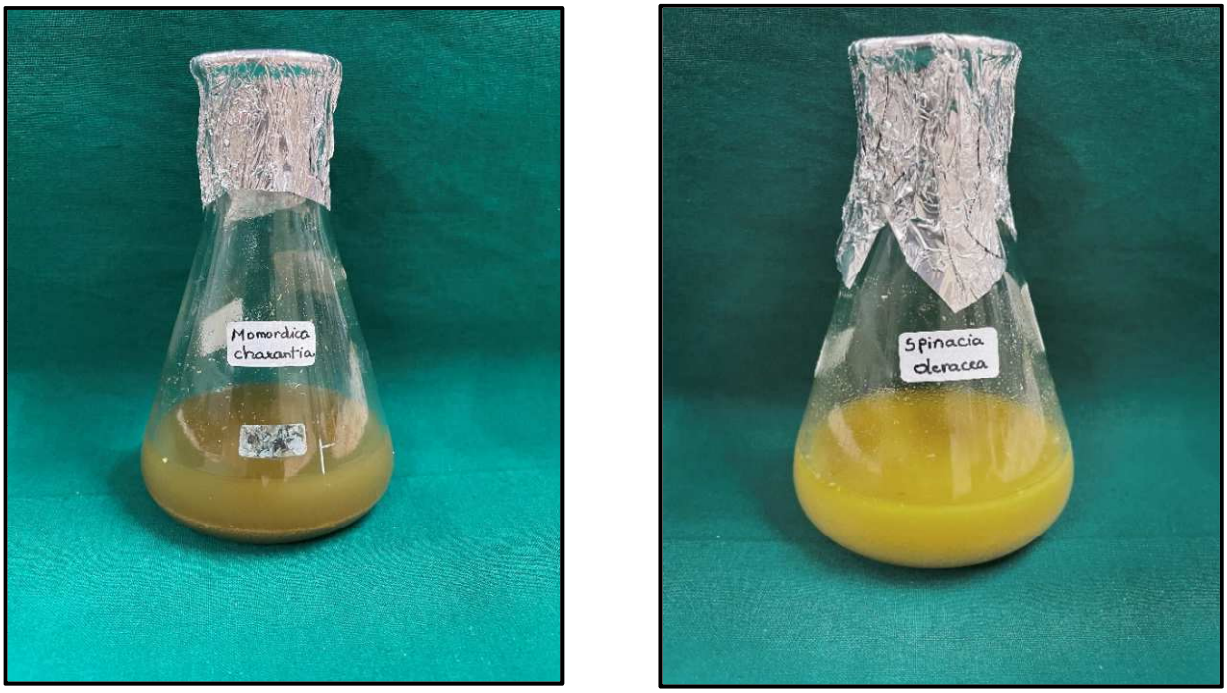


(a) **(b)**

Figure No. 8(a) and 8(b): Photograph showing *Momordica charantia* and *Spinacia oleracea* kept for shed drying

(a) **(b)**

Figure No. 9(a) and 9(b): Photograph showing grounded *Momordica charantia* and *Spinacia oleracea*.



(a) **(b)**

Figure No. 10(a) and 10(b): Photograph showing extraction of ethanolic extract of *Momordica charantia* and *Spinacia oleracea.*

Figure No. 11: Photograph showing rotary incubator used in the study.

(a)

(b)

Figure No. 12(a) and 12(b): Photograph showing filtration of ethanolic extract of *Momordica charantia* and *Spinacia oleracea.*

(a) **(b)**

Figure No. 13(a) and 13(b): Photograph showing ethanolic extract of *Momordica charantia* and *Spinacia oleracea.*

B) MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION DETERMINATION

Minimum Inhibitory Concentration (MIC) was determined against strains of *Streptococcus mutans, Lactobacillus acidophilus and Porphyromonas gingivalis.* Inoculum of standard strains of organisms was prepared as per 0.5 McFarland Standard.

Methodology for Minimum Inhibitory Concentration (MIC) Determination by Resazurin Method:[43]

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. Resazurin method was used for determination of Minimum Inhibitory Concentration. The ethanolic extract of *Momordica charantia* and *Spinacia oleracea* was weighed and dissolved in 0.5 ml of Dimethyl sulfoxide present in micro centrifuge tube (Eppendorf) and was vortexed. The Standard Operating Protocols of vertical laminar flow were followed. 96 well culture plates were taken and 100 µl of the freshly prepared microbiological media was added in specified wells using micropipette. 100 µl of the prepared working solution of the ethanolic extracts was added in the first well and then serially diluted to requisite concentrations up to the 12^{th} well. Excess diluted extract was pipetted and discarded from the 12^{th} tube. Broth and extract solution were thoroughly mixed using micropipette. 10 µl of inoculum of bacteria (MTCC strains of *Streptococcus mutans* & *Lactobacillus acidophilus*) was added in each well except positive control. The 96 well plates were then incubated for 24 hours at 37 degree Celsius. 20 µl of 0.015% freshly prepared resazurin solution was added to each well. The 96 well plates were then incubated for 1-4 hours at 37 degrees. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent

resorufin (pink) which can be further reduced to hydroresorufin. The concentration at which resazurin was just reduced to resofurin by colour change from to blue to slight pink was taken as Minimum Inhibitory Concentration [Figure No. 14,15,16].

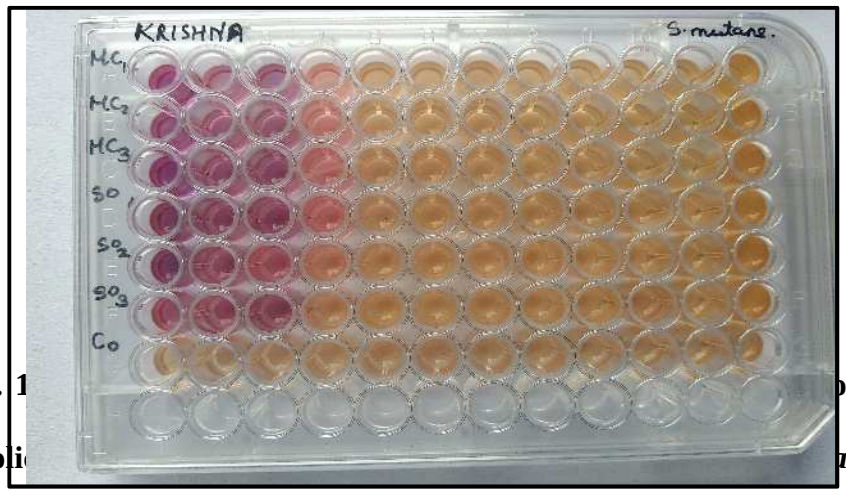


Figure No. 1 **on (MIC)** **for ethanoli** ***a*** **against** ***Streptococcus mutans.***

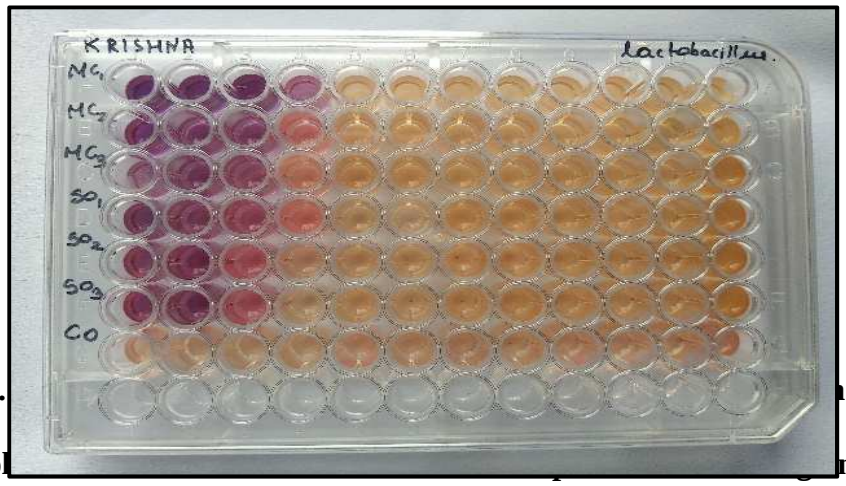


Figure No. **(MIC)** **for ethanol** **nst** ***Lactobacillus acidophilus.***

Figure No. 16: Photograph showing Minimum Inhibitory Concentration (MIC) for ethanolic extract of *Momordica charantia* and *Spinacia oleracea* against *Porphyromonas gingivalis*.

Methodology for Minimum Bactericidal Concentration Determination (MBC) by Agar Plate Streaking Method:[43]

Minimum Bactericidal Concentration is the lowest concentration of an antimicrobial agent required to kill a microorganism. Agar plate streaking method was used for determination of Minimum Bactericidal Concentration.

Microbiological media was prepared by adding 5.2 gm of Brain Heart Infusion Agar into 100 ml of distilled water in a conical flask. This solution was autoclaved for 15-20 minutes and then poured into glass plates. These plates were then cooled down to room temperature under UV light.

The Minimum Bactericidal concentration (MBC) was determined by streaking the supernatant solutions from the micro tubes used for Minimum Inhibitory Concentration of each organism in Brain Heart Infusion broth media using a sterile streaking loop in laminar air flow chamber (Yorco, York Scientific Industries, India) and incubating them for 24 hours in incubator (Yorco, York Scientific Industries, India) at 37 ^{0}C. The

lowest concentration at which there is no growth of bacteria is seen in the culture plate was taken as Minimum Bactericidal Concentration.

The Minimum Bactericidal Concentration (MBC) for ethanolic extract of *Momordica charantia* and *Spinacia oleracea* was carried out against test organisms i.e. *Streptococcus mutans* [Figure Nos. 17,20], *Lactobacillus acidophilus* [Figure Nos. 18, 21], *Porphyromonas gingivalis* [Figure Nos. 19,22] respectively**.**

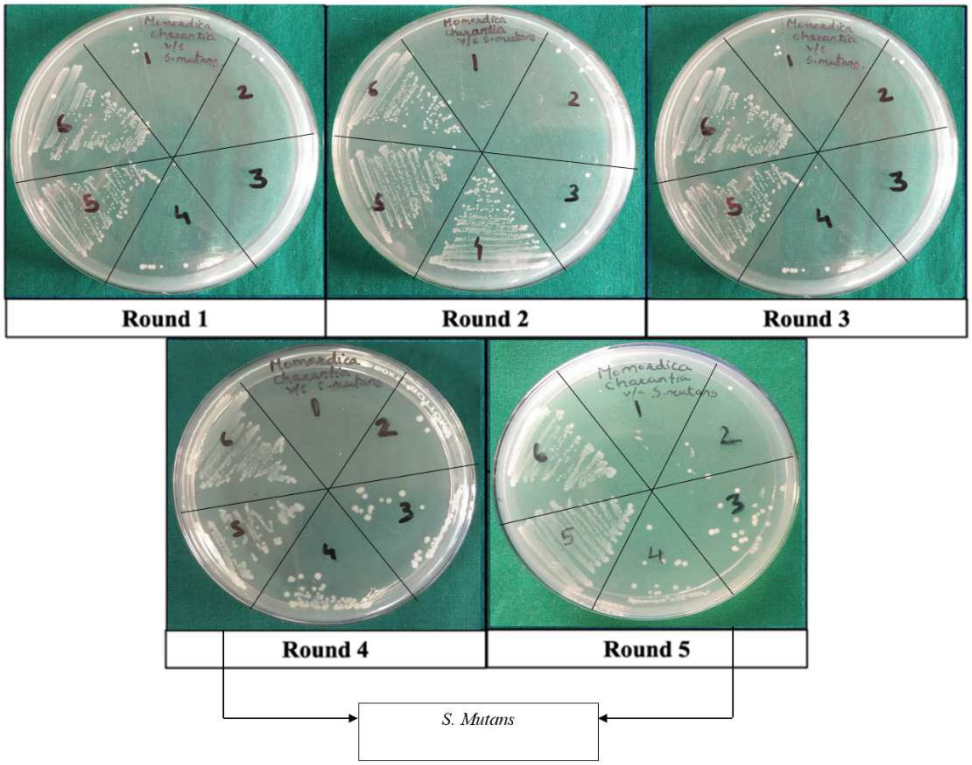


Figure No. 17: Photograph showing Minimum Bactericidal Concentration (MBC) for *Momordica charantia* against *Streptococcus mutans.*

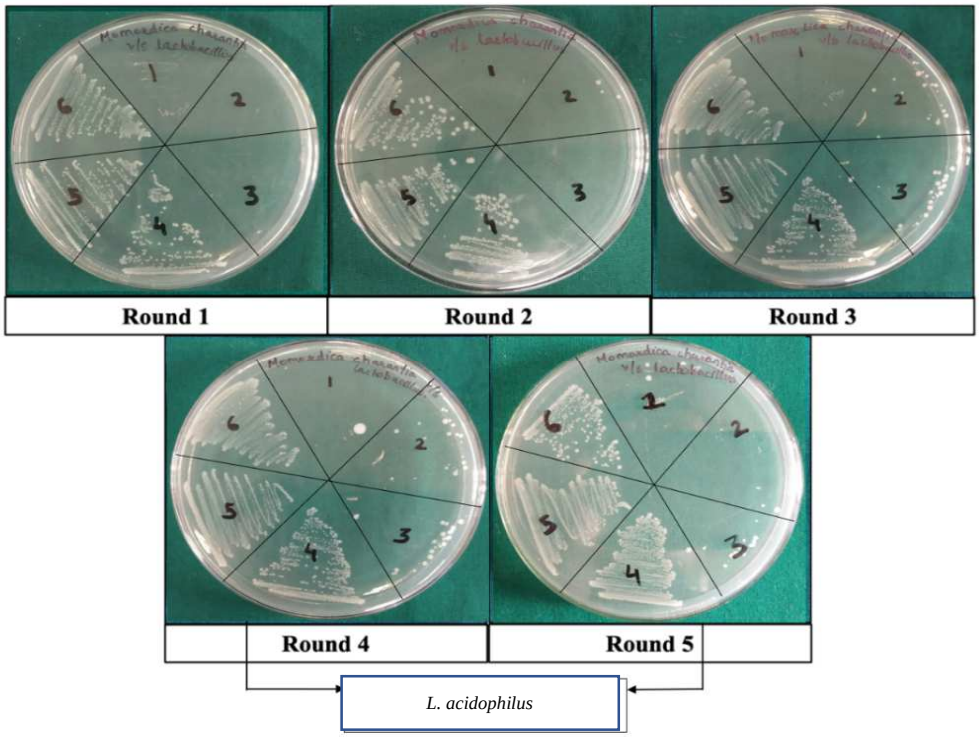


Figure No. 18: Photograph showing Minimum Bactericidal Concentration (MBC) for *Momordica charantia* against *Lactobacillus acidophilus.*

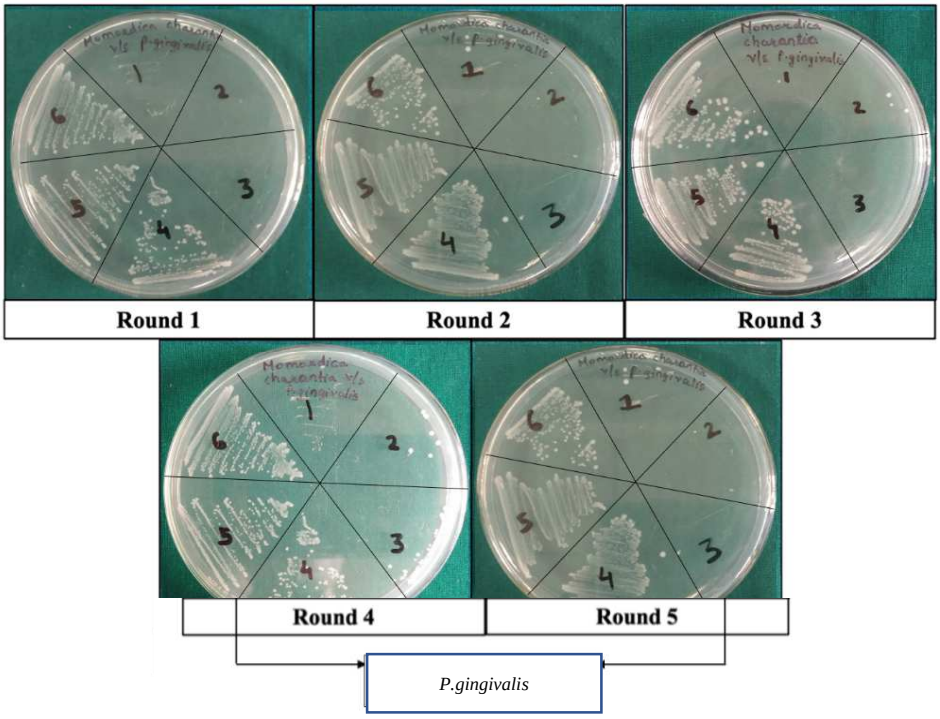


Figure No. 19: Photograph showing Minimum Bactericidal Concentration (MBC) for *Momordica charantia* against *Porphyromonas gingivalis*

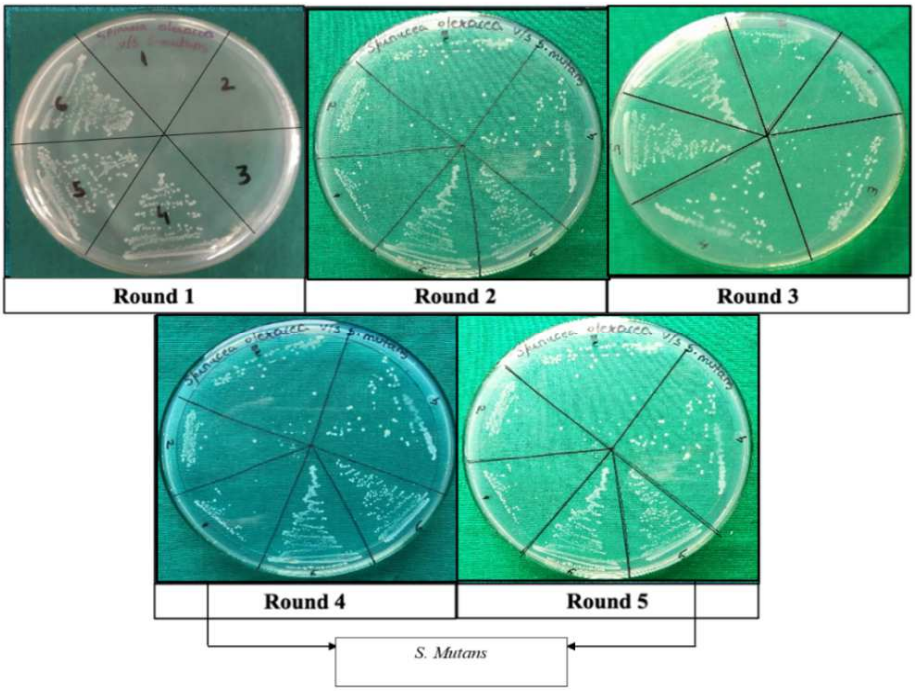


Figure No. 20: Photograph showing Minimum Bactericidal Concentration (MBC) for *Spinacia oleracea* against *Streptococcus mutans.*

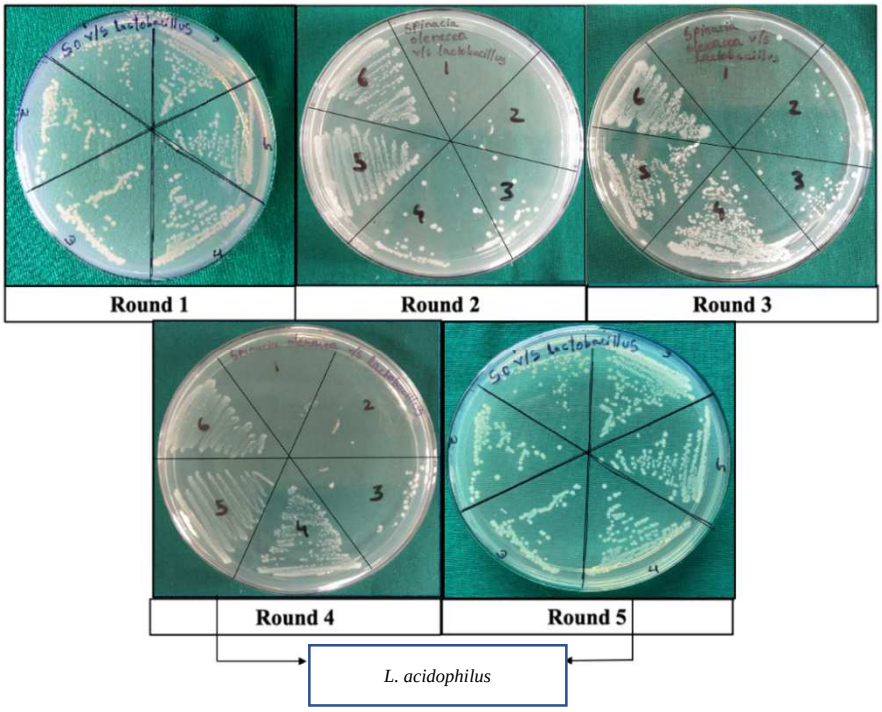


Figure No. 21: Photograph showing Minimum Bactericidal Concentration (MBC) for *Spinacia oleracea* against *Lactobacillus acidophilus*

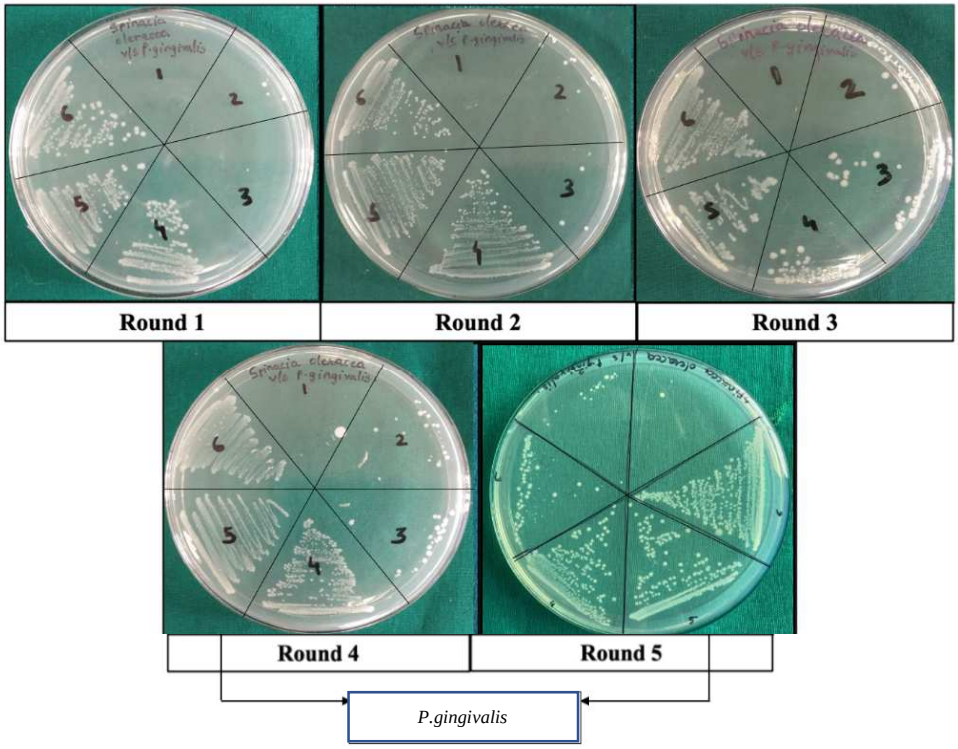


Figure No. 22: Photograph showing Minimum Bactericidal Concentration (MBC) for *Spinacia oleracea* against *Porphyromonas gingivalis.*

Preparation of *Momordica charantia* and *Spinacia oleracea* mouthwash:

The mouthwash was prepared in the KLE Academy of Higher Education and Research's College of Pharmacy, Department of Pharmaceutics, Belagavi.

Based on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), 0.25g of extract of *Momordica charantia* and *Spinacia oleracea* was used to prepare *Momordica charantia* and *Spinacia oleracea* mouthwash. To overcome any changes in the property of extract while adding other components for mouthwash preparation. Initially, in 50 ml of distilled water sodium benzoate; methyl and propyl parabens were dissolved with aid of magnetic stirrer (KI-66-02, Kumar Sales Corporation, Maharashtra, India) [Figure no. 23] for 15 min at room temperature to form solution 1. Then, in 50 ml of distilled water xylitol and glycerin were dissolved

to get solution 2. Finally, solution 1 and solution 2 were filtered through Whatman filter paper no.40, filtrate was mixed with *Momordica charantia* and *Spinacia oleracea* extract separately for each mouthwash and stirred in magnetic stirrer for 30 minutes at room temperature. The final mouthwash was poured into two separate bottles with tight lid and stored at room temperature. The final mouthwash obtained was clear and 25 ml in quantity [Figure no. 24].

Table No. 1: Table showing composition of formulation of *Momordica charantia mouthwash and Spinacia oleracea mouthwash*

Sl.No.	Material	Function	Formulation
1	Herbal extract	Antibacterial	62.5 mg/25ml
2	Xylitol	Sweetening agent	2% w/w
3	Glycerin	Humectant	5% w/w
4	Sodium benzoate	Preservative (Bacteriostatic)	0.1% w/w
5	Methyl paraben	Preservative (Bactericidal)	0.05% w/w
6	Propyl paraben	Preservative (Bactericidal)	0.01% w/w
7	Distilled water	Vehicle	100ml

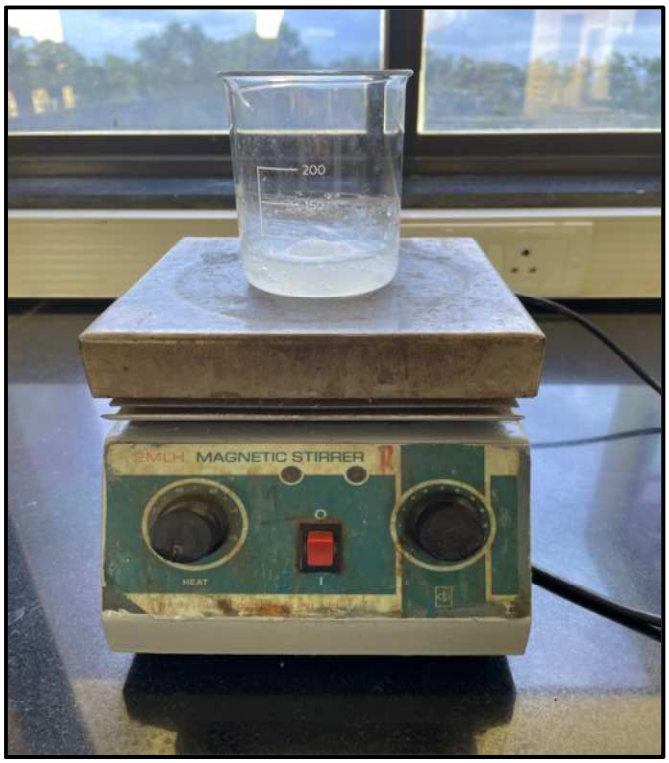


Figure No. 23: Photograph showing mixture kept on magnetic stirrer.

Figure No. 24: Photograph showing *Momordica charantia mouthwash and Spinacia oleracea mouthwash*

D) CYTOTOXICITY ASSAY[44]

Cytotoxicity Assay Principle: This is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g., DMSO, Isopropanol) and the released, solubilized formazan reagent is measured with spectrophotometer. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Procedure: 5 mg of MTT reagent was added to 1 ml of Phosphate Buffer Saline (PBS – pH 7.4) and kept aside. Detachment of cells from flask was carried out by manually shaking them. Cells were centrifuged at 3500 rpm for 4 minutes. A small pellet was formed at the bottom and the supernatant media was discarded [Figure No. 25]. 5 ml of fresh media was added and mixed thoroughly.

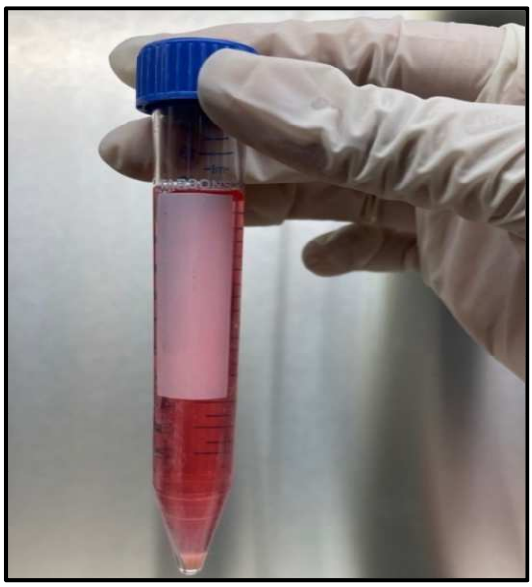


Figure No. 25: Photograph showing formation of pellet at bottom during manual detachment of cells.

50 µl of 1 × 10^5 cells/ml cell suspension of the extract were seeded into each well in a 96 well micro titre plate and final volume was made up to 150 µl by adding DMEM media (Dulbecco Modified Eagle Medium)**.** DMEM media is a modification of Basal Medium Eagle (BME) that contains four-fold concentrations of amino acids and vitamins. It also includes glycine, serine and ferric nitrate along with high concentration of glucose [Figure No. 26].

Seeding of cells was done followed by counting. They were incubated for 24 hours in in presence of 5 % CO2, at 37^0C into CO_2 incubator.

Figure No. 26: Photograph showing tissue culture plate with seeded fibroblast cells in DMEM media.

After 24 hours, 100 µl of test compounds were added to the wells and incubated for 24 hours in presence of 5 % CO2, at 37^0C into CO_2 incubator [Figure No. 27]. 20µl of 5 mg/ ml MTT reagent was added to each well [Figure No. 28]. The plate was kept for 4 hours incubation. The plate was covered with aluminium foil, since MTT reagent is photosensitive.

Figure No. 27: Photograph showing tissue culture plate with addition of test compounds.

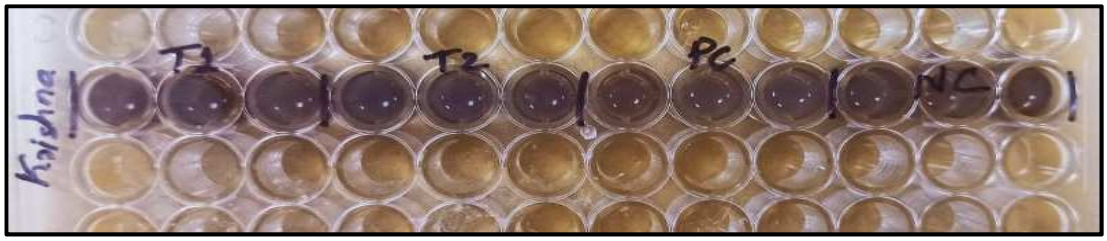


Figure No. 28: Photograph showing tissue culture plate after addition of MTT reagent.

The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 200 µl of DMSO (dimethyl sulfoxide) was added to dissolve the crystals formed. The absorbance of this coloured solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The degree of light absorption depends on the solvent. The optical density (OD) was measured at wavelength of 570 nm. The surviving fibroblasts were also observed under microscope [Figure No. 29a, 29b, 29c].

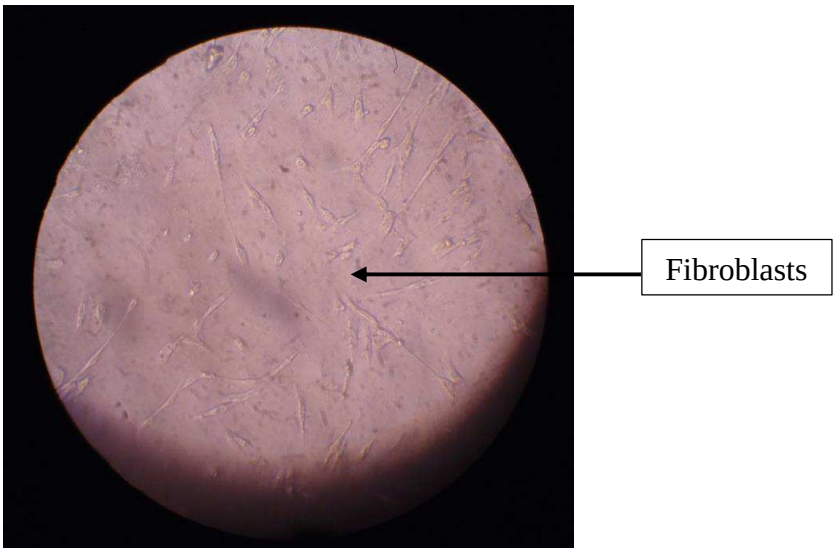


Figure No. 29a: Photograph showing microscopic picture of the surviving fibroblasts with *Chlorhexidine* mouthwash.

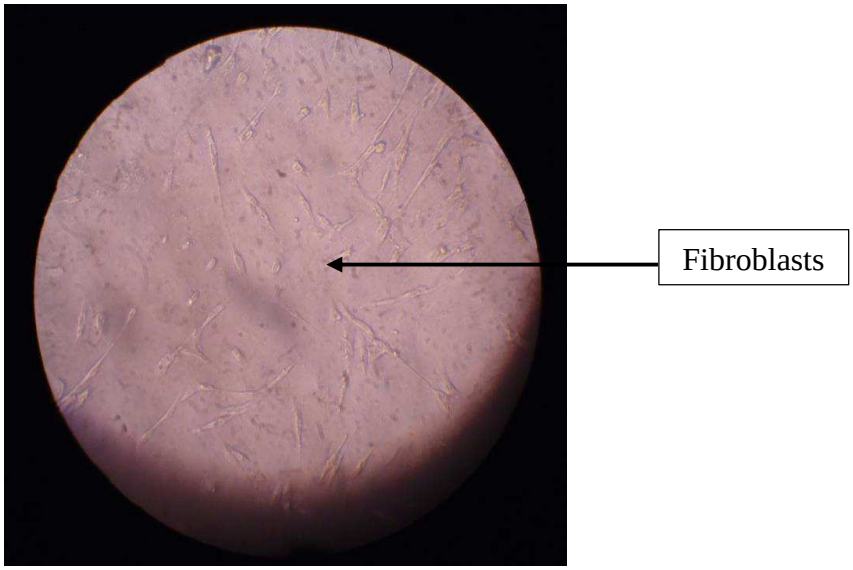


Figure No. 29b: Photograph showing microscopic picture of the surviving fibroblasts with *Momordica charantia* mouthwash.

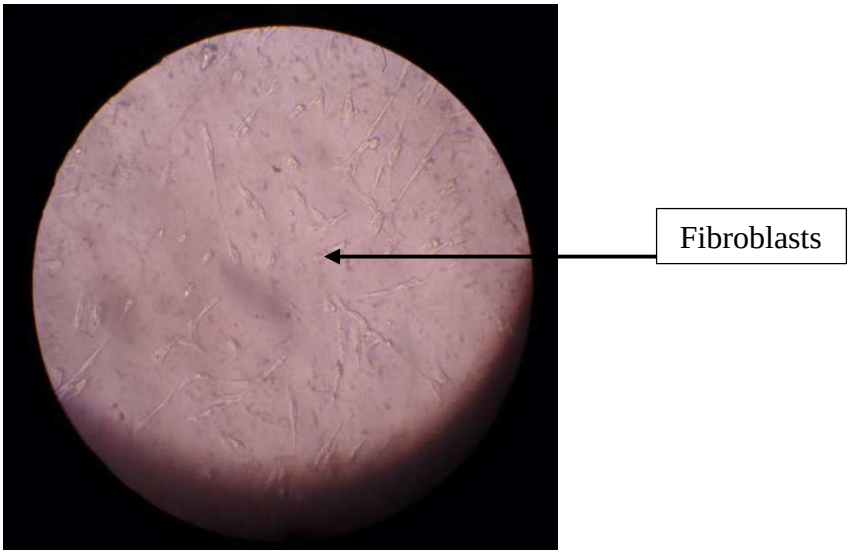


Figure No. 29c: Photograph showing microscopic picture of the surviving fibroblasts with *Spinacia oleracea* mouthwash.

E) ANTIBACTERIAL SUSCEPTIBILITY TESTING:

Antibacterial susceptibility for Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea* mouthwash (Group III) was tested against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* by Agar Well Diffusion Method and Time Kill Assay.

Procedure for antimicrobial susceptibility using Agar Well Diffusion Method: [45]

Microbiological media was prepared by adding 5.2 gm of Brain Heart Infusion Agar into 100 ml of distilled water in a conical flask. This solution was autoclaved for 15-20 minutes and then poured into glass plates. These plates were then cooled down to room temperature under the UV light.

The Standard Operating Protocols of vertical laminar flow were followed. Agar plates were inoculated with the standardized inoculum of the test microorganisms i.e., *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis*. Three wells with a diameter of 6 to 8 mm, equidistant from each other were punched aseptically following which the test compounds i.e., Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash were introduced into the wells. The agar plates were then incubated for 24 hours. The test compounds diffused in the agar medium and inhibited the growth of the bacteria. A zone of inhibition appeared on the agar plate, whose dimensions were recorded using a standardised scale [Figure No. 30-32].

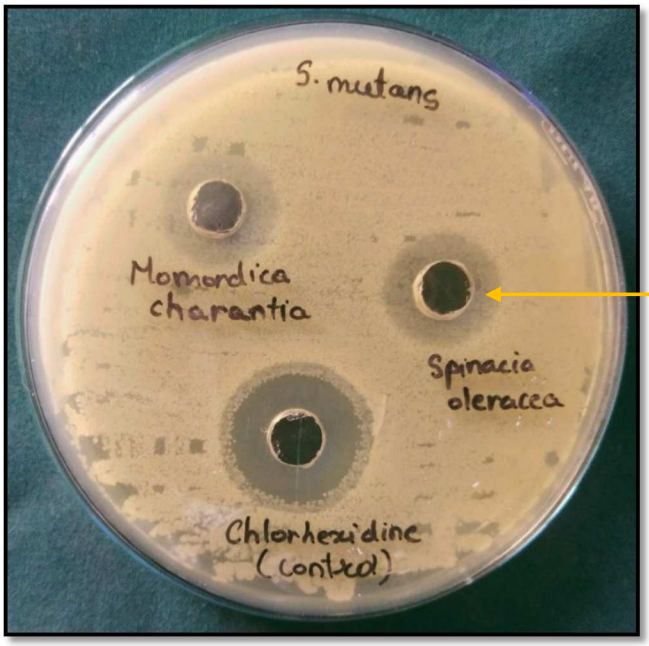


Figure No. 30: Photograph showing Zone of Inhibition of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash, against *Streptococcus mutans*.

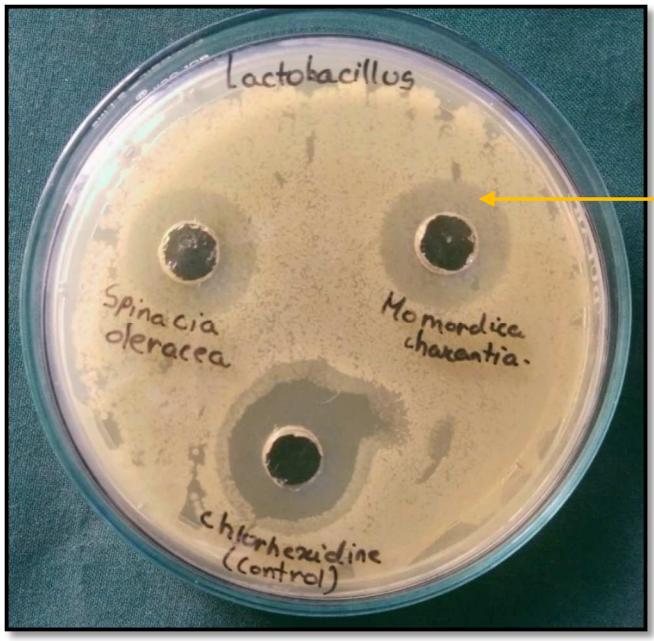


Figure No. 31: Photograph showing Zone of Inhibition of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash against *Lactobacillus acidophilus.*

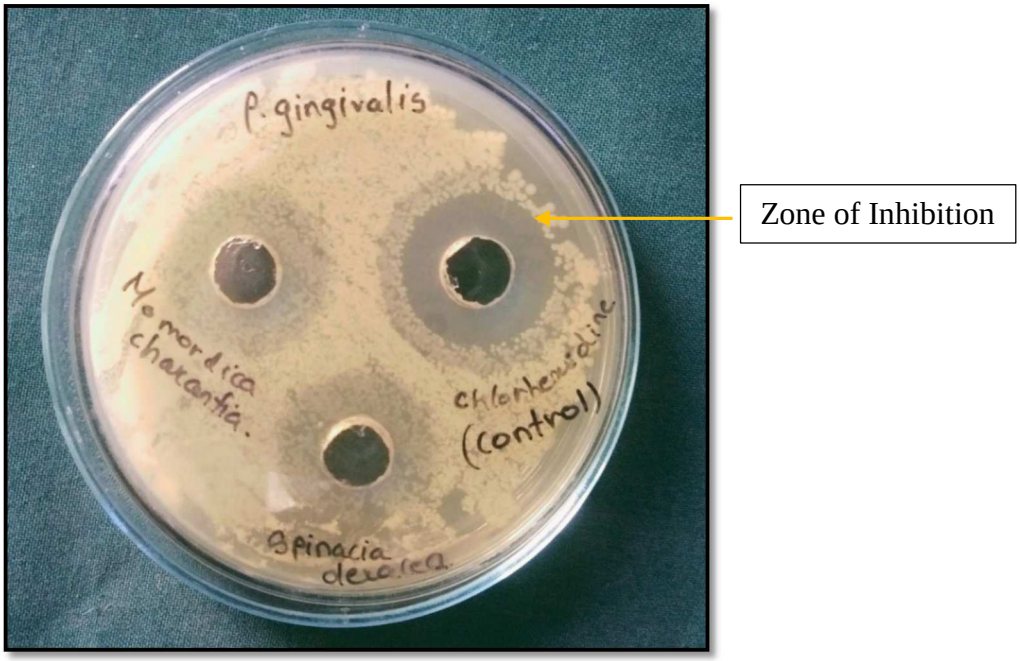


Figure No. 32: Photograph showing Zone of Inhibition of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash, against *Porphyromonas gingivalis*

Procedure for antimicrobial susceptibility using Time Kill Assay:[45]

Time Kill Assay was performed by counting the viability of the bacterial strains at different time intervals under the effect of antimicrobial agents. A solution of test compounds was prepared by adding 1 ml of prepared Brain Heart Infusion Broth with 1 ml of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash respectively in separate test-tubes. 2 ml of aseptic broth was taken as negative control [Figure No. 33, 34, 35].

To obtain the time kill curve, the growth rate of bacterial strains was counted at different time intervals starting i.e., at 0, 2, 4, 6, 8 and 24 hours.

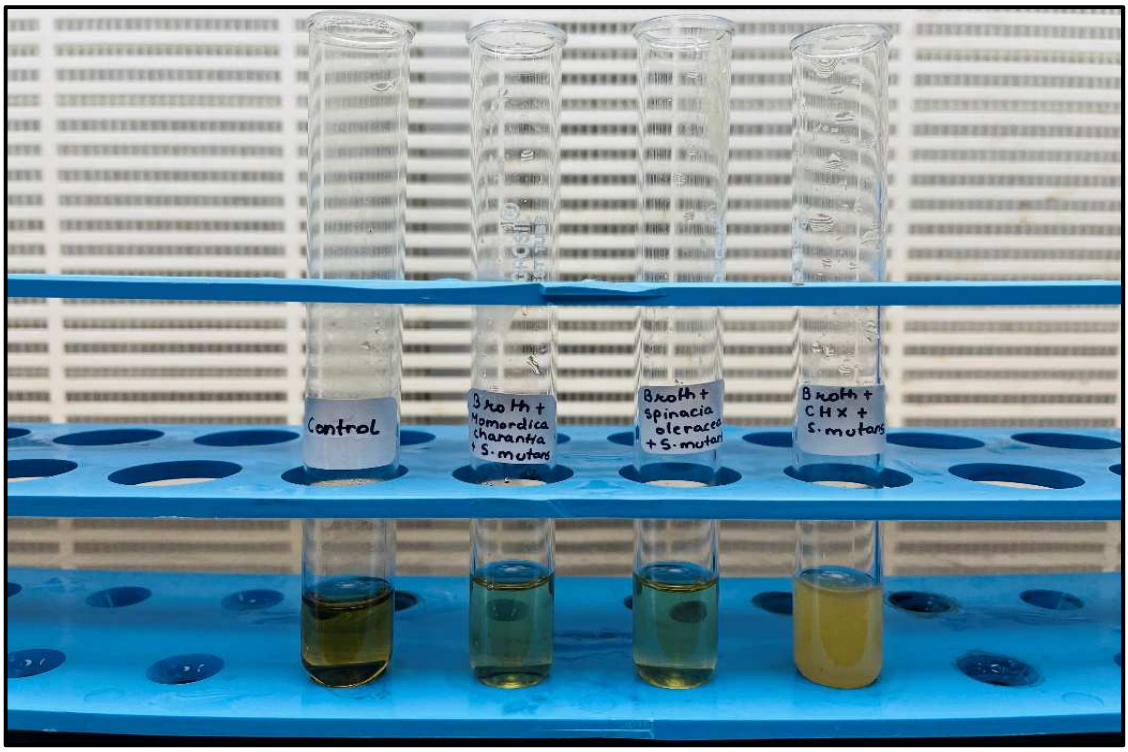


Figure No. 33: Photograph showing prepared stock solutions of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash against *Streptococcus mutans*.

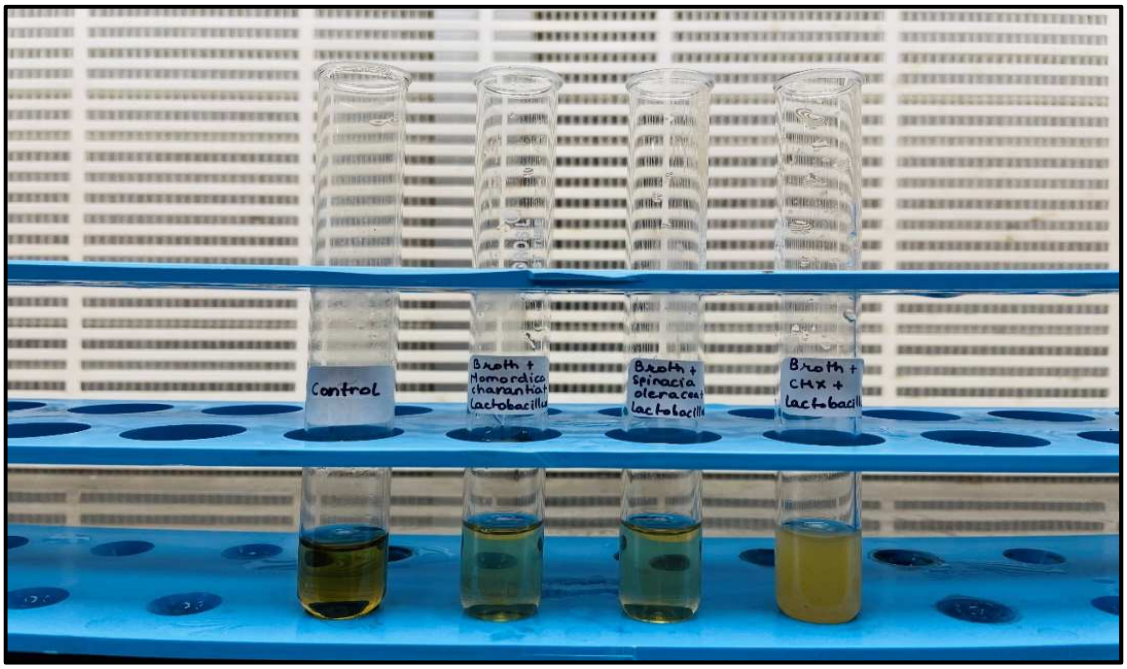


Figure No. 34: Photograph showing prepared stock solutions of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash against *Lactobacillus acidophilus.*

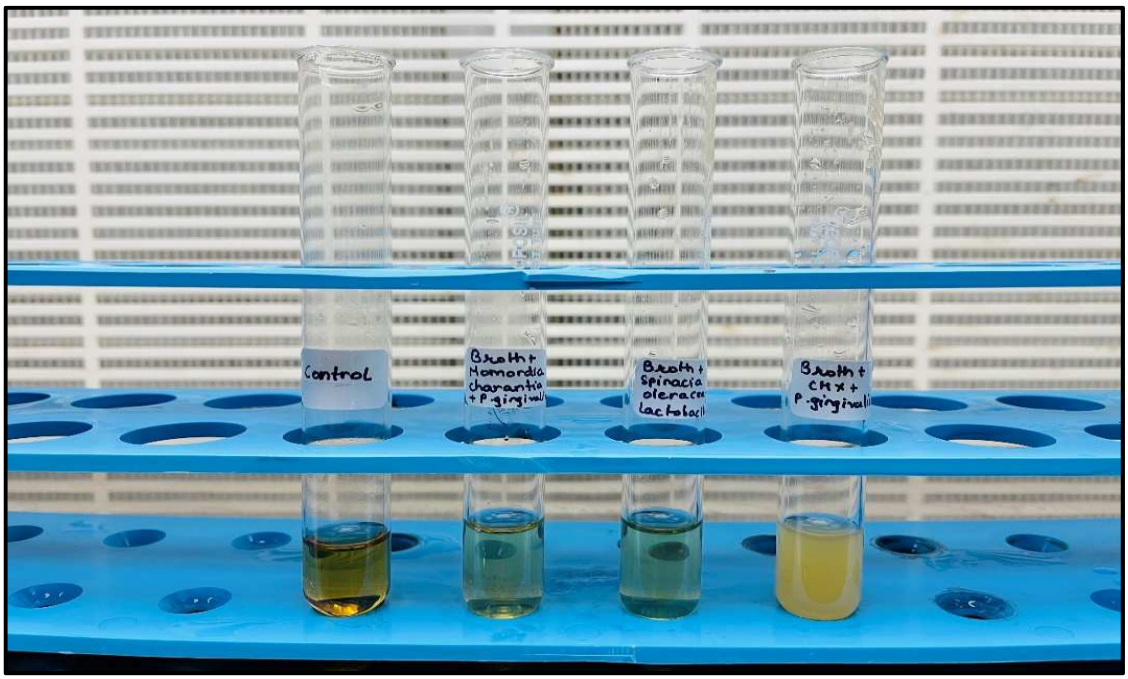


Figure No. 35: Photograph showing prepared stock solutions of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash against *Porphyromonas gingivalis.*

STATISTICAL ANALYSIS

The results were tabulated and entered on the excel sheet. Then the results were subjected to the following statistical tests using IBM SPSS software (version 20.0, Bangalore).

The results were tabulated and entered on the excel sheet. Then the results will be subjected to the statistical tests.

Statistical analysis for Antibacterial tests was done using:

- Fischer exact test for comparison between two groups
- Two-way Repeated Measures ANOVA for comparison of antibacterial effectiveness of two groups
- Chi square test
- Dependent ‘t’ test and Independent ‘t’ test

RESULTS

TABLES, GRAPHS AND OBSERVATIONS

The present in-vitro study was designed to evaluate and compare the antibacterial efficacy of Chlorhexidine mouthwash, *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations of ethanolic extracts of *Momordica charantia and Spinacia oleracea* were determined against *Streptococcus mutans* and *Lactobacillus acidophilus* and *Porphyromonas gingivalis.* Based on the obtained values, preparation of Herbal mouthwash was carried out. The three study groups, namely, Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea* mouthwash (Group III) were subjected to cytotoxicity test, antibacterial susceptibility tests.

The respective data was entered in the Excel sheet and master charts were prepared accordingly. The data was further subjected to statistical analysis using standardized tests that are Chi square test, Two Way ANOVA, Fisher exact test, Dependent ‘t’ test and Independent ‘t’ test respectively.

Table 2: Table showing master chart of the Minimum Inhibitory Concentration (MIC) of *Momordica charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Streptococcus mutans* at various concentrations.

Streptococcus mutans										
Minimum Inhibitory Concentration (MIC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	S	R	R	R	R	R	R	R	R
4	S	S	R	R	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R	R	R
Average MIC value for *Momordica charantia* v/s *Streptococcus mutans* is 2.5mg/ml										
Minimum Inhibitory Concentration (MIC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	S	R	R	R	R	R	R	R	R
4	S	S	R	R	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R	R	R
Average MIC value for Spinacia oleracea v/s *Streptococcus mutans* is 2.5mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml = milligram/ milliliter.

Table 3: Table showing master chart of the Minimum Inhibitory Concentration (MIC) of *Momordica charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Lactobacillus acidophilus* at various concentrations.

Lactobacillus acidophilus										
Minimum Inhibitory Concentration (MIC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	S	R	R	R	R	R	R	R	R
4	S	S	R	R	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R	R	R
Average MIC value for *Momordica charantia* v/s *Lactobacillus* acidophilus is 2.5mg/ml										
Minimum Inhibitory Concentration (MIC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	R	R	R	R	R	R	R	R	R
4	S	R	R	R	R	R	R	R	R	R
5	S	R	R	R	R	R	R	R	R	R
Average MIC value for *Spinacia oleracea* v/s *Lactobacillus acidophilus* is 4mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml =milligram/ milliliter.

Table 4: Table showing master chart of the Minimum Inhibitory Concentration (MIC) of Momordica *charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Porphyromonas gingivalis* at various concentrations.

Porphyromonas gingivalis										
Minimum Inhibitory Concentration (MIC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	S	R	R	R	R	R	R	R	R
4	S	S	R	R	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R	R	R
Average MIC value for *Momordica charantia* v/s *Porphyromonas gingivalis* is 2.5mg/ml										
Minimum Inhibitory Concentration (MIC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
3	S	R	R	R	R	R	R	R	R	R
4	S	R	R	R	R	R	R	R	R	R
5	S	R	R	R	R	R	R	R	R	R
Average MIC value for *Spinacia oleracea* v/s *Porphyromonas gingivalis* is 4mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml =milligram/ milliliter.

Table 5: Comparison of sensitivity patterns of *Momordica charantia* extract and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* using Fisher exact test.

Organisms	***Momordica charantia***	**%**	***Spinacia oleracea***	**%**	**Total**	**%**
S. mutans						
Sensitive	2	20.00	2	20.00	4	20.00
Resistance	8	80.00	8	80.00	16	80.00
Fisher exact test, p=1.0000						
L. acidophilus						
Sensitive	2	20.00	1	10.00	3	15.00
Resistance	8	80.00	9	90.00	17	85.00
Fisher exact test, p=1.0000						
P. gingivalis						
Sensitive	2	20.00	1	10.00	3	15.00
Resistance	8	80.00	9	90.00	17	85.00
Fisher exact test, p=1.0000						
Total	10	100.00	10	100.0	20	100.0

Graph No. 1: Graphical representation of the comparison of sensitivity patterns of *Momordica charantia* extract and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* using Fisher exact test.

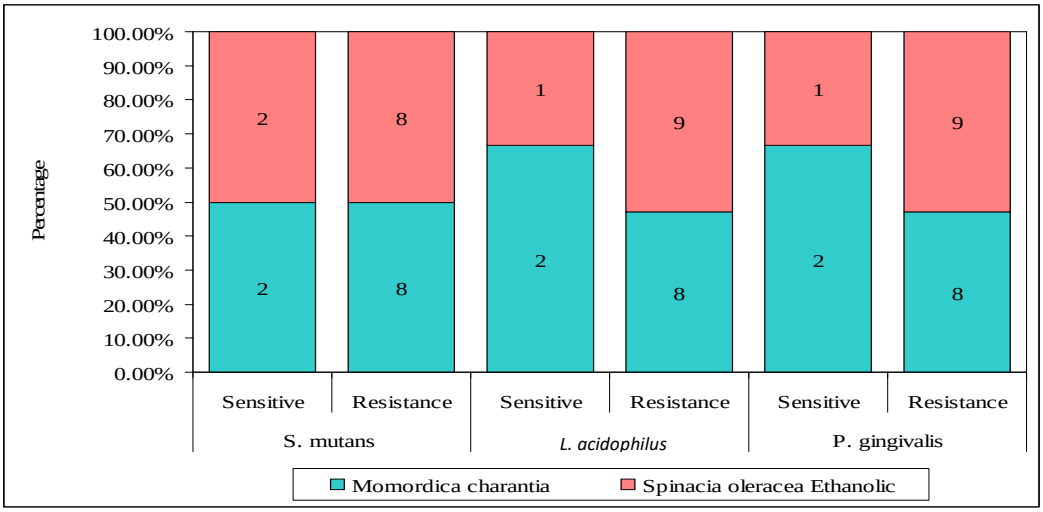


Table No. 5 and Graph No. 1 shows comparison of *Momordica charantia* extract and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* with respect to their sensitivity patterns using Fisher exact test. 20 percent of *Streptococcus mutans* were sensitive to both *Momordica charantia* extract and *Spinacia oleracea* extract with statistically insignificant difference (p=1.0). 20 percent of *Lactobacillus acidophilus* organism were sensitive to *Momordica charantia* extract when compared to 10 percent of *Spinacia oleracea* extract with statistically insignificant difference (p=1.0). 20 percent of *Porphyromonas gingivalis* organism were sensitive to *Momordica charantia* extract when compared to 10 percent of *Spinacia oleracea* extract with statistically insignificant difference (p=1.0). The sensitivity of *Momordica charantia* extract was found to be similar to *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* even though there were no statistically significant (p=1.0) differences.

Table 6: Table showing master chart of the Minimum Bactericidal Concentration (MBC) of *Momordica charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Streptococcus mutans* at various concentrations.

Streptococcus mutans										
Minimum Bactericidal Concentration (MBC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	NG	G	G	G	G	G	G	G	G
2	NG	NG	G	G	G	G	G	G	G	G
3	NG	NG	G	G	G	G	G	G	G	G
4	NG	NG	G	G	G	G	G	G	G	G
5	NG	NG	G	G	G	G	G	G	G	G
Average MBC value for *Momordica charantia* v/s *Streptococcus mutans* is 5 mg/ml										
Minimum Bactericidal Concentration (MBC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	NG	G	G	G	G	G	G	G	G
2	NG	NG	G	G	G	G	G	G	G	G
3	NG	NG	G	G	G	G	G	G	G	G
4	NG	NG	G	G	G	G	G	G	G	G
5	NG	NG	G	G	G	G	G	G	G	G
Average MBC value for *Spinacia oleracea* v/s *Streptococcus mutans* is 5 mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml = milligram/ milliliter.

Table 7: Table showing master chart of the Minimum Bactericidal Concentration (MBC) of *Momordica charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Lactobacillus acidophilus* at various concentrations.

Lactobacillus acidophilus										
Minimum Bactericidal Concentration (MBC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	NG	G	G	G	G	G	G	G	G
2	NG	NG	G	G	G	G	G	G	G	G
3	NG	NG	G	G	G	G	G	G	G	G
4	NG	NG	G	G	G	G	G	G	G	G
5	NG	NG	G	G	G	G	G	G	G	G
MBC value for *Momordica charantia* v/s *Lactobacillus acidophilus* is 5mg/ml										
Minimum Bactericidal Concentration (MBC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	G	G	G	G	G	G	G	G	G
2	NG	G	G	G	G	G	G	G	G	G
3	NG	G	G	G	G	G	G	G	G	G
4	NG	G	G	G	G	G	G	G	G	G
5	NG	G	G	G	G	G	G	G	G	G
Average MBC value for *Spinacia oleracea* v/s *Lactobacillus acidophilus*. is 10 mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml =milligram/ milliliter.

Table 8: Table showing master chart of the Minimum Bactericidal Concentration (MBC) of *Momordica charantia* ethanolic extract, *Spinacia oleracea* ethanolic extract against *Porphyromonas gingivalis* at various concentrations.

Porphyromonas gingivalis										
Minimum Bactericidal Concentration (MBC)										
MCEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	G	G	G	G	G	G	G	G	G
2	NG	G	G	G	G	G	G	G	G	G
3	NG	G	G	G	G	G	G	G	G	G
4	NG	G	G	G	G	G	G	G	G	G
5	NG	G	G	G	G	G	G	G	G	G
Average MBC value for *Momordica charantia* v/s *Porphyromonas gingivalis* is 10 mg/ml										
Minimum Bactericidal Concentration (MBC)										
SOEE mg/ml	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1	NG	G	G	G	G	G	G	G	G	G
2	NG	G	G	G	G	G	G	G	G	G
3	NG	G	G	G	G	G	G	G	G	G
4	NG	G	G	G	G	G	G	G	G	G
5	NG	G	G	G	G	G	G	G	G	G
Average MBC value for *Spinacia oleracea* v/s *Porphyromonas gingivalis* is 10 mg/ml										

MCEE= *Momordica charantia* Ethanolic Extract, SOEE= *Spinacia oleracea* Ethanolic Extract.

S= Sensitive; R=Resistance; mg/ml =milligram/ milliliter.

Table 9: Comparison of sensitivity patterns of *Momordica charantia* and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* using Fisher exact test.

Organisms	***Momordica charantia***	**%**	***Spinacia oleracea***	**%**	**Total**	**%**
S. mutans						
Growth	8	80.0	8	80.00	16	80.00
No growth	2	20.0	2	20.00	4	20.00
Fisher exact test, p=1.0000						
L.acidophilus						
Growth	8	80.0	9	90.00	17	85.00
No growth	2	20.0	1	10.00	3	15.00
Fisher exact test, p=1.0000						
P. gingivalis						
Growth	9	90.0	9	90.00	18	90.00
No growth	1	10.0	1	10.00	2	10.00
Fisher exact test, p=1.0000						
Total	10	100.0	10	100.00	20	100.00

Graph No. 2: Graphical representation of comparison of sensitivity patterns of *Momordica charantia* and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* using Fisher exact test.

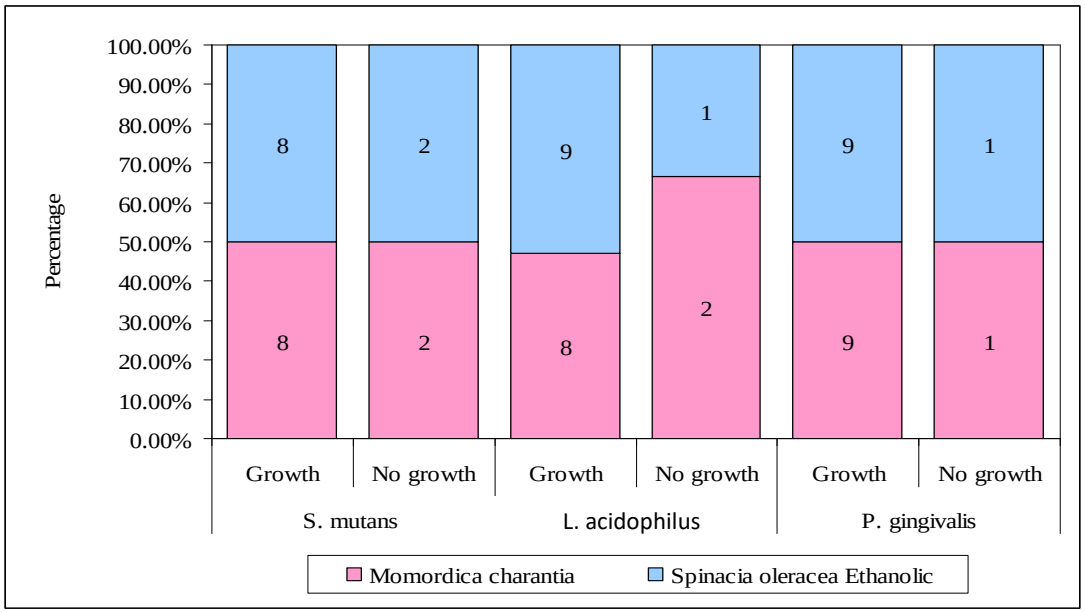


Table No. 9 and Graph No. 2 shows comparison of sensitivity patterns of *Momordica charantia* extract and *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* using Fisher exact test. 80 percent of *Streptococcus mutans* were sensitive to both *Momordica charantia* extract and *Spinacia oleracea* extract with statistically insignificant difference (p=1.0). 80 percent of *Lactobacillus acidophilus*. organism were sensitive to *Momordica charantia* extract when compared to 90 percent of *Spinacia oleracea* extract with statistically insignificant difference (p=1.0).

90 percent of *Porphyromonas gingivalis* organism were sensitive to both *Momordica charantia* extract and *Spinacia oleracea* extract with statistically insignificant difference (p=1.0).

The sensitivity of *Momordica charantia* extract was found to be similar to *Spinacia oleracea* extract against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas*

gingivalis even though there was no statistically significant (p=1.0) differences.

Table 10: Comparison of Antibacterial susceptibility (in mm) of three groups of mouthwashes against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis* organisms

Sl. No.	Mouthwashes	*S.mutans*	*L. acidophilus*	*P. gingivalis*
1	Chlorhexidine mouthwash	20	21	21
2	*Momordica charantia* mouthwash	16	20	19
3	*Spinacia oleracea* mouthwash	18	17	17

Graph No. 3: Graphical representation of Comparison of Antibacterial susceptibility (in mm) of three groups of mouthwashes against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis* organisms

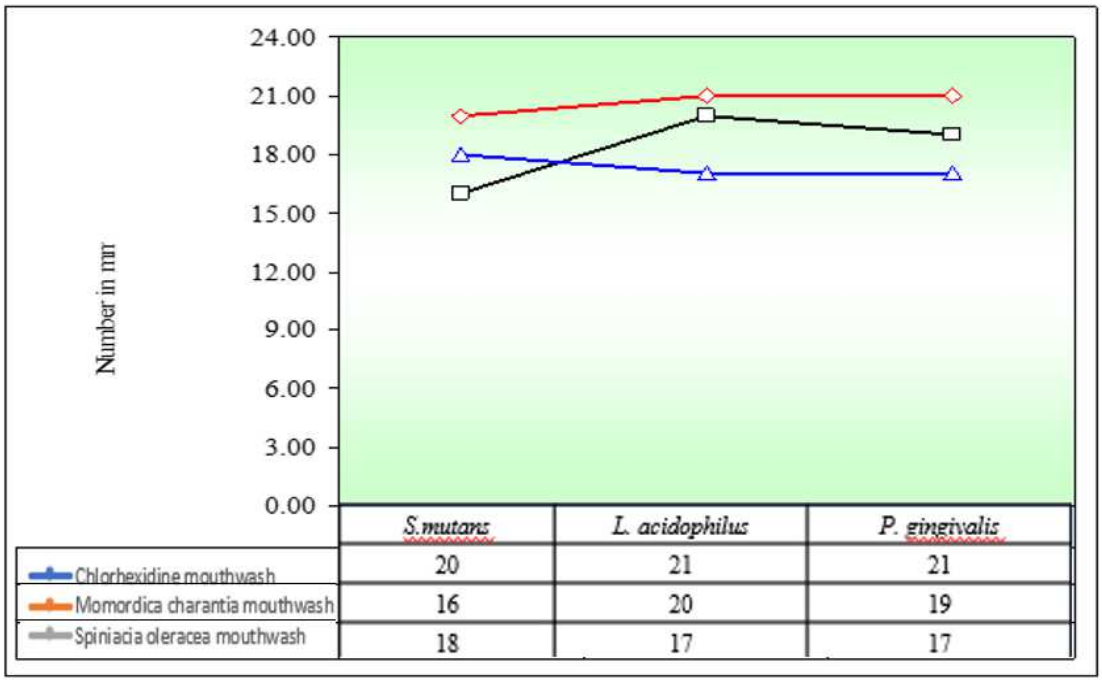


Table No. 10 and Graph No. 3 shows comparison of Antibacterial susceptibility (in mm) of three groups of mouthwashes Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) with *Streptococcus mutans*, *Lactobacillus acidophilus* and *Porphyromonas gingivalis* organism.

Zone of inhibition in mm denotes that all three groups of mouthwashes Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash, have antibacterial efficacy and there is no statistically significant difference among the three groups of mouthwashes.

Table 11: Summary and comparison of MTT Assay results of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash by Kruskal Wallis ANOVA

Mouthwash	Mean	SD	Mean rank	CV	% of viability
Chlorhexidine	0.56	0.11	10.50	19.42	97.8
***Momordica charantia* mouthwash**	0.44	0.06	7.67	13.28	106.0
***Spinacia oleracea* mouthwash**	0.37	0.05	3.83	12.08	108.7
Negative control	0.37	0.08	4.00	20.85	100.0
H-value	7.1396				
P-value	0.0676				

Graph No. 4: Graphical representation showing comparison of MTT Assay of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash.

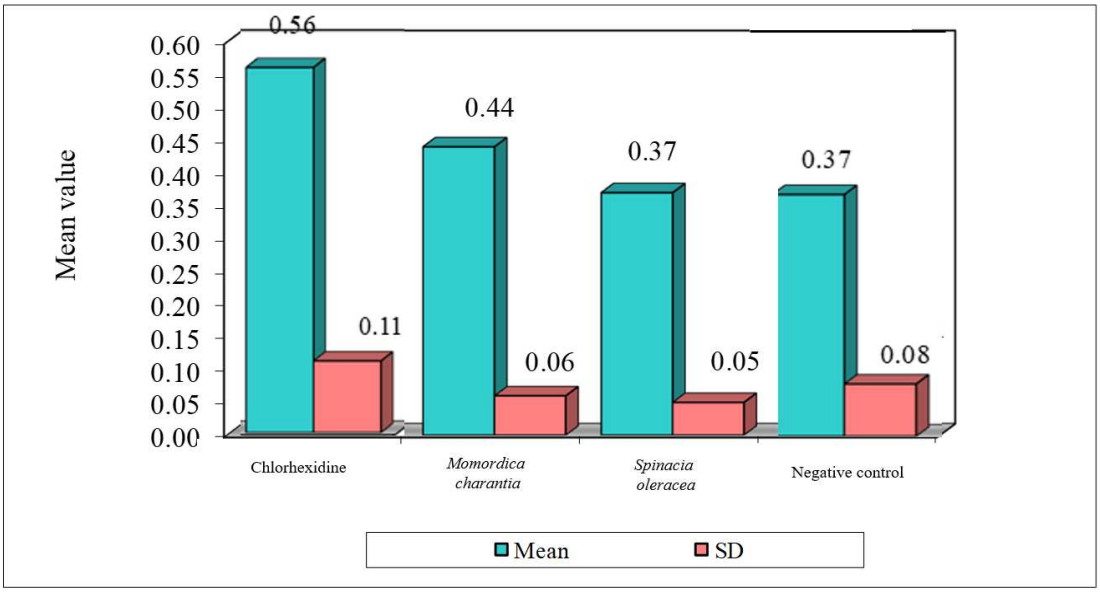
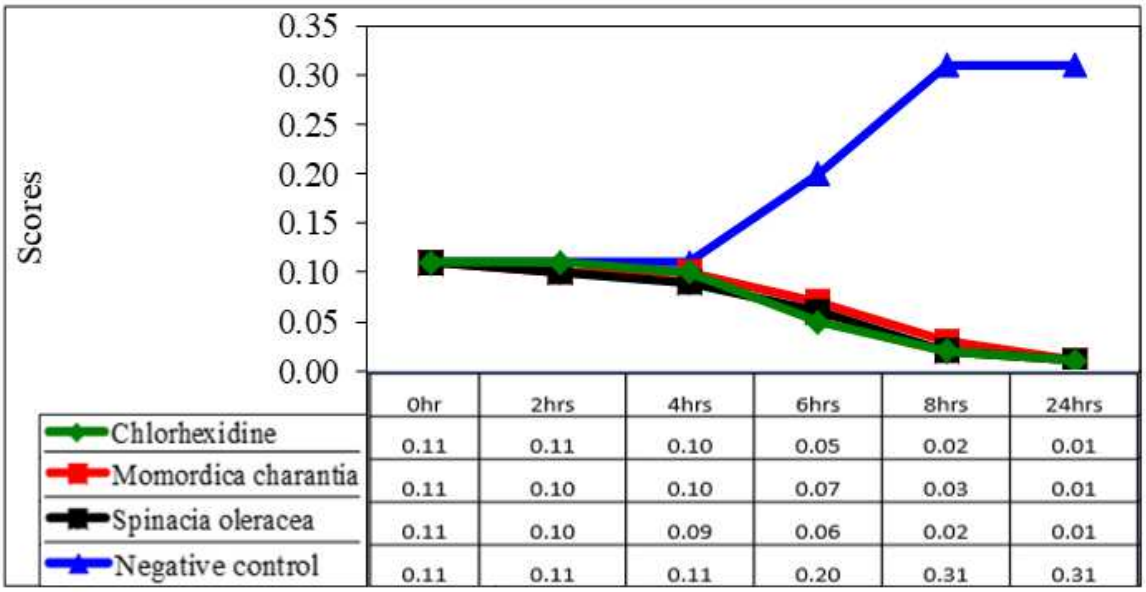


Table No.11 and Graph No. 4 shows cytotoxic evaluation by MTT Assay, it was observed that the viability of L929 mouse fibroblast was observed to be 97.8% for Chlorhexidine mouthwash, 106% for *Momordica charantia* mouthwash, 106% for *Spinacia oleracea* mouthwash which indicates that all three mouthwashes are biocompatible.

Table No. 12: Table showing the antibacterial susceptibility with respect to Time Kill Assay scores of three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Streptococcus mutans* at various time intervals.

Groups	0hr	2hrs	4hrs	6hrs	8hrs	24hrs
Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Momordica charantia	0.11	0.10	0.10	0.07	0.03	0.01
Spinacia oleracea	0.11	0.10	0.09	0.06	0.02	0.01
Negative control	0.11	0.11	0.11	0.20	0.31	0.31

Graph No. 5: Graphical representation showing the antibacterial susceptibility with respect to Time Kill Assay scores of three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Streptococcus mutans* at various time intervals.



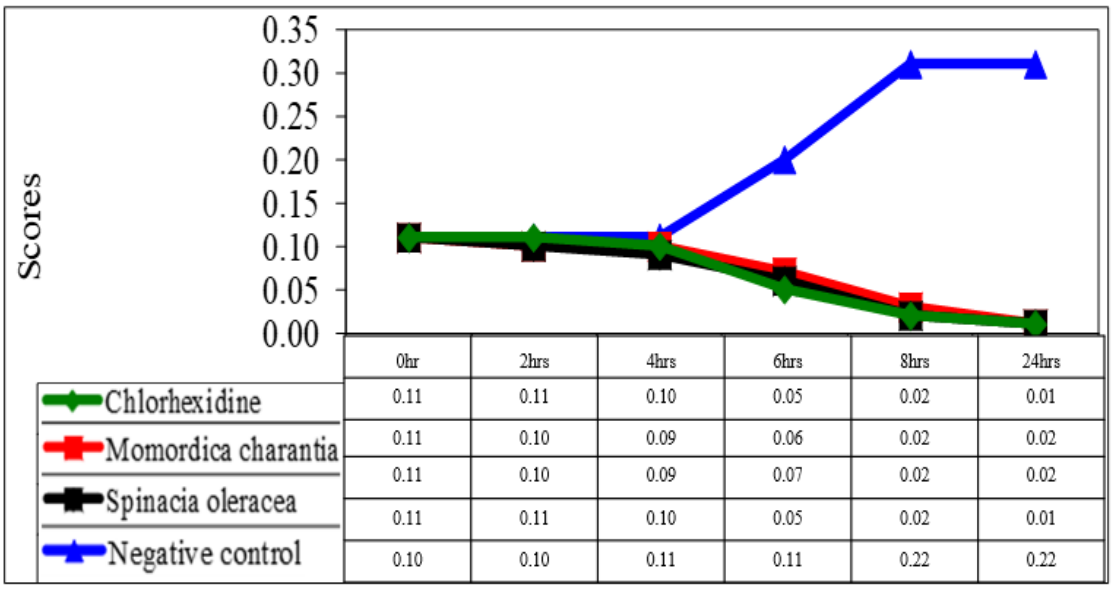
	0hr	2hrs	4hrs	6hrs	8hrs	24hrs
Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Momordica charantia	0.11	0.10	0.10	0.07	0.03	0.01
Spinacia oleracea	0.11	0.10	0.09	0.06	0.02	0.01
Negative control	0.11	0.11	0.11	0.20	0.31	0.31

Table No. 12 and Graph No. 5 denotes comparison of Chlorehexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash with Time Kill Assay scores against *Streptococcus mutans*. The Log CFU/ ml for all groups is determined at time 0 and at subsequent time points upto 24 hrs. Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash exhibited bacteriostatic effect upto 4hrs as log CFU/ml remained roughly same as the starting CFU/ml concentration however, after 4 hrs the three test compounds exhibited bactericidal effect reducing the starting log CFU/ml by greater than 1 log. The negative control exhibited very little antimicrobial effect as bacteria in presence of this compound grew over time.

Table No. 13: Table showing the antibacterial susceptibility with respect to Time Kill Assay scores in three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Lactobacillus acidophilus* at various time intervals

Groups	0hr	2hrs	4hrs	6hrs	8hrs	24hrs
Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Momordica charantia	0.11	0.10	0.09	0.06	0.02	0.02
Spinacia oleracea	0.11	0.10	0.09	0.07	0.02	0.02
Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Negative control	0.10	0.10	0.11	0.11	0.22	0.22

Graph No. 6: Graphical representation showing the antibacterial susceptibility with respect to Time Kill Assay scores in three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Lactobacillus acidophilus* at various time intervals



	0hr	2hrs	4hrs	6hrs	8hrs	24hrs
Chlorhexidine	0.11	0.11	0.10	0.05	0.02	0.01
Momordica charantia	0.11	0.10	0.09	0.06	0.02	0.02
Spinacia oleracea	0.11	0.10	0.09	0.07	0.02	0.02
Negative control	0.11	0.11	0.10	0.05	0.02	0.01
	0.10	0.10	0.11	0.11	0.22	0.22

Table No. 13 and Graph No. 6 denotes comparison of Chlorehexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash with Time Kill Assay scores against *Lactobacillus acidophilus*

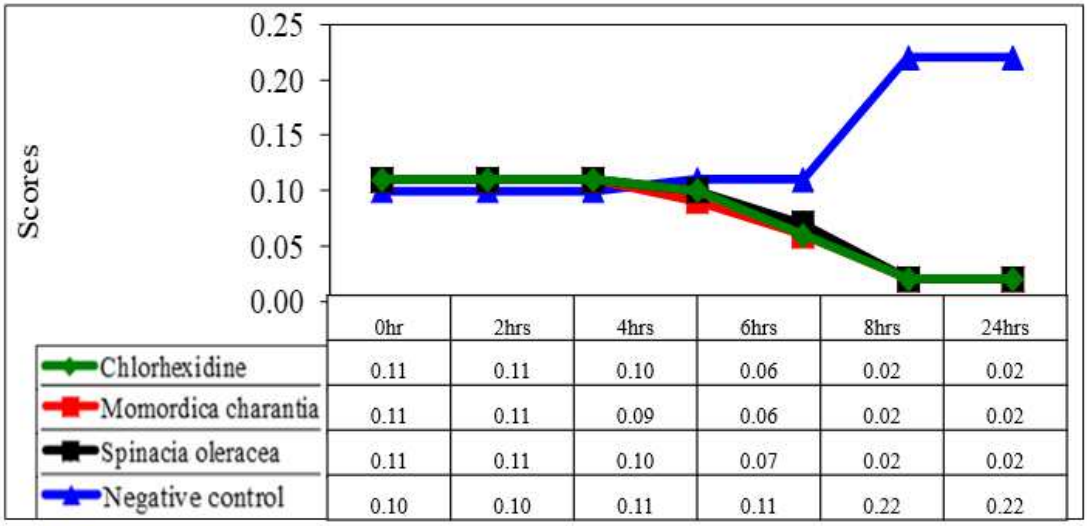
Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash exhibited bacteriostatic effect upto 4hrs as log CFU/ml remained roughly same as the starting CFU/ml concentration however, after 4 hrs the three test compounds exhibited bactericidal effect reducing the starting log CFU/ml by greater than 1 log.

The negative control exhibited very little antimicrobial effect as bacteria in presence of this compound grew over time.

Table No.14: Table showing the antibacterial susceptibility with respect to Time Kill Assay scores in three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Porphyromonas gingivalis* at various time intervals

Groups	**0hr**	**2hrs**	**4hrs**	**6hrs**	**8hrs**	**24hrs**
Chlorhexidine	0.11	0.11	0.10	0.06	0.02	0.02
Momordica charantia	0.11	0.11	0.09	0.06	0.02	0.02
Spinacia oleracea	0.11	0.11	0.10	0.07	0.02	0.02
Negative control	0.10	0.10	0.11	0.11	0.22	0.22

Graph No. 7: Graphical representation showing the antibacterial susceptibility with respect to Time Kill Assay scores in three groups namely Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea mouthwash* (Group III) against *Porphyromonas gingivalis* at various time intervals



	0hr	2hrs	4hrs	6hrs	8hrs	24hrs
Chlorhexidine	0.11	0.11	0.10	0.06	0.02	0.02
Momordica charantia	0.11	0.11	0.09	0.06	0.02	0.02
Spinacia oleracea	0.11	0.11	0.10	0.07	0.02	0.02
Negative control	0.10	0.10	0.11	0.11	0.22	0.22

Table No. 14 and Graph No. 7 denotes comparison of Chlorehexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash with Time Kill Assay scores against *Porphyromonas gingivalis*. Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash exhibited bacteriostatic effect upto 4hrs as log CFU/ml remained roughly same as the starting CFU/ml concentration however, after 6 hrs the three test compounds exhibited bactericidal effect reducing the starting log CFU/ml by greater than 1 log. The negative control exhibited very little antimicrobial effect as bacteria in presence of this compound grew over time. It was seen that all three mouthwashes, Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash have no statistically significant difference in their antimicrobial efficacy and are equally efficacious against *Streptococcus mutans*, *Lactobacillus acidophilus,* and *Porphyromonas gingivalis.*

DISCUSSION

"Nature is one of the most underutilized treasures in life. It has the power to unburden hearts and reconnect to that inner place of peace."

- Janice Anderson

Herbal plants have been an integral part of human development and civilization since hundreds of decades. With the development of ayurveda, these herbal plants have been relied upon by over 80% of the world population for their basic health care needs.[5] Herbal products have recently been used as an alternative to commercially available synthetic preparations to prevent dental caries. In spite of advancements in dental sciences and technology, majority of population suffers from dental caries and periodontal problems among which most of the dental problems have been seen in developing countries.[6] It is seen that about 89% of Indian population suffer from dental caries out of which 72% are seen in people of rural areas who cannot afford a proper dental treatment.[46] Since last two decades there has been an increase surge in search for development of different herbal drugs.[8]

Streptococcus mutans is the main bacteria responsible for initiation of dental caries, it is Facultative, Gram-positive anaerobic organism prevalent of adhesion, acidogenicity, acid tolerance which are the main factors that favors cariogenicity. Acidogenic bacteria such as *Streptococcus mutans*, *Lactobacillus acidophilus* have ability to produce lactic acid and acetic acid which helps to lower plaque pH below 5.5 leading to demineralization of tooth structure followed by development of dental caries.[47]

The aim of present in vitro study is to evaluate and compare antibacterial efficacy of Chlorhexidine and *Momordica charantia*, *Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis.*

Momordica charantia is a medicinal plant which belongs to family Cucurbitaceae. Found in tropical and subtropical areas in world mainly in India, Asia, South America.[48] Some common names being Bitter gourd in English, Paakharkaai in Tamil, Karela in Hindi, Hagalakayi in Kannada.[49] Due to jagged appearance of leaves, the plant is called *Momordica* in Latin which means to bite.

Table No.15: Taxonomy of Momordica charantia

TAXONOMY	
Kingdom	Plantae
Sub-kingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Traqueofita
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Momordica
Species	Charantia

Table No. 16: Botanical description of *Momordica charantia*

Stem	Stem is round and well branched with internodes at 5-6 cm, thin, corrugated in the axilla of leaf the tendrils appear to be unbranched.
Leaves	Palmately-lobed, alternating, rounded edge with 3–7 lobes deeply separated and with quite small marginal points. They are distributed individually in petioles 1.5–5 cm long and have no stipules.
Flowers	Solitary, pubescent and with 5 yellow petals and 5 central stamens. The male flowers have thinner stems and larger petals than the female flowers and, while the male flower sepals are oval-elliptical, those of the female flowers are narrow and oblong lanceolate
Fruit	Pendular discoid with ovoid shape, 2 to 10 cm in length, covered with broken or continuous longitudinal ridges and warts. The young fruit is white or emerald green that turns orange when ripe, and its white pulp becomes scarlet during ripening.
Seed	8–15 mm long, rectangular squares, corrugated on the margin, sculpted on both sides, but covered with a white pulp when green and red when ripe.

The fruits and leaves contain alkaloids, glycoside, saponin like substance, rennin which is an aromatic volatile oil mucilage.[6] We have used *Momordica charantia* fruit in our study as it contains a number of bioactive substances that have been identified in the literature; they fall under the categories of carbohydrates, proteins, lipids, etc. The main components being trans nerolidol and alpha momorcharin which are anti-microbial in nature and have bactericidal effect against *Streptococcus mutans* and

Lactobacillus acidophilus An in vitro study was conducted to determine anti-microbial and anti-oxidant components present in methanolic extract of *Momordica charantia,* where they found that the fruit extract contain plumericin, MAP-30, charantin, seed oil, alpha -momocharin, trans-nerolidol which have significant anti-microbial activity that could prevent caries.[6] Previous research on phytochemicals have demonstrated the bioactive elements and their associated functions (Table No. 17)

Table No.17: Functions of active bioactive components of *Momordica charantia*

Major Bioactive Components	Functions	Distribution
Polysaccharides	Antioxidants, anti-diabetic, anti-tumor, enhances immunity	Fruit
Trans - nerolidol	Antimicrobial	fruit
Alpha - momorcharin	Antimicrobial	fruit
Terpenoids	Anticancer, antioxidant, antidiabetic, hypoglycemic, cancer chemoprevention	Stem, leave, fruit
Peptides and proteins	RNA N-glycosidase, polynucleotide adenosine glycosidase (PAG), DNase-like, phospholipase, superoxide dismutase, antimicrobial	Seed
Lipid	Antitumor, antioxidant	Seed
Saponins	antihyperglycemic, hypolipidmic,	Fruit, seed

Momordica charantia has been used to cure a variety of illnesses since antiquity, it is still frequently employed as a therapeutic agent in the aforementioned Latin American and Asian nations. An outline of its typical mechanism of its pharmacological activity is provided below.[6]

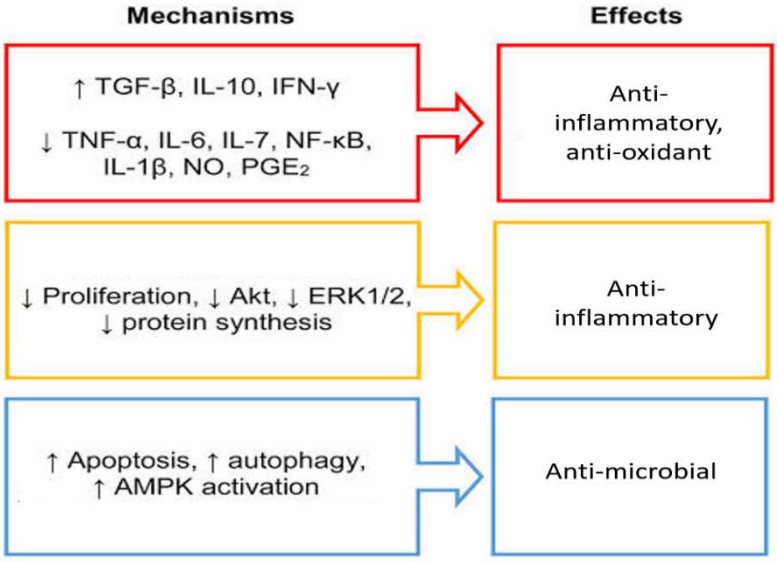


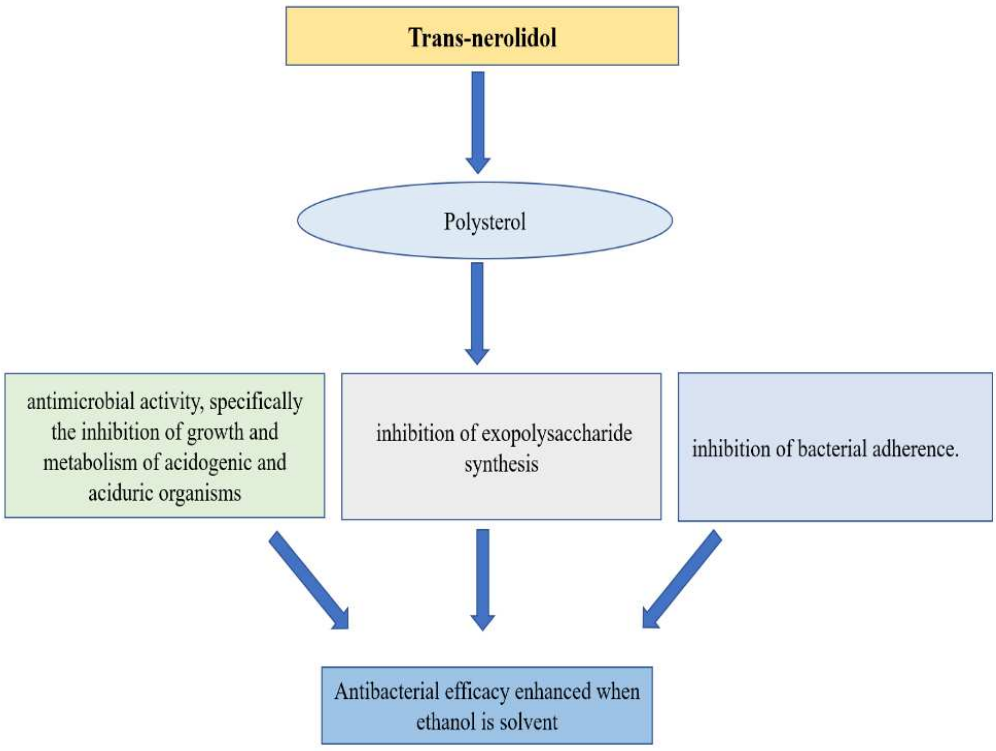
Figure No. 36: Mechanism of active components in *Momordica charantia*

The active components of *Momordica charantia* plant are responsible for its biological and pharmacological activities including anti-diabetic, anti-oxidant, anti-viral, anti-ulcerative, immunomodulatory, etc. It is known to contain compounds such as momorchanins, momordenol, momordicilin, momordicius, momordin, mmordicinin, momordolol, charantia, charaine, cycloartenols, diosgenin, gentisic actid and multiflorenol.[5]

Any natural product's active ingredient must be extracted using a solvent that can dissolve the components of interest. Ethanol, methanol, and water are the most often utilised solvents for the extraction of the components for testing antimicrobial activity. Due of its toxicity, methanol was not utilised. Since it proved difficult to dissolve in distilled water, extract was produced using ethanol as the primary solvent.[38]

Our study aimed to isolate the active component using ethanol as solvent present in the fruit namely trans nerolidol and alpha momorcharin and use it further to prepare the mouthwash. Study conducted in Thailand using ethanolic extract of *Momordica charantia* have isolated the active components trans nerolidol and alpha momorcharin and found the effectiveness in inhibition of growth of *Streptococcus mutans* to an appreciable level mainly by reducing the adhesion of *Streptococcus mutans* to teeth.[39]

The mechanism by which it inhibits the development of caries have been depicted in flowchart. (Figure no. 36). An in-vitro study was conducted to study the anti-microbial effectiveness of *Momordica charantia* extract on *Streptococcus mutans* and *Lactobacillus acidophilus* where they found that the anti-bacterial effectiveness can be attributed to trans-nerolidol and alpha momorcharin with mechanism of action depicted in flowchart.[6] (Figure no. 37,38)



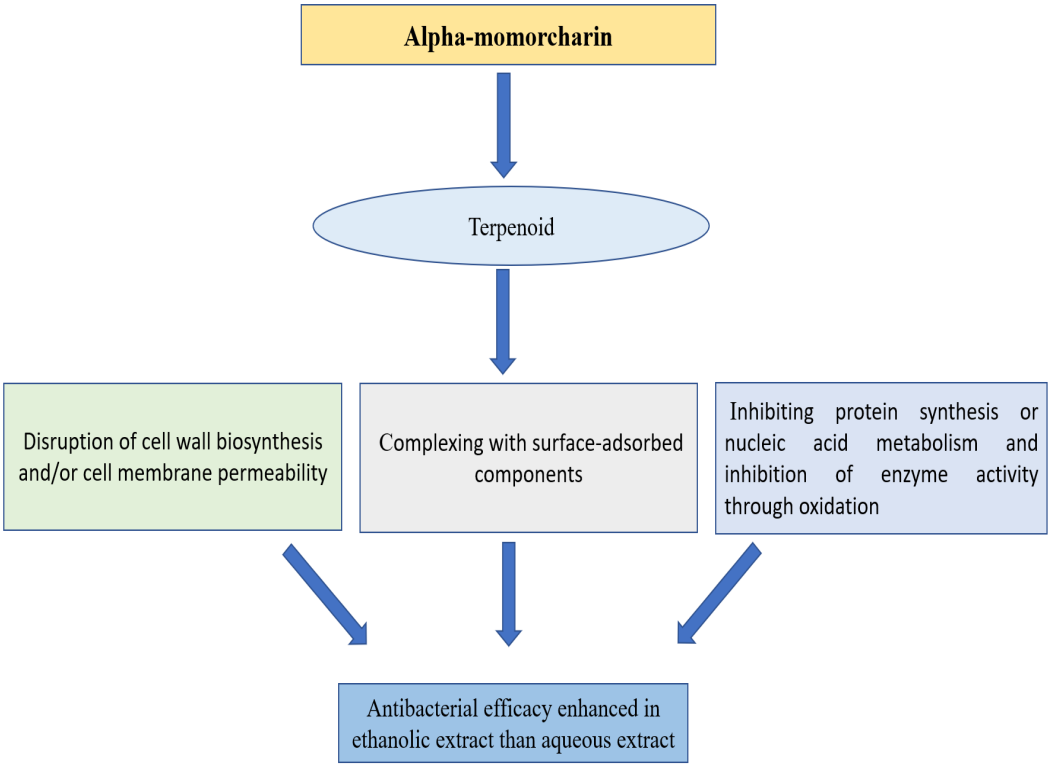


Figure No. 38: Mechanism of active component alpha-momorcharin in inhibition of dental caries.[36]

Our study showed comparable results to an invitro study where they have shown that ethanolic extract of *Momordica charantia* contains higher concentration of active anti-microbial agents which had inhibitory effect on *Streptococcus mutans* and *Lactobacillus acidophilus*[8]

The second herb used in our study was *Spinacia oleracea*. Studies have conducted to determine the anti-microbial effect of *Spinacia oleracea* in past few decades. However, there was dearth of literature pertaining to effectiveness against organism causing dental caries. Thus, a study was conducted with an effort to expand the spectrum of anti-bacterial activity of aqueous and ethanolic extracts of *Spinacia oleracea* leaves against *Streptococcus mutans* and *Lactobacillus acidophilus*[9]

Spinacia oleracea from the Chenopodiaceae family, is frequently referred to as "Spinach." It is an upright herb that is between 30 and 60 centimetres tall. It is grown as a leafy vegetable all over the world. This plant's many parts are utilised in traditional Indian medicine as there are many therapeutic effects of plant. Spinacia oleracea is called spinach in English, Palak in Hindi and Marathi, Keerai in Tamil, Soppu in Kannada. It is widely grown in Asia, northern Europe, United states and is leafy annual of amaranth family.[40]

Spinacia oleracea contains significant amounts of vitamins A, C, and E, magnesium, manganese, folate, betaine, iron, vitamin K, Calcium, vitamin B6, folic acid, copper, protein, vitamin B2, vitamin B6, omega-3 fatty acids, phosphorus, zinc, niacin, and selenium. Spinach also has a lot of antioxidants components such flavonoids, polyphenols, carotenoids with anti-inflammatory properties, antineoplastic, and antimutagenic potential, as along with chemopreventive effects.[10] We have used Spinacia oleracea leaves in our study as they contain Sliver nanoparticles which have inhibitory action against *Streptococcus mutans* and *Lactobacillus acidophilus.*

Even though many researchers have examined the antibacterial effectiveness of spinach on many species, significant studies haven’t been carried to check its efficacy on inhibition of caries. Thus, our study assessed the anti-microbial action of the plant.

Table No.18: Taxonomy of *Spinacia oleracea*

TAXONOMY	
Kingdom	Plantae
Sub-kingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Caryophyllanae
Order	Caryophyllanaes
Family	Amaranthaceae
Genus	Spinacia
Species	Oleracea

Table No.19: Botanical description of *Spinacia oleracea*. [9]

Stem	Thin, corrugated in the axilla of leaf the tendrils appear to be unbranched.
Leaves	Simple leaves are somewhat triangular, ovate and may be flat or puckered.
Flowers	Flowers are inconspicuous and produce dry fruits
Fruit	Plant produces dry fruits on unusual basis

Previous studies on phytochemicals have shown the presence of the bioactive components and the related roles they play. (Table No. 20)

Table No. 20: Functions of active bioactive components of *Spinacia oleracea.*[40]

Major Bioactive Components	**Functions**	**Distribution**
Polphenols	Antioxidants, anti-tumor, enhances immunity	All parts of plants.
Alkaloids	Anti-bacterial	Stem, leaves
Silver nanoparticles	Anti-caries action	Leaves
Flavonoids	Anticancer, antioxidant, chemoprevention	Stem, leave, fruit
Carotenoids	anti-tumour, immune suppression, antimicrobial	Seed, dry fruits
Vitamins	Antitumor, antioxidant	All parts
Terpenoids	antihyperglycemic, antiviral	Fruit, root,
Tannins	immune enhancement, anti-oxidants	Stem
Glycosides	Antioxidant, anti-inflammation, immune enhancement	Dry fruits,leaves
Organic acids	Antimicrobial	Leaves, roots

The preliminary results of an in-vitro study demonstrating the antimicrobial activity of *Spinacia oleracea* extracts against *Streptococcus mutans* and *Lactobacillus acidophilus* raise the possibility that *Spinacia oleracea* may be used as a preventive measure for dental caries and possibly other oral infections like gingival and periodontal issues.[40]

When compared to other aqueous and methanolic extracts of Pumpkin, Suran, and Ghuiya, Spinach (methanolic) extract was more effective against bacterial strains (*Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis, Bacillus cereus, Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Enterobacter agglomerans, Streptococcus aureus, Pseudomonas aeruginosa, Candida albicans, Penicillium chrysogenum, Enterococcus faecalis, Klebsiella pneumoniae, Bacillus sphaericus, Bacillus thuringiensis, and Cryptococcus*).[50] Aqueous and ethanolic *Spinacia oleracea* extract had an antibacterial action against *Streptococcus mutans, Lactobacillus acidophilus*, according to the findings of an in-vitro investigation.[41] Silver nanoparticles (AgNPs), a naturally occurring antimicrobial substance, are responsible for the antibacterial activity. AgNPs are said to have antiangiogenesis, anti-inflammatory, and antiplatelet activity in addition to their antibacterial capabilities. It has been proved that AgNPs, total phenolics, and flavonoids have a variety of medicinal uses.[40]

Our study thus used ethanolic extract with active component Sliver nanoparticles (AgNPs) to prepare mouthwash and check its anti-bacterial activity against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis*. The mechanism of action of Silver nanoparticles (AgNPs) inhibiting dental caries have been shown in flowchart. (Figure no.39)

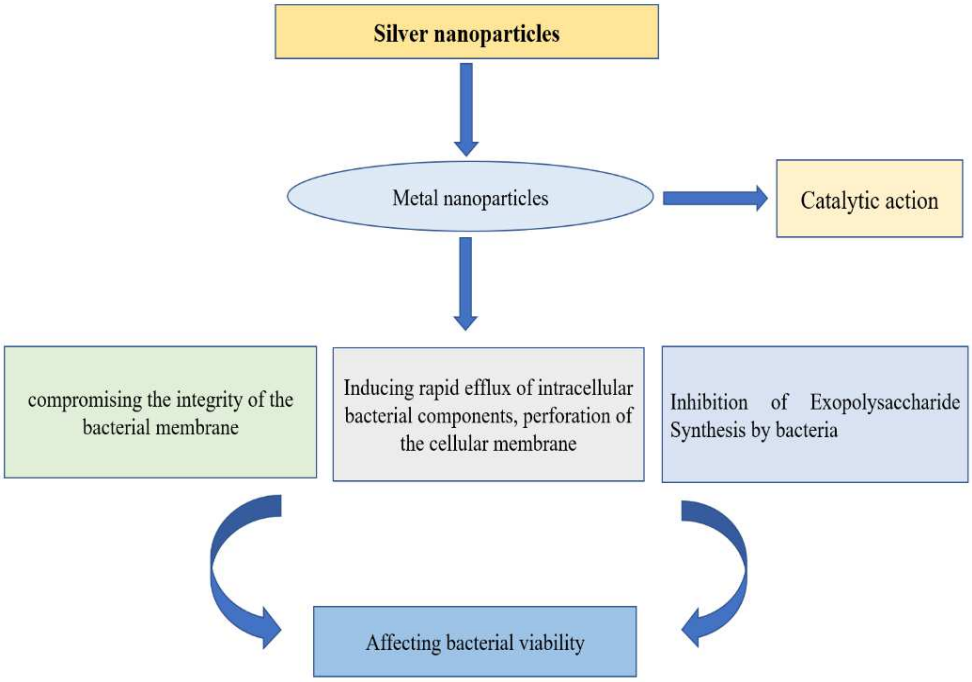


Figure No.39: Mechanism of active component Silver nanoparticles in inhibition of dental caries:

Apart from anti- microbial action, it is regarded as a beneficial nutritional source of lutein, folate, nonheme iron, and vitamin A. It is thought to be an antioxidant, anti-inflammatory, anti-pyretic, anti-aging, and bacterial inhibitor.[10] So, this in vitro experiment was planned to investigate how it affects *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis.*

After preparation of ethanolic extract of *Momordica charantia* and *Spinacia oleracea* our next step was to find out its antibacterial efficacy by carrying Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract. A preliminary study was done to evaluate the MIC and MBC of the extract of *Momordica charantia* and *Spinacia oleracea* against the test organisms, it was found that the ethanolic extract had the best inhibition. *Momordica charantia*

extract and *Spinacia oleracea* extract was prepared as an ethanolic extract to obtain the maximum number of bioactive components present in it. This could be attributed to the fact that trans-nerolidol, alpha momorcharin and Silver nanoparticles which are the phytochemicals of the interest are easily soluble in ethanol. The results of the preliminary study were consistent with the in-vitro studies conducted using ethanol as solvent where the active ingredient present in extract showed higher efficacy in ethanol as solvent rather than methanol, saline, water as solvents.[6,5]

The MIC was determined using the Risazurin technique and MBC was found by sub-culturing them on antibiotic-free medium. Due to the high initial inoculum produced by the relatively large volume of broth in each of the tubes, this approach has the benefits of producing a quantitative result (the MIC) and allowing for the analysis of a significant number of bacterial cells. The laborious manual process of producing the two-fold solutions for each test, the need for a sizable number of reagents and space, and the potential for mistakes when preparing antibiotic concentrations are the main drawbacks.[43]

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibiotic that, under specific circumstances, inhibits a microorganism from growing visibly is known as a Minimum Inhibitory Concentration (MIC). This in vitro measure is utilised in clinical practise to categorise the tested microorganism as clinically sensitive, intermediate, or resistant to the test substance. The Minimum Inhibitory Concentration (MIC) can be determined using a number of techniques, including the Risazurin method and the broth dilution method. The present in vitro study followed the Risazurin method as it the most commonly used method for assessing the bacterial susceptibility. In this study, the Minimum Inhibitory

Concentration (MIC) for 10mg/ml of *Momordica charantia* extract was 2.5 mg/ml against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* respectively. For 10mg/ml of Spinacia oleracea extract the Minimum Inhibitory Concentration (MIC) was 2.5mg/ml against *Streptococcus mutans* and 4mg/ml against *Lactobacillus acidophilus* and *Porphyromonas gingivalis* respectively. The results of our study were comparable to two in-vitro studies.[40,41]

In one study conducted to determine the anti-microbial activity of medicinal plants on *Streptococcus mutans* where 100mg/ml of ethanolic extract was used to determine MIC. It was seen that at 100mg/ml, MIC of both *Spinacia oleracea* and *Momordica charantia* was 25mg/ml.[40] In a similar study conducted to study in-vitro anti-microbial activity of *Spinacia oleracea* against *Streptococcus mutans* and *Lactobacillus acidophilus*, where at 100 µg/mL of ethanolic extract, MIC of *Spinacia oleracea* was found at 25 µg/mL against *Streptococcus mutans* and 50 µg/mL against *Lactobacillus acidophilus*[41] But the limitation of Minimum Inhibitory Concentration (MIC) method is that it does not give an indication of the mode of action (cidal or static) of the antimicrobial agent.[43] So, to evaluate the mode of action of *Momordica charantia extract* and *Spinacia oleracea* extract, Minimum Bactericidal Concentration (MBC) was carried out. As per the literature search done by us there were no other studies that could be used to compare our results for *Porphyromonas gingivalis.*

The second anti-bacterial test carried was the Minimum Bactericidal Concentration (MBC) which is the smallest concentration at which an antimicrobial medication is bactericidal is known as the Minimum Bactericidal Concentration (MBC). Re-culturing (subculturing) broth dilutions that prevent a bacterial organism from growing is how it is discovered (i.e., those at or above the MIC). The Minimum

Bactericidal Concentration (MBC) is the lowest antimicrobial broth dilution that stops an organism's development on an agar plate. It is implied that there are only nonviable organisms present if the organism fails to develop on the plate.[43] The Minimum Bactericidal Concentration (MBC) in this investigation for *Momordica charantia* extract was 5mg/ml against *Streptococcus mutans* and *Lactobacillus acidophilus* While it was 10mg/ml against *Porphyromonas gingivalis* and for *Spinacia oleracea* extract it was 5mg/ml against *Streptococcus mutans*, 10 mg/ml against *Lactobacillus acidophilus* and *Porphyromonas gingivalis* respectively, since no bacterial growth was seen at such concentrations. The results of MBC were comparable to the study conducted to determine the anti-microbial activity of medicinal plants on *Streptococcus mutans* where at 100mg/ml of ethanolic extract concentration, MBC for *Spinacia oleracea* was 50mg/ml and *Momordica charantia* was 100mg/ml.[40]

Based on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), 62.5mg of extract of *Momordica charantia* and *Spinacia oleracea* was used to prepare separate *Momordica charantia* and *Spinacia oleracea* mouthwash so that it will be effective against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Porphyromonas gingivalis.* To overcome any changes in the property of extract while adding other components for mouthwash preparation. As per the literature search done by us there were no other studies that could be used to compare our MBC results for *Porphyromonas gingivalis*.[6]

The positive control that we used in our study was Chlorhexidine mouthwash. Two 4-chlorophenyl rings, two biguanide groups, and a core hexamethylene chain make up the symmetrical cationic compound Chlorhexidine. Strong in base, it is most stable when present as salts.[7] Due to its high-water solubility, the digluconate salt is used for its preparation frequently. Chlorhexidine is effective against ychloreast, fungi,

facultative anaerobes, and aerobes are just a few of the gram-positive and gram-negative species.

Chlorhexidine which is a bi-cationic positively charged molecule attaches to the negatively charged sulfates and phosphate ions on the bacterial cell wall. It gets absorbed on bacterial cell wall altering the integrity of bacterial cell membrane. With increased concentration of Chlorhexidine there is progressive damage to cell membrane as it binds to the phospholipids in the inner membrane causing leakage of low molecular weight compounds like postassium ions. It causes coagualation and precipitation of cytoplasmic components by formation of phosphate complexes which include adenosine triphosphate and nucleic acids causing death of bacteria. At higher doses Chlorhexidine is bactericidal due to cytoplasmic precipitation or coagulation, which is most likely brought on by protein cross-linking. Because Chlorhexidine releases slowly, the bactericidal impact is assumed to be less significant than the bacteriostatic effect.[51]

Chlorhexidine also attaches to the hydroxyapatite in dental enamel, the pellicle on the surface of the tooth, salivary proteins, bacteria, and extracellular polysaccharides of bacterial origin because of its cationic characteristics. A third to a half of the Chlorhexidine that is still present in the mouth is phosphate group-bound. It binds to mucous membrane surface coats in the mouth primarily. As the concentration of Chlorhexidine in the mouth diminishes, the adsorbed Chlorhexidine gradually releases for up to 24 hours. Thus, it was assumed that Chlorhexidine would lessen bacterial colonization of the tooth surfaces. Despite being efficient, it has several undesirable side effects, including tooth discoloration, erosion of the oral mucosa, and altered taste perception.[52]

By attaching to a particular sodium receptor molecule in the taste buds, which is distinct from receptors for sweet, bitter, and sour stimuli, Chlorhexidine may also cause taste abnormalities. The mechanism by which Chlorhexidine prevents development of caries has been depicted in the flowchart. (Figure No. 40)

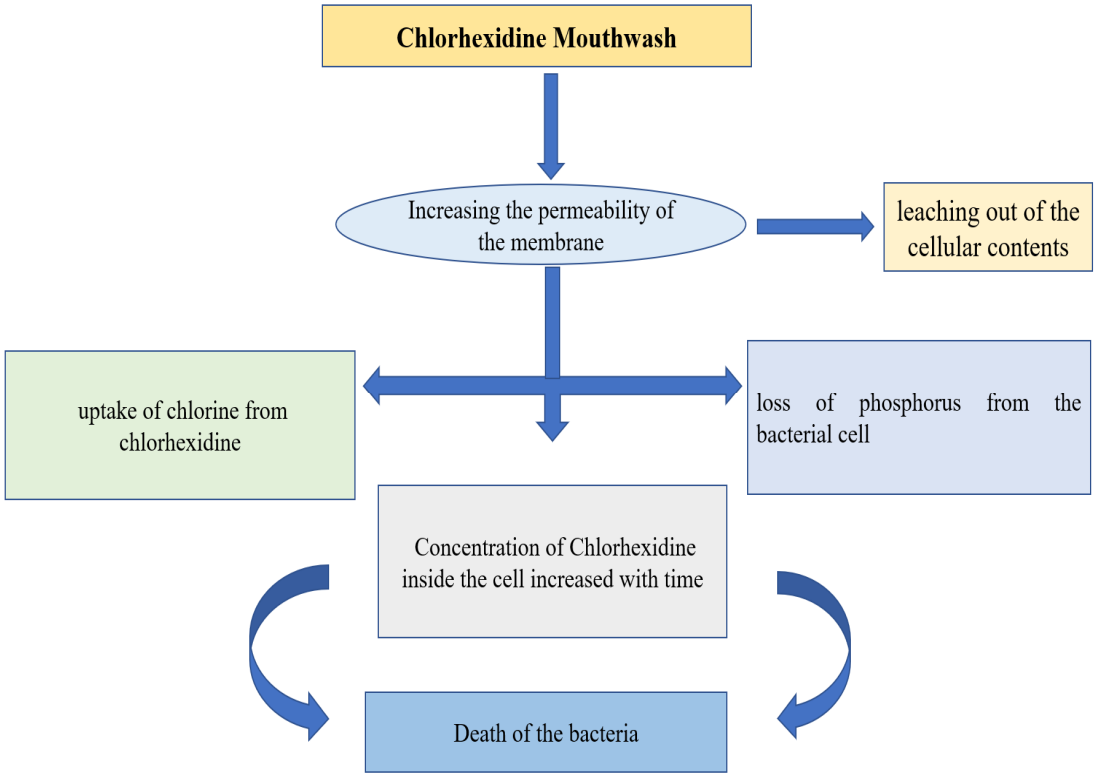


Figure No. 40: Mechanism of action of Chlorhexidine.[7]

Whereas, trans nerolidol, alpha momorcharin, Silver nanoparticles provoke irreversible permeability changes in cell membrane, causing cells to lose the ability to maintain membrane potential and leakage of cytoplasm macromolecules including nucleotide.[54]

Next important step was to determine the biocompatibility of mouthwash. In dental practise, a variety of biomaterials are used. A crucial step in determining the biocompatibility of all biomaterials is determining cytotoxicity using a variety of cytotoxicity testing methodologies. The toxicological characteristics of *Momordica*

charantia and *Spinacia oleracea* extracts have been examined in a number of investigations.[44,42,]

In our study, Cytotoxic evaluation by MTT assay, it was observed that the viability of L929 mouse fibroblast was observed to be 97.8% for Chlorhexidine mouthwash, 106% for *Momordica charantia* mouthwash, 106% for *Spinacia oleracea* mouthwash which indicates that all three mouthwashes appear to be non-toxic to the L929 mouse fibroblast against which the cytotoxic activity had been tested. According to the literature search done, we found that this study was first of its kind and no such test has been carried to determine cytotoxicity of *Momordica charantia* and *Spinacia oleracea* mouthwash.

In order to determine antibacterial efficacy of prepared mouthwash, Antibacterial susceptibility test was performed. Antibacterial susceptibility for Chlorhexidine mouthwash (Group I), *Momordica charantia* mouthwash (Group II), *Spinacia oleracea* mouthwash (Group III) was tested against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* by Agar Well Diffusion Method and Time Kill Assay.[45]

In order to check whether the two mouthwashes *Momordica charantia* mouthwash (Group II) and *Spinacia oleracea* mouthwash (Group III) have synsergistic or antagonistic effect with each other we also tried to combine the two mouthwashes and check its anti-bacterial efficacy by comparing the zones of inhibition with Chlorhexidine mouthwash. against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.* It was seen that the antibacterial efficacy was enhanced when *Momordica charantia* mouthwash was combined with *Spinacia oleracea* mouthwash, which showed its synergistic effect and

was equally efficacious to Chlorhexidine mouthwash with zone of inhibition of 20mm against *Streptococcus mutans* (Figure No. 41a) and was more than Chlorhexidine mouthwash with zone of inhibition of 23mm against *Porphyromonas gingivalis.* (Figure No. 41b) However, the combined mouthwashes showed comparable antibacterial efficacy when compared to Chlorhexidine mouthwash against *Lactobacillus acidophilus* with zone of inhibition of 20mm. (Figure No. 41c) No such studies have been carried where they have compared anti-bacterial efficacy of Chlorhexidine mouthwash and *Momordica charantia* and *Spinacia oleracea* mouthwash.

(a) **(b)** **(c)**

Figure No. 41(a), (b), (c): Figure showing Zone of Inhibition of Chlorhexidine mouthwash, combination of *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash, against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis.*

Time Kill Assay was carried out to study the influence of antimicrobial drugs at various time intervals, the comparison was done of Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash with Time Kill Assay scores against *Streptococcus mutans, Lactobacillus acidophilus,*

Porphyromonas gingivalis. It was seen that Chlorhexidine mouthwash, *Momordica charantia* mouthwash, *Spinacia oleracea* mouthwash, exhibited bacteriostatic effect up to 4hrs as log CFU/ml remained roughly same as the starting CFU/ml concentration however, after 6 hrs the three test compounds exhibited bactericidal effect reducing the starting log CFU/ml by greater than 1 log. The negative control exhibited very little antimicrobial effect as bacteria in presence of this compound grew over time. It was seen that all three mouthwashes Chlorhexidine mouthwash, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash have no statistically significant difference in their antimicrobial efficacy.[45]

Chlorhexidine mouthwash and *Momordica charantia* extract mouthwash, Spinacia oleracea extract mouthwash show almost similar Time Kill Assay after 24 hours, hence *Momordica charantia* mouthwash, Spinacia oleracea mouthwash can be comparable to Chlorhexidine mouthwash.[29]

Herbal extracts for tooth cleansing and as an antimicrobial plaque agent have been successfully used in dentistry. Herbal mouth rinses are gaining special attention in the recent times because they are non-chemical and non-synthetic. Studies on the antimicrobial and anticariogenic properties of *Momordica charantia* and *Spinacia oleracea* are scarce in literature. In the current study, *Momordica charantia* and *Spinacia oleracea extract* were chosen as it is an herbal product which has shown several health benefits including antibacterial, antioxidant and other beneficial biological properties.[5,6]

The study results should be interpreted in the light of few limitations that it was an in vitro study. In future, an in vivo study could be carried by testing

antibacterial property of *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash against other pathogenic oral micro-organisms too.

However, it has substantial future implications that *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash can be promoted as an herbal alternative as it has equal antibacterial efficacy against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis* in comparison to 0.2% Chlorhexidine mouthwash.

CONCLUSION

The following conclusions are drawn from the present study:

1. The mean Minimum Inhibitory Concentration of *Momordica charantia* was 2.5mg/ml against *Streptococcus mutans*, 2.5mg/ml against *Lactobacillus acidophilus* and against *Porphyromonas gingivalis was* 2.5mg/ml. *Spinacia oleracea* .had a Minimum Inhibitory Concentration of 2.5mg/ml against *Streptococcus mutans*, 4mg/ml against *Lactobacillus acidophilus* and against *Porphyromonas gingivalis* was 4mg/ml which shows that both herbal mextracts had good antibacterial efficacy against *Streptococcus mutans, Lactobacillus acidophilus, Porphyromonas gingivalis.*

2. The mean Minimum Bactericidal Concentration of *Momordica charantia* was 5mg/ml against *Streptococcus mutans*, 5mg/ml against *Lactobacillus acidophilus* and against *Porphyromonas gingivalis was* 10mg/ml. *Spinacia oleracea* had a Minimum Inhibitory Concentration of 5mg/ml against *Streptococcus mutans*, 10mg/ml against *Lactobacillus acidophilus* and against *Porphyromonas gingivalis* was 10mg/ml denoting their potential antimicrobial action against test micro-organisms.

3. During cytotoxicity evaluation, fibroblasts showed 97.8% for Chlorhexidine mouthwash, 106% viability for *Momordica charantia* mouthwash, 106% for *Spinacia oleracea* mouthwash. This signifies the non-toxic nature of the study groups.

4. *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash showed comparable zone of inhibition against test organisms, i.e., *Streptococcus mutans*, *Lactobacillus acidophilus* and *Porphyromonas gingivalis.*
5. Chlorhexidine mouthwash, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash gave comparable results during Time Kill Assay by showing similar time dependent bactericidal effect by the end of 24 hours.
6. Chlorhexidine mouthwash, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash showed comparable antibacterial efficacy at various time intervals as evaluated using Time Kill Assay.

SUMMARY

The present study was conducted with the aim to evaluate and compare of Chlorhexidine mouthwash and *Momordica charantia, Spinacia oleracea* mouthwash against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis.*

After the collection of raw herbs, they were powdered and ethanolic extract was prepared by maceration. The MIC and MBC of ethanolic extracts of *Momordica charantia* and *Spinacia oleracea* was determined against *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis* using Resazurin method and Agar Plate Streaking method respectively. These values were used for the preparation of Herbal mouthwash. The cytotoxicity of both the study groups i.e., Chlorhexidine mouthwash, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash was evaluated against fibroblast cells using MTT assay. Further, a comparison of antibacterial effectiveness of both the study groups was done using Agar Well Diffusion test and Time Kill Assay.

The sensitivity of all the extracts were found to be comparable against the test organisms, i.e., *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis. Momordica charantia* extract showed MIC of 2.5mg/ml and MBC of 5mg/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus,* its MIC was 2.5mg/ml and MBC was 5mg/ml and against *Porphyromonas gingivalis* its MIC was 2.5mg/ml and MBC was 10mg/ml. *Spinacia oleracea* had a MIC of 2.5mg/ml and MBC of 5mg/ml against *Streptococcus mutans*. It showed MIC of 4mg/ml and MBC of 10mg/ml against *Lactobacillus acidophilus* and against *Porphyromonas gingivalis* had MIC of 4mg/ml and MBC of 10mg/ml respectively.

The cytotoxicity evaluation using MTT assay was observed that the viability of L929 mouse fibroblast was 97.8% for chlorhexidine mouthwash, 106% for *Momordica charantia* mouthwash, 106% for *Spinacia oleracea* mouthwash and, signifying the non-toxic nature of the study groups.

On comparison of antibacterial effectiveness using Agar Well Diffusion test, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash showed comparable zone of inhibition against test organisms, i.e., *Streptococcus mutans, Lactobacillus acidophilus* and *Porphyromonas gingivalis*. While the combination of *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash showed greater zone of inhibition than chlorhexidine mouthwash. The three study groups i.e., Chlorhexidine mouthwash, *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash gave comparable results during Time Kill Assay by showing similar time dependent bactericidal effect.

These results were obtained showing good antibacterial efficacy of *Momordica charantia* mouthwash and *Spinacia oleracea* mouthwash advocates its use for prevention of dental caries in children.

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ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
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FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No.: 1471

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of antibacterial efficacy of Chlorhexidine mouthwash & momordica charantia, Spinacia oleracea mouthwash against Streptococcus mutans, Lactobacillus spp & Porphyromonas gingivalis - an in vitro study *Submitted by*

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

Department of Pediatric & Preventive dentistry *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II

AUTHENTICATION CERTIFICATE FROM ICMR

राष्ट्रीय पारम्परिक चिकित्साविज्ञान संस्थान
ICMR-NATIONAL INSTITUTE OF TRADITIONAL MEDICINE
(पूर्वक्षेत्रीयआयुर्विज्ञानअनुसंधानकेन्द्र Formerly Regional Medical Research Centre)
Nehru Nagar, Belagavi-590 090

Dr. Harsha Hegde
Scientist-E
harshah@icmr.gov.in

भारतीयआयुर्विज्ञानअनुसंधानपरिषद
INDIAN COUNCIL OF MEDICAL RESEARCH
स्वास्थ्यअनुसंधानविभाग, स्वास्थ्यऔरपरिवारकल्याणमंत्रालय, भारतसरकार
Department of Health Research,
Ministry of Health & Family Welfare, Govt. of India

Date: 13-10-2021

AUTHENTICATION

This is to authenticate that the plant materials brought by II-MDS, Dept. of Pediatric and Preventive Dentistry, KLE VK Institute of Dental Sciences, Belagavi, are identified as ***Spinacia oleracea*** **L. (Asclepiadaceae)** and ***Momordica charantia*** **L. (Cucurbitaceae).** The herbarium specimens of the same have been deposited in our herbaria with accession numbers RMRC-1649 and RMRC-1650 respectively.

Harsha Hegde
Scientist-E

ANNEXURE III:

BIOSTATISTICS CERTIFICATE

K L E
VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES
A Constituent college of
K.L.E. Academy of Higher Education and Research
J.N.M.C. Campus, Nehru Nagar Belagavi -590010 Karnataka, India.
Department of Pediatric and Preventive Dentistry

BIOSTATISTICS CLEARANCE CERTIFICATE

This is to certify that the Biostatistics art of Dissertation/ Research work of **Postgraduate student** under the guidance of M.D.S. *(Ph.D)* **Reader, Department of Pediatric and Preventive Dentistry** entitled **"Comparative evaluation of anti-bacterial efficacy of Chlorhexidine mouthwash and *Momordica charantia*, *Spinacia oleracea* mouthwash against *Streptococcus mutans*, *Lactobacillus spp.* and *Porphyromonas gingivalis* - An in vitro study."** has been done under my guidance and considered satisfactory.

Place: Belagavi

Date: 29/08/2022

Name and Signature of Biostatistician

(Dr. S.B. Javali)

ANNEXURE IV:

PLAGARISM REPORT

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) **Placed in Category 'A' by MHRD (GoI)**

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Date : 19.9.2022 Serial No. : 109

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : POSTGRADUATE STUDENT
Batch & Year : 2020-2023
Department : PEDIATRIC AND PREVENTIVE DENTISTRY

The soft copy of Research Work / ~~Manuscript~~ by ..... entitled "COMPARATIVE EVALUATION OF ANTI-BACTERIAL EFFICACY OF CHLORHEXIDINE MOUTHWASH AND MOMORDICA CHARANTIA, SPINACIA OLERACEA MOUTHWASH AGAINST STREPTOCOCCUS MUTANS, LACTOBACILLUS S.P.P AND PORPHYROMONAS GINGIVALIS - AN IN VITRO STUDY"
under the guidance of .....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....10.....%, which is **within** / ~~**not within**~~ the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi