COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY AND REMINERALIZATION POTENTIAL OF ACIDULATED PHOSPHATE FLUORIDE GEL WITH HERBAL DENTAL GEL CONTAINING *ZINGIBER OFFICINALE*, *SALVADORA PERSICA* AND *CINNAMOMUM ZEYLANICUM* – AN IN VITRO STUDY.

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ABSTRACT

AIM: The aim of the study is to evaluate and compare the antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel Containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

METHOD: Ethanolic extracts of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* were prepared. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of these extracts were determined against *Streptococcus mutans* and *Lactobacillus acidophilus* using Resazurin method and Agar Plate Streaking method respectively. Herbal Dental gel was formulated and its cytotoxicity was evaluated using MTT assay. Antibacterial susceptibility of Acidulated Phosphate Fluoride gel and Herbal Dental gel was assessed using Agar Well Diffusion method and Time Kill Assay. Remineralizing potential was evaluated using stereomicroscope.

RESULTS: *Zingiber officinale* showed MIC of 11.25 + 2.80 μl/ml and MBC of 27.50 ± 13.69 μl/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus,* its MIC was 10.94 + 9.38 μl/ml and MBC was 22.50 ± 5.59 μl/ml. Salvadora persica had a MIC of 1.56 + 0.96 μl/ml and MBC of 13.75 ± 6.85 μl/ml against *Streptococcus mutans*. It showed MIC of 2.50 + 0.86 μl/ml and MBC of 20.00 ± 6.85 μl/ml against *Lactobacillus acidophilus*. *Cinnamomum zeylanicum* had MIC of 11.25 + 2.80 μg/ml and MBC of 27.50 ± 13.69 μg/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus*, its MIC was 20.00 + 6.85 μg/ml and MBC was 35.00 ± 13.69 μg/ml. Herbal Dental gel showed better antibacterial efficacy as depicted by the zone of inhibition of 20 mm and 21 mm obtained against *Streptococcus mutans* and *Lactobacillus acidophilus* respectively compared to 11.50

mm and 16.50 mm zone of inhibition obtained by Acidulated Phosphate Fluoride gel. Herbal Dental gel also showed better remineralization potential than Acidulated Phosphate Fluoride gel after time interval of 24 hors and 48 hours as depicted with a statistically significant ‘p’ value of 0.0061 and 0.0219 respectively. In cytotoxicity test, the fibroblasts showed 100% viability in presence of both the study groups which highlights its non-toxicity and safety.

CONCLUSION: The results of our study conclude that Herbal Dental gel is safe and non-toxic, having anticariogenic potential due to its good antibacterial action and remineralization potential as compared to conventional Acidulated Phosphate Fluoride gel.

KEYWORDS: Antibacterial, Dental Caries, Fluoride, Herbal, Remineralization

LIST OF ABBREVIATIONS

Sl. No.	Abbreviation	Expanded form
1.	APF	Acidulated Phosphate Fluoride
2.	BHI	Brain Heart Infusion Agar/Broth
3.	Ca	Calcium
4.	CAM	Complementary and Alternative Medicine
5.	CFU	Colony Forming Units
6.	cm	Centimeters
7.	CO_2	Carbon dioxide
8.	CV	Coefficient of Variation
9.	CZ	*Cinnamomum zeylanicum*
10.	d	Mean difference
11.	DMEM	Dulbecco Modified Eagle Medium
12.	DMFS	Decayed, Missing and Filled Surfaces
13.	DMFT	Decayed, Missing and Filled Teeth
14.	DMSO	Dimethyl Sulfoxide
15.	ECC	Early Childhood Caries
16.	ECOHIS	Early Childhood Oral Health Impact Scale
17.	et al	et alia
18.	etc.	et cetera
19.	F	Fluoride
20.	FTT	Failure To Thrive
21.	H^+	Hydrogen ion
22.	h/hrs	hours

23.	HCl	Hydrochloric acid
24.	i.e.	Id est
25.	IRB	Institutional Review Board
26.	IQ	Intelligence Quotient
27.	mm	millimeters
28.	mL	milliliters
29.	MBC	Minimum Bactericidal Concentration
30.	MIC	Minimum Inhibitory Concentration
31.	MTCC	Microbial Type Culture Collection
32.	MTT	3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide
33.	n	Sample Size
34.	nm	nanometer
35.	No.	Number
36.	OD	Optical Density
37.	PBS	Phosphate Buffer Saline
38.	pH	Potential of Hydrogen
39.	ppm	Parts per million
40.	QLF	Quantitative Light Induced Fluorescence
41.	rpm	Rotations per minute
42.	SDF	Silver Diamine Fluoride
43.	SD	Standard Deviation
44.	SE	Standard Error
45.	Sl.	Serial

46.	SP	*Salvadora persica*
47.	SPSS	Statistical package for the Social Sciences
48.	T3	Triiodothyronine
49.	T4	Thyroxine
50.	TSH	Thyroid Stimulating Hormone
51.	UV	Ultraviolet
52.	WHO	World Health Organization
53.	Z	Standard Score
54.	ZO	*Zingiber officinale*
55.	α	Alpha
56.	β	Beta
57.	μg	Microgram
58.	μm	Micrometer
59.	%	Percentage

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INTRODUCTION

"Mother nature is the true artist and our job as cooks is to allow her to shine"

- Marco Pierre White

According to World Health Organization (WHO), oral health is a human right and is an integral part of the general health and essential for overall well-being of a child. Poor oral health may have a profound effect on general health as several oral diseases are associated with chronic disorders. Despite substantial improvement in the oral health of the populations in numerous countries, global concern still persists as oral diseases affect nearly 3.5 billion people worldwide.[1,2]

According to Global Burden of Disease, dental caries is the most common oral health condition worldwide affecting 60–90% of school-aged children. More than 530 million children suffer from dental caries of primary teeth alone making it a major public health concern.[1,2] However, in spite of the foremost progress and advances in the field of preventive dentistry and with globalization on the rise, the ever-increasing rate of caries in children has become a matter of concern for pediatric dentists.

A rational approach to the control of dental caries involves an understanding of the nature of disease itself. It is a localized multifactorial disease occurring due to complex interaction over time between the acid producing microorganisms, fermentable carbohydrates and a susceptible host or tooth surface. The most dominant etiological agent is *Streptococcus mutans* which is involved with the initiation of decay followed by *Lactobacillus acidophilus* which is associated with progression of the lesion.[3] These bacteria reside in bacterial communities known as dental plaque which accumulates on the tooth surface. The process of decay occurs due to

dissolution of mineralized tooth structure, primarily hydroxyapatite, by organic acids derived from bacterial fermentation of sucrose and other dietary carbohydrates. These minerals are normally replenished by the saliva between meals. However, when fermentable foods are eaten frequently, the low pH in the plaque is sustained and a net loss of mineral from the tooth occurs, leading to cavitation.[4]

The focus of interventions for treatment of dental caries should aim to prevent and delay development of caries and increase resistance to carious lesions. Other key aspects of caries control are behavioral interventions with children along with their caretakers to promote use of disease transmission- reducing and resistance-enhancing agents. Mitigating the transmission of disease and enhancement of resistance can be achieved through use of fluorides, sugar substitutes, mechanical barriers such as pit-and-fissure sealants, and antimicrobials.[5]

Fluoride has been termed as gold standard in remineralization of incipient caries lesion and is widely recognized for reducing the prevalence of dental caries. It can be delivered systematically and topically. Topical delivery of fluoride can be carried out in forms of varnish, gel, mouth rinse, and toothpaste. In the presence of calcium and phosphate ions, topical fluoride ions act as catalyst and promote their diffusion in tooth enamel remineralizing the crystalline structures in the lesion. These rebuilt crystalline surfaces are composed of fluoridated hydroxyapatite and fluorapatite and are less soluble than the original mineral rendering them more resistant to acid attack.[6]

Another mechanism by which fluoride acts is by interfering with the formation of dental plaque induced by microorganisms. Fluoride diffuses inside the bacterial cells and acidifies the cell cytoplasm. It also interferes with enzyme activities,

hindering the metabolic processes inside the bacterial cell.[6] This non-invasive treatment of early carious lesions using fluoride is a major advance in the clinical management of the disease.

However, with progressive research and scientific evidence, it has been deduced that fluoride is a double-edged sword because of both beneficial and damaging effects.[7] Prolonged excessive intake of fluoride has been associated with fluorosis with dental fluorosis being the most common clinical manifestation. It is a degenerative and progressive disorder which can also affect other organs such as the bones, thyroid, kidney, liver, lungs and brain.[8] Prolonged excessive intake of fluoride during pregnancy has been associated with mental problems in the developing fetus.[9] Moreover, even with the extensive use of fluoride in our day to day lives, dental caries still dominates the spectrum of oral health diseases. Henceforth, it is the need of an hour to return back to our roots and search for an effective and safe alternative.

During the last few decades, an uprising interest has been observed in the study of herbal extracts and their use in different gamut of healthcare. India is well known for its ample use of herbal products for curing various health ailments since ancient times. The knowledge and understanding on medicinal plants have been acquired over several centuries and is based on different medicinal systems such as Ayurveda, Unani and Siddha. As per the data obtained by the World Health Organization, nearly 80% of the world's population is dependent on traditional medicine for their primary healthcare needs representing its global dimension and its use still continues to expand rapidly across the world.[10]

Herbal extracts have been widely used in dentistry as anti-inflammatory, antimicrobials, antiseptics, antioxidants, antifungals, antibacterials, antivirals and

analgesics. Few herbs such as Neem, Propolis, Turmeric, Aloe Vera, Clove, Miswak, Ginger, Cinnamon, Thyme and Peppermint have been highlighted in the recent past that show medicinal properties and are extensively used in day to day lives.[10]

One of the commonly used herbs is *Zingiber officinale,* more commonly known as ginger. It belongs to the family *Zingiberaceae*, in the order Zingiberales of monocotyledons. It is well known for its medicinal uses such as antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, anti-tumorigenic, anti-apoptotic, anti-hyperglycemic, anti-lipidemic, anti-emetic, antiplatelet, antipyretic and immunomodulation.[11]

Another commonly used herb is *Salvadora persica*, which is most commonly known as miswak. It belongs to the family *Salvadoracea*. It has a wide array of healthcare effects including anticonvulsant, antibacterial, antimycotic, analgesic, antifertility, carminative, diuretic and astringent effects. It has also been used as a dental care tool since ancient times due to the ability of its fibers to reach in between teeth and richness of its phytoconstituents.[12]

Cinnamomum zeylanicum is also one of the most commonly used herb belonging to the family *Lauracea.* It is widely used as an indigenous spice and is found in almost every household. It has numerous properties such as antioxidant, anti-inflammatory, antilipemic, antidiabetic, antimicrobial, and anticancer.[13]

Owing to innumerable benefits with these herbs, incorporating them in our daily oral health maintenance would prove worthful. According to the existing literature, the synergistic effect pertaining to these herbs hasn't been explored. When literature search was carried out, there were no studies found which incorporated the use of these herbal ingredients in the form of a dental gel. Hence, we planned this study to evaluate the antibacterial efficacy and remineralization potential of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* by combining them as Herbal Dental gel and comparing it with commercially available Acidulated Phosphate Fluoride gel.

AIM AND OBJECTIVES

AIM OF THE STUDY:

The aim of the study was to evaluate and compare the antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel Containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

OBJECTIVES OF THE STUDY:

1. To determine the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus*.
2. To evaluate the cytotoxicity of Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* on fibroblasts.
3. To evaluate the antibacterial effectiveness of Acidulated Phosphate Fluoride gel and Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus.*
4. To evaluate the remineralization potential of Acidulated Phosphate Fluoride gel and Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum.*
5. To compare the antibacterial effectiveness of Acidulated Phosphate Fluoride gel with Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica*

and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus.*

6. To compare the remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum.*

RESEARCH HYPOTHESIS

NULL HYPOTHESIS:

There is no difference in the antibacterial effectiveness and remineralization potential of Acidulated Phosphate Fluoride gel as compared to Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

ALTERNATIVE HYPOTHESIS:

There is a difference in the antibacterial effectiveness and remineralization potential of Acidulated Phosphate Fluoride gel as compared to Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

REVIEW OF LITERATURE

LITERATURE IN RELATION TO EARLY CHILDHOD CARIES

Dental caries is a major global burden and one of the most common preventable childhood diseases. A systematic review and meta-analysis was carried out to determine the prevalence of dental caries in both primary and permanent dentition in children worldwide. The review included 164 studies published between 1995 to 2019. Out of this, 81 articles reported the prevalence of dental caries in primary teeth and 83 articles reported its prevalence in permanent teeth. After the quantitative and qualitative analysis, it was found that the prevalence of dental caries in primary teeth in children in the world with a sample size of 80,405 was 46.2%. In respect to permanent teeth, the prevalence of dental caries in children in the world with a sample size of 1,454,871 was 53.8%. These results indicate that the worldwide prevalence of dental caries in children was found to be high.[14]

Early childhood caries (ECC) is a potential source of pain and infection resulting in reduced quality of life for children as well as their families. It may result in increased emergency consultations, absenteeism from school, as well as influence on dietary patterns leading to poor nutritional intake thereby influencing the physical growth and development of child. It is also considered as a strong predictor of dental caries experiences in the succeeding dentition. A cross sectional study was carried out with an objective to quantify the relative association between dental caries experience and the oral health-related quality of life among a population of preschool children. Caries prevalence among the sample was found to be 39.7% and mean Early Childhood Oral Health Impact Scale (ECOHIS) score was 2. With an increase in child caries

experience, an association was seen with worsening child and family quality of life. The ECOHIS scores were found to be 3.0 times greater for children with dmft scores higher than 5 as compared to children with dmft scores as 0. The study concludes that dental caries can have a deleterious impact on child and family quality of life.[15]

Dental caries results from interactions over time between bacteria that produce acid, a substrate which the bacteria metabolize, and host factors that include teeth and saliva. A systematic review was carried out to determine the risk factors for early childhood caries. The systematic review included 89 studies published until 2019 that evaluated 1,352,097 individuals. All preschool children, from birth until six years of age regardless of gender, race, health status, geographical location, or socioeconomic status were included. A total of 123 risk factors were identified. Meta-analysis revealed that the strongest risk factors were presence of dentinal caries and high levels of *Streptococcus mutans*. Almost all the studies in this area observed an increase in the caries experience with increased salivary *Streptococcus mutans* levels. A strong correlation was also observed between *Lactobacillus acidophilus* count and caries, the higher the DMF index, the higher the number of children harboring a high *Lactobacillus acidophilus* count. Since dental caries is a multifactorial process, the detection of its risk factors should be done as early as possible so that the most adequate measures for its prevention can be quickly implemented.[16]

LITERATURE IN RELATION TO PREVENTIVE DENTISTRY

"Prevention is not waiting for unfortunate things to happen, its preventing things from happening in the first place." With the transposition in paradigm towards the most prevalent disease i.e., dental caries, preventive dentistry is now the sound foundation for all the dentistry aspects. Due to this preventive philosophy, the dentistry for children now focuses to a very large extent on inculcating sound dental practices in each child patient for a healthy dentition to last for life. Preventive dental care for children and adolescents has historically focused on fluoride therapy, oral hygiene and home care maintenance, simple dietary advice, and the placement of fissure sealants for prevention of tooth decay and other dental conditions.

An overview of systematic reviews was carried out to systematically evaluate the available scientific evidence on the effectiveness of methods for prevention of early childhood caries. The main findings from the included studies demonstrated that oral health education promoted better oral health as children whose mothers received dental health education through home visits; received oral health education during pregnancy, referral and screening by a multidisciplinary team presented lower occurrence of dental caries. The use of fluoride varnish was also associated with the prevention or reversal of enamel demineralization, thereby reducing the occurrence of caries. However, these findings were supported by moderate evidence. It was also observed that the use of prenatal fluoride had no caries preventive effect. Multiple applications of 38% Silver Diamine Fluoride (SDF) were found to inhibit the progression of caries in primary teeth. Daily toothbrushing with fluoridated toothpaste was also considered as one of the best preventive practices based on moderate quality of evidence. However, evidence regarding the concentration of fluoride in the toothpaste was controversial. Findings

showed that toothpaste containing less than 1,000 ppm of fluoride was less efficient in affecting the mean dmft. Chlorhexidine in the form of varnish or gel, probiotic bacteria, casein phosphopeptide-amorphous calcium phosphate, and sealants did not demonstrate reliable effects on the occurrence of early childhood caries. However, it was found that young children's preventive dental visits lead to a reduction in the number of restorative dental appointments and expenditure on dental care during the first years of life, and resulted in lower impact of caries in the long run.[17]

LITERATURE IN RELATION TO FLUORIDES

Fluoride remains the most effective agent in the prevention of dental caries. The relation between the proper use of fluoride and the improved dental health is well established. The effects can be achieved by either systemic administration or topical application. Systemic benefits of fluoride can be achieved through the intake of foods containing natural levels of fluoride, water that contains natural fluoride or to which fluoride has been added and dietary fluoride supplements. Topical benefits are available through these sources also as a result of their contact with the teeth as well as from fluoridated toothpastes, fluoride mouth rinses and other concentrated form of fluoride that are self-administered or applied professionally. Professionally applied fluorides are applied by a dental professional during dental visits and are available in the form of a gel, foam or rinse.

Acidulated phosphate fluoride (APF) gel is frequently used for topical application by professionals. It can be administered in the form of a solution, foam or gel. A randomized clinical trial was carried out to evaluate the caries preventive effects of 1.23 % APF gel and APF foam. The trial enrolled 661 children aged between 6-7 years which were randomly allocated to three groups. The experimental groups

received six monthly APF foam and APF gel application respectively. The mean caries increment of smooth surfaces of permanent first molars after a 24 month follow up period was 0.16 and 0.17 in the foam group and in the gel group, respectively, while that of the control group was 0.27. The mean DMFS increment of all surfaces in the foam and gel group was also found to be lower than that in the control group. These findings advocate that application of APF is associated with lower incidence of caries.[18]

In a similar context, a community intervention trial was carried out to assess the feasibility of APF gel as a caries-preventive agent in a high-risk group of school going children aged between 9 and 16 years. 257 children having three or more incipient or cavitated primary or secondary carious lesions were included in the study. In the experimental group, 1.23% APF gel was applied at baseline and at 6 months followed by oral health education. Follow-up examination was performed at 6 and 12 months and the caries status were recorded by Decayed, Missing, Filled Teeth (DMFT) and Decayed, Missing, Filled Surfaces (DMFS) indices. No statistically significant difference in DMFT and DMFS values was observed, but a significant difference was seen in presence of incipient carious lesions between the experimental and control groups. In the experimental group, the percentage of caries reduction was 76.5% on the occlusal surface, 85.5% on the buccal surface and 83.3% on the lingual surface. These results suggest that application of APF gel is an effective preventive measure in reversing incipient carious lesions.[19]

However, it has been found that fluoride has a potential to cause major adverse health problems, while having only a modest dental caries prevention effect. A review article suggested that fluoride exposure has a complex relationship in relation to dental caries. The effect of fluoride in the remineralization process of enamel is not dependent

on fluoride, but is critically dependent on calcium and magnesium content of enamel. Among undernourished children with low calcium and magnesium in enamel, fluoride ingestion and contact with teeth present histologically as hypo-calcification or hypoplasia, which may paradoxically make such individuals more vulnerable to dental caries. Thus, it may increase dental caries risk in malnourished children due to calcium depletion and enamel hypoplasia, while offering modest caries prevention in otherwise well-nourished children. Another adverse effect of fluoride is that it causes brittleness of enamel. At low friction loads, enamel hydroxyapatite and fluorapatite appear to wear in a similar manner. However, when subjected to high friction loads, fluorapatite enamel wears calamitously, rendering the surface prone to fracture, whereas hydroxyapatite enamel does not as it is more adaptable to remodelling.[20]

Fluorosis, caused by ingestion of excessive amount of fluoride through food or water, is also a major public health problem. A study was undertaken to quantify the dental fluorosis burden among school going children and to find factors associated with dental fluorosis. The study included a total of 1026 children aged between 12-17 years. Among them, 64.3% of children were detected with dental fluorosis. Out of them, more than 50% children presented with either severe or moderate fluorosis. A strong positive correlation was observed between the amount of fluoride content in drinking water in the respective area and the prevalence of dental fluorosis.[21]

Fluoride also possesses a number of potential and established adverse effects including cognitive impairment, hypothyroidism, skeletal fluorosis, enzyme and electrolyte derangement, and even cancer. A systematic review and meta-analysis was carried out to investigate the effects of fluoride on neurobehavioral development of children. It included 27 epidemiological studies published over a time span of 22 years.

The qualitative and quantitative analysis reported strong indications that fluoride may adversely affect cognitive development in children. Children who lived in areas with high fluoride exposure were found to have lower IQ scores than those who lived in low-exposure areas. The average loss in intelligence quotient was reported as a standardized weighted mean difference of 0.45, which would be approximately equivalent to seven IQ points for commonly used IQ scores with a standard deviation of 15. The mechanism by which fluoride interferes with child neurodevelopment are associated with damage to nervous cells. These results advocate fluoride as a developmental neurotoxicant that impacts brain development.[22]

Another systematic review and meta-analysis was carried out to determine the association between fluoride exposure and neurological disorders. A total of twenty-seven studies performed on humans exposed to fluoride were included. Twenty-six studies reported alterations related to Intelligence Quotient (IQ) while one study reported the incidences of headache, insomnia, lethargy, polydipsia and polyuria. Ten studies were included in meta-analysis, which reported impairment of IQ for individuals under high fluoride exposure. It has been found that chronic exposure to fluoride in the prenatal and neonatal periods is potentially toxic to the metabolism and physiology of neuronal and glial cells. This leads to changes in processes related to memory and learning. This is attributed to the ability of fluoride to cross the placental and blood–brain barriers, especially in developing individuals, as they have greater permeability of this barrier and possess immature defense mechanisms. Fluoride can also interact with the Ras protein, thereby influencing the membrane ion channels leading to changes in ion flow and nerve cell volume, which can cause metabolic disturbances, changes in cell function and modification of transmission of nerve impulses.[23]

Fluoride is also known to interfere with thyroid gland function and cause degenerative changes in the central nervous system. To gain a better understanding of the problem, a study was carried out to determine the fluoride status and the free T4, free T3, and thyroid stimulating hormone (TSH) levels of children with and without dental fluorosis. A sample of 90 children aged between 7 to 18 years exhibiting dental fluorosis and consuming fluoridated water were selected through a school dental fluorosis survey. 21 children of the same age group and socioeconomic status, who did not exhibit dental fluorosis and resided in non-endemic areas were enrolled in the control group. It was found that 47% of children with average water fluoride level of 4.37 ppm reported evidence of clinical hypothyroidism attributable to fluoride. They found borderline low T3 levels among all children exposed to fluoridated water. The mechanisms through which fluoride exacerbates hypothyroidism include competitive binding with iodine, as well as obstruction of synthesis of T3 and T4. These findings suggest that children with or even without dental fluorosis from exposure to excess fluoride may show thyroid hormone derangements that may not be clinically observable until late stages.[24]

Another review article claimed that fluoridation of water or incorporation of fluorides in tooth paste is controversial. The author stated that as storehouse of fluoride is mainly the bones, it results in increased bone density thereby changing the internal architecture, making it osteoporotic and more prone to fractures. Its other effects include disruption of the synthesis of collagen leading to the breakdown of collagen in bone, tendon, muscle, skin, cartilage, lungs, kidney and trachea, causing disruptive effects on various tissues in the body. It also inhibits antibody formation, disturbing the immune system of the child making them prone to malignancy. Thus, consumption of fluoride has been reported to increase the occurrence of cancer. It has been categorized

as a protoplasmic poison and any additional ingestion of fluoride by children is considered undesirable [Figure No. 1].[25]

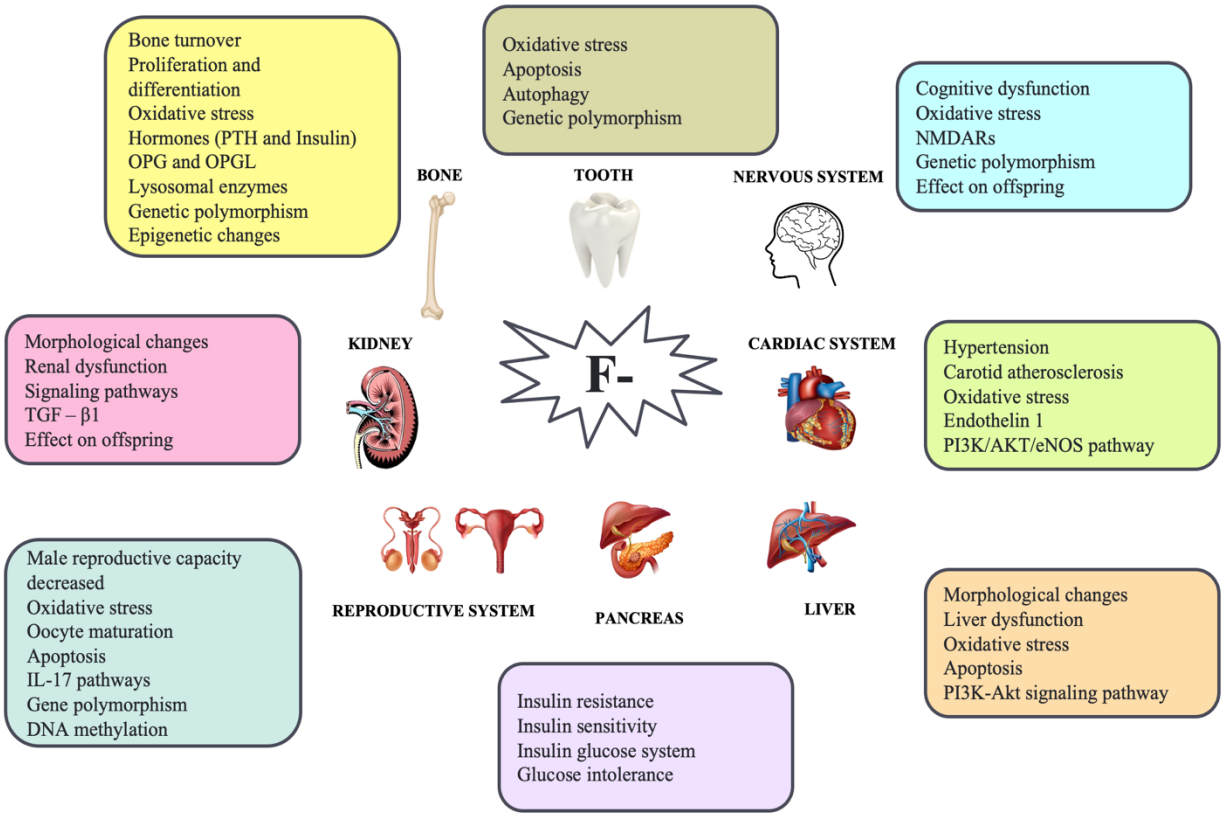


Figure No. 1: Figure showing effects of fluoride on different tissues and organs of the body.

LITERATURE IN RELATION TO USE OF HERBALS IN PREVENTIVE DENTISTRY

Herbal extracts have been a part of traditional medicine in India and other countries since thousand years. Various review articles have evaluated the emerging trends of use of herbals in dentistry. The authors have summarized the various herbals extracts used for reducing inflammation, as antimicrobial plaque agents, for preventing release of histamine, as antiseptics, antioxidants, antimicrobials, antifungals, antibacterials, antivirals and analgesics [Table No. 1].[10,26]

Table No. 1: Table showing commonly used herbs in dentistry.

Herbs	Use In Oral healthcare
Myrrh (*Commiphora Myrrha*)	Helps to promote healing in cases of pyorrhea and combat halitosis.
Prickly Ash (*Zanthoxylum*)	Increases salivary flow and relieves toothache.
Peppermint (*Mentha Piperita*)	Used for toothache and to relieve gum inflammation.
Red clover (*Trifolium Pretense*)	Healing of irritated and diseased gums. Also has antibiotic properties on gums that are abscessed or sore and inflamed from root canal therapy or other dental procedures.
Rosemary (*Rosmarinus officinalis*)	Potent antibacterial and antioxidant agent. Used for treatment of gum disease and bad breath.
Sanicle (*Sanicula Europaea*)	Used as a powerful antioxidant and to heal septic wounds.

Shepherd's Purse (*Capsella Bursa- pastoris*)	Helps stop bleeding after tooth extraction.
Tree tea oil (*Melaleuca Alternifolia*)	Used to heal sore, inflamed gums and to relieve inflammation. It has mild solvent action, and hence can be used in root canal treatment for dissolving the necrotic pulp tissue.
Thyme (*Thymus Vulgaris*)	Treatment of oral herpes. Antibacterial action against *Streptococcus mutans*.
Violets (*Clematis Virginca*)	Relieves pain and tenderness from sores caused by oral cancer and is helpful in soothing canker sores and cold sores.
Wintergreen (*Gaultheria Procumbens*)	Astringent and antiseptic action.
Yarrow (*Achillea Millefolium*)	Used to treat hemorrhages, ulcers and to improve blood clotting.
Bloodroot (*Sanguinaria Canadensis*)	Used in gingival and periodontal disease.
Chamomile (*Matricaria Recutita*)	Anti-inflammatory and antispasmodic effect. Used in gingival and periodontal disease.
Sage (*Salvia Officinalis*)	Antibacterial, antifungal, and antiviral activity.

LITERATURE IN RELATION TO *ZINGIBER OFFICINALE*

Zingiber Officinale belongs to the family *Zingiberaceae* and is more commonly known as ginger or adrak. It is believed to be a native of south-eastern Asia. It grows well in tropical and subtropical areas perennially and is cultivated through rhizomes. The rhizomes are full of aroma and can reach a size of 30-90 cm comprising of thick lobes with ring-like scars. It is widely used as a spice, natural additive, and in herbal remedies.

Figure No. 2: Figure showing *Zingiber officinale.*

Zingiber officinale is a vital plant possessing several remedial and nutritional properties. To evaluate the antimicrobial activity of *Zingiber officinale* against *Streptococcus mutans*, a study was conducted wherein rhizomes of ginger were collected and essential oil was extracted from them. The oil was further purified and recrystallisation was performed to obtain zerumbome with 98% purity. The antimicrobial activity was assessed using microdilution method in which bacterial

growth was evaluated by measuring the turbidity. The Minimum Inhibitory Concentration (MIC) was found to be 250 µg/ml. Further, Minimum Bactericidal Concentration (MBC) was determined by counting the number of surviving cells. It was found to be 500 µg/ml. A time kill curve assay was performed to determine the speed of cidal activity. After 24 hours of exposure, 70.62% reduction in bacterial growth was observed at concentration of 500 µg/ml. It was also observed that 99.99 % bacteria were killed at the time interval of 48-72 hour. The cytotoxicity activity was assessed using MTT assay and it was observed that it showed no cytotoxic effect on normal mammalian cells up to a concentration of 100 µg/ml.[27]

In a similar context, an in vitro study was carried out to determine the antimicrobial activity of *Zingiber officinale* against *Streptococcus mutans* and *Lactobacillus acidophilus.* Bacterial species were isolated from two hundred fifty extracted carious teeth. Antibiotic sensitivity test was employed wherein the mean zones of inhibition were measured. The diameter of the zone of inhibition was found to be 20.0 $\pm$ 0.5 mm against both the organisms at a concentration of 500 mg/ml. These results highlight the potential of ginger as a strong antimicrobial agent against caries causing microorganisms. These properties are due to presence of various phytochemicals in ginger [Table No. 2].[28]

Table No. 2: Table showing phytoconstituents of *Zingiber officinale* with their benefits.

Sl. No.	Phytoconstituents	Properties
1	Gingerol	Antioxidant, anti-inflammatory, analgesic and anti-microbial activity.
2	Paradol	Anti-oxidant, anti-microbial and anti-cancerous activity.
3	Shogaol	Anti-oxidant and anti-inflammatory activity.
4	Zingerone	Antioxidant, anti-inflammatory and anti-bacterial activity.
5	Zerumbone	Anti-microbial activity.
6	1-Dehydro-(10) gingerdione	Regulation of inflammatory genes.
7	Terpenoids	Induce Apoptosis by activation of p53.
8	Flavonoids	Antioxidant activity.

The antimicrobial property of *Zingiber officinale* is also dependent on the extraction method employed. An in-vitro study was carried out to screen the antibacterial activity of the n-hexane, ethyl acetate, methanol, and aqueous extracts of *Zingiber officinale* against *Streptococcus mutans*. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration was determined using broth microdilution method. Minimum Inhibitory Concentration of n-hexane, ethyl acetate and methanol extracts of *Zingiber officinale* was found to be 800 µg/ml, whereas it was 1 mg/ml for water extract. Furthermore, the Minimum Bactericidal Concentration value of all

extracts was 2 mg/ml. Agar diffusion method was used to determine the susceptibility of *S. mutans* against these extracts. Methanol extract showed the highest antibacterial activity in the concentration of 2 mg/mL, with an inhibition zone of 21.7 mm. Biofilm formation inhibition of *Zingiber officinale* was also assessed by the microdilution method. Methanolic extract showed greatest inhibition of biofilm at concentration of 15-31 µg/mL.[29]

A similar study was designed to detect the inhibitory effect of various extracts of *Zingiber officinale* towards the virulence of *Streptococcus mutans.* Initially, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration was determined using microdilution method which was found to be 256 µg/ml. The kinetics of the antimicrobial effect of crude extract and methanolic extract was determined and it was found that these extracts killed *S. mutans* in a time as well as dose dependent manner. At a concentration of 128 µg/ml, crude extract showed a decrease of more than 5-log CFU/ml after 24 hours of incubation, whereas methanolic extract showed a reduction of 4 - log CFU/ml. Sucrose-dependent and sucrose-independent adherence assay was performed and it was found that both the extracts displayed a strong anti-adherence activity by inhibiting the sucrose-dependent as well as sucrose-independent adherence. Crude extract at 128 µg/ml was found to reduce sucrose dependent and sucrose independent adherence by 78% and 73% respectively, whereas methanolic extract at the same concentration reduced it by 72% and 70% respectively. Both the extracts also inhibited biofilm formation in a concentration dependent manner. A decline in the percentage of adherent cells was seen with every successive growth phase under various concentrations of extracts. Both the extracts also inhibited the glycolytic acid production and F-ATPase activity of *S. mutans* at a concentration of 128 µg/ml. These extracts also resulted in downregulation of virulent genes of *S. mutans* such as

relA, gtfC, brpA and comDE. These findings reflect a prospective role of *Zingiber officinale* as a potential therapeutic agent against virulence traits of *S. mutans* [Figure No. 3].[30]

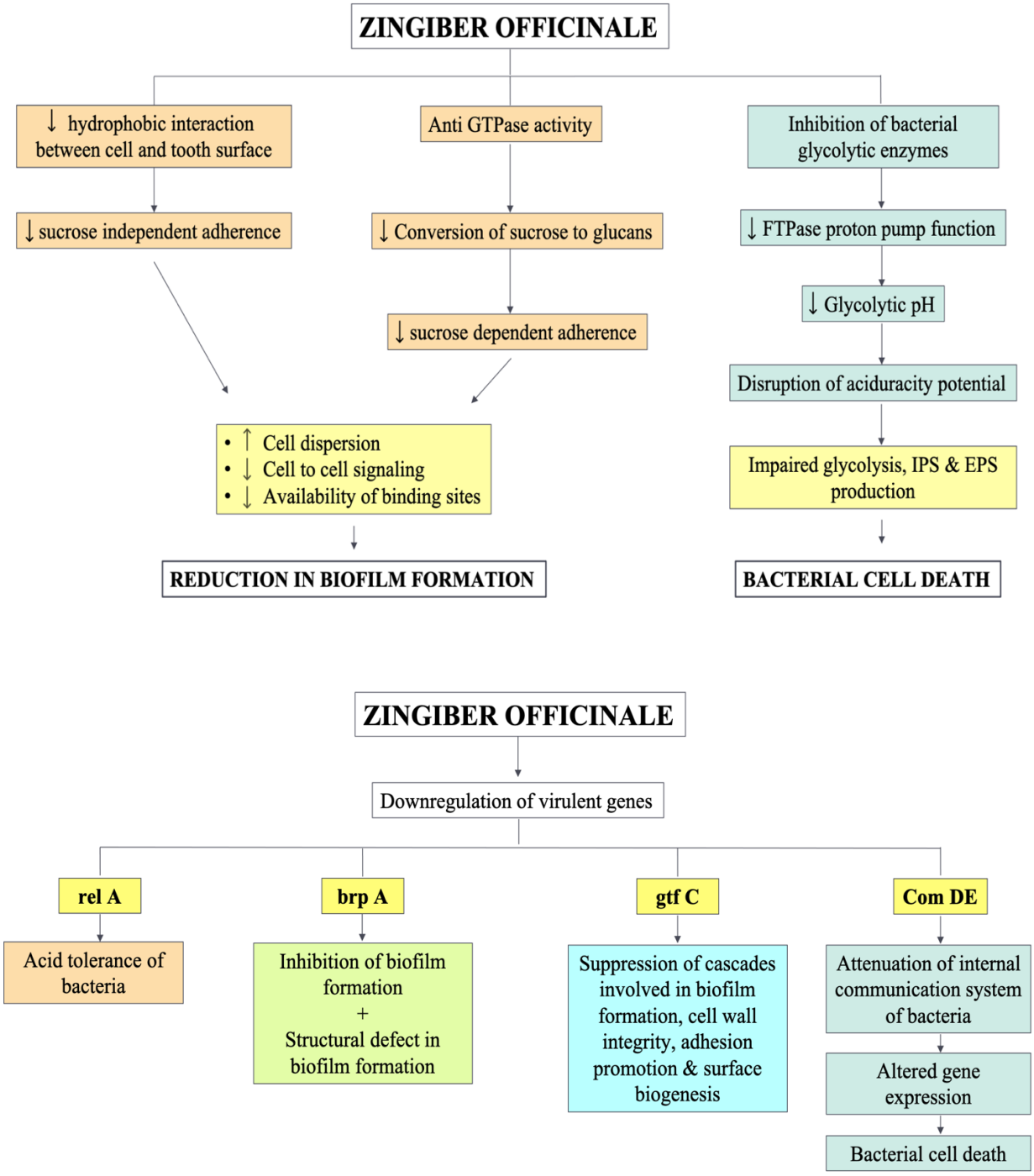


Figure No. 3: Figure illustrating mechanism of action of *Zingiber officinale*.

Another study was conducted to evaluates ginger's biochemical profile using qualitative and quantitative analysis and its bioactive potentials using antioxidant and antimicrobial assays against *Streptococcus mutans* and *Lactobacillus spp.* Solvents like ethanol, chloroform, ethyl acetate n-hexane, n-butanol, and distilled water were used for the extraction procedure. Distilled water and ethanolic extracts showed the highest antioxidant activity at different tested concentrations. Among the tested bacterial isolates, *Streptococcus mutans* showed sensitivity against all tested extracts. Ethanolic extracts and ethyl acetate extracts showed the highest antibacterial activity against *Lactobacillus spp*. The chemical analysis of ginger reveals that it contains almost 400 different compounds. However, it was observed that ethanolic extracts had the highest polyphenols content. Maximum concentration of Quercetin, which is an anti-biofilm agent with broad spectrum antibacterial properties, was found in the fraction of water and ethanol. The ethanolic extract of *Zingiber officinale* was also found to be rich in tannins, alkaloids, saponins, flavonoids, phenols, steroids, terpenoids and carboxylic acids. These findings highlight the antibacterial and antioxidant potential of ethanolic extract of *Zingiber officinale*.[31]

In accordance to the available literature highlighting the antimicrobial efficacy of ethanolic extract, an in vitro study was performed to assess the antibacterial efficacy of ethanolic extract of ginger against *Streptococcus mutans*. Two different culture methods were performed in the study. In the first method, simultaneous culture of bacterial suspension and the extract in agar medium was performed. In the second method, bacteria was cultured in the mixture of agar medium and diluted extract. The respective mixtures were transferred to plates on which the bacteria were inoculated. The concentration of the extract in the first plate showing inhibitory effect on bacterial growth was taken as MIC and the minimum concentration showing no bacterial growth

was taken as MBC. The minimum inhibitory concentration of ginger extract was found to be 0.02 mg/ml and minimum bactericidal concentration was found to be 0.04 mg/ml. This study advocates the antimicrobial potency of ethanolic extract of ginger against *Streptococcus mutans*.[32]

In addition to the antibacterial effect, ginger is also being explored as a potent remineralization agent. A study was conducted to evaluate the remineralization potential of mixture of *Zingiber officinale* and *Apis mellifera* on artificially demineralized human enamel using Quantitative Light Induced Fluorescence (QLF). A window measuring 4x4 mm was created on the buccal surface of each tooth and the samples were then subjected to demineralization process for a period of 96 hours. Remineralization was then carried out with application of the mixture twice daily for a period of 21 days. QLF readings were then recorded at the end of demineralization, Day 7, Day 14 and Day 21. A reduction in fluorescence was observed after the 14th day, however, a statistically significant fluorescence gain was observed at 21st day of remineralization cycle. This study highlights the role of ginger in remineralization of enamel.[33]

A similar study was conducted to evaluate the effectiveness of herbal medicaments such as ginger, rosemary and honey on remineralization of initial enamel lesion. These medicaments were applied three times a day on demineralized enamel specimens for a time span of twenty-one days. Assessment of mineral content was carried out using FluoreCam and QLF. Among all treatment materials, combination of ginger and honey group showed the greatest remineralization with statistically significant values. These findings advocate the use of ginger as a potent remineralizing agent.[34]

LITERATURE IN RELATION TO *SALVADORA PERSICA*

Salvadora persica, more commonly known as Miswak is a small ever-green tree belonging to the family *Salvadoraceae*. It is also known as Jaal, Pilu, Goni mara and mirjoli in various regional languages. The *Salvadora persica* tree is usually found in the arid regions of India [Figure No. 4]. It is believed to have varied usefulness and has been traditionally used for epilepsy, scurvy, cough, rheumatism etc.

Figure No. 4: Figure showing *Salvadora persica* tree with miswak sticks.

To determine the anticariogenic potential of *Salvadora persica*, a study was carried out to evaluate the incidence of caries among children using miswak sticks. A study population consisting of 240 children was taken which was equally divided into two groups consisting of study group and control group. Their teeth were examined at the beginning of the study and DMF scores were recorded for each participant. There was no significant difference in DMF scores between both the groups at the start of the study. The study group was trained to use miswak sticks twice daily. The control group was instructed to use toothbrush twice daily. The participants were recalled after every 3 months and the instructions were repeated at each visit. After 2 years of intervention, the DMF scores of all the participants were recorded. An increase in caries index was observed in non-miswak group indicating the anticariogenic effect of miswak. It was also observed that dropout rate was more in non-miswak group which indicates that participants using miswak were more interested in brushing their teeth. These findings suggest that miswak can be used as an oral hygiene aid.[35]

In a similar context, an in-vivo study was carried out to determine the effect of miswak toothpaste and mouthwash against commercially available toothpastes and normal saline. A sample size of 40 was selected and randomly divided into 4 groups. Pre intervention salivary samples were collected. All participants were then instructed to use their specified mode of intervention. Salivary samples were collected immediately after intervention. A third sample was collected after 2 weeks of continuously using the specified methods of cleansing. *Streptococcus mutans* and *Lactobacillus spp.* count was assessed in salivary samples. It was observed that in miswak toothpaste group there was no significant reduction in *S. mutans* count immediately but a highly significant decrease was observed after 2 weeks. However, miswak toothpaste showed significant reduction in *Lactobacilli* count both immediately

and 2 weeks post intervention. Miswak mouthwash also showed a highly significant decrease in both the bacteria at all time intervals. Also, miswak mouthwash was significantly more effective in reducing both the bacteria against miswak toothpaste. On the other hand, there was no significant reduction in growth of both the bacteria in ordinary toothpaste group. These results indicate that miswak products are more effective in reducing caries. These effects of miswak can be attributed to the presence of various phytoconstituents [Table No. 3].[36]

Table No. 3: Table showing phytoconstituents of *Salvadora persica* with their benefits.

Sl. No.	Phytoconstituents	Properties
1	Sulphur	Antibacterial effect
2	Chlorides, fluorides	Enamel remineralization
3	Vitamin C	Tissue healing and repair
4	Tannins	Reduce plaque and gingivitis
5	Silica	Removing stains
6	Alkaloids	Antibacterial activity
7	Benzyl isothiocyanate	Prevent cariogenic and genotoxic compounds
8	Essential oils	Flow of saliva and buffering pH
9	Butanediamide	Antimicrobial agent
10	N-benzyl-2-phenylacetamide	Antimicrobial agent

In order to determine the efficacy of various extraction methods employed, a study was done to evaluate the antibacterial effect of ethanolic and hexane extracts of miswak against *Streptococcus mutans*. 10 % hexane and ethanolic extracts were freshly prepared. Antimicrobial activity was tested using well diffusion method. Minimum Inhibitory Concentration was determined using broth dilution assay. Ethanolic extract showed minimum inhibitory concentration and minimum bactericidal concentration of 8 mg/ml. Hexane extract showed minimum inhibitory concentration of 4 mg/ml and minimum bactericidal concentration of 8 mg/ml against *S. mutans*. Zone of microbial inhibition was also measured and 8 mm inhibitory zone was observed for ethanolic extract and 6 mm for hexane extract at 50 mg/ml concentration. At a concentration of 100 mg/ml, 16 mm zone of inhibition was obtained for ethanolic extract and 13 mm zone of inhibition was obtained for hexane extract. These results highlight the effectiveness of ethanolic extract of miswak against *S. mutans*.[37]

Owing to this, an in-vitro study was carried out to compare the antimicrobial activity of aqueous and ethanolic extracts of *Salvadora persica* against *Streptococcus mutans, Streptococcus mitis, Lactobacillus acidophilus, Candida albicans* and *Prevotella intermedia*. The aqueous and ethanolic extracts were prepared in concentrations of 200 µg/ml and 400 µg/ml. Antibacterial activity was assessed using agar diffusion method where zone of inhibition was measured. The tested bacteria showed no zone of inhibition except for *S. mutans* at both the concentrations of aqueous extract. 400 µg/ml of aqueous extract showed a 13 mm zone of inhibition for S. mutans. However, significant zones of inhibition were observed in ethanolic extract. *S. mutans* and *L. acidophilus* gave an inhibitory zone of 17.33 mm and 10.67 mm respectively at concentration of 400 µg/ml. The results of this study showed that ethanolic extracts of *Salvadora persica* possess better antimicrobial activity than other extracts.[38]

In context to above stated findings, a study was carried out to compare the antimicrobial activity of water, ethanol and hexane extracts of miswak against commercial toothpastes. The bacteria tested were *S. mutans*, *L. acidophilus*, *E. coli* and *S. aureus*. Chloride content and metal content of dried powder of miswak was analyzed using Mohr's method. The miswak content profile showed highest level of chloride in water extract. Lowest level of mineral content was seen in ethanolic extract of miswak. Salivary samples were also collected to determine the role of miswak on salivary pH. An increase in salivary pH was observed following the use of miswak. Total bacterial count was also measured from the collected saliva samples after the use of miswak at the concentration of 100 µg/ml. 42.5% bacterial reduction was observed with water extracts, 41.3% reduction was seen in ethanolic extract and hexane extract showed 19.4% bacterial reduction. The commercially available toothpastes showed a bacterial reduction of 13.4% and 11.2%. Further, disc diffusion assay was used where zone of inhibition was measured to detect the antimicrobial activity. The results showed that water extract had the greatest antimicrobial activity against all test organisms. The hexane extract showed the least antimicrobial activity. However, ethanolic extract were found to be the most effective against *S. mutans*. The results concluded that miswak possesses a broad antimicrobial activity against both gram negative and gram positive bacteria [Figure No. 5].[39]

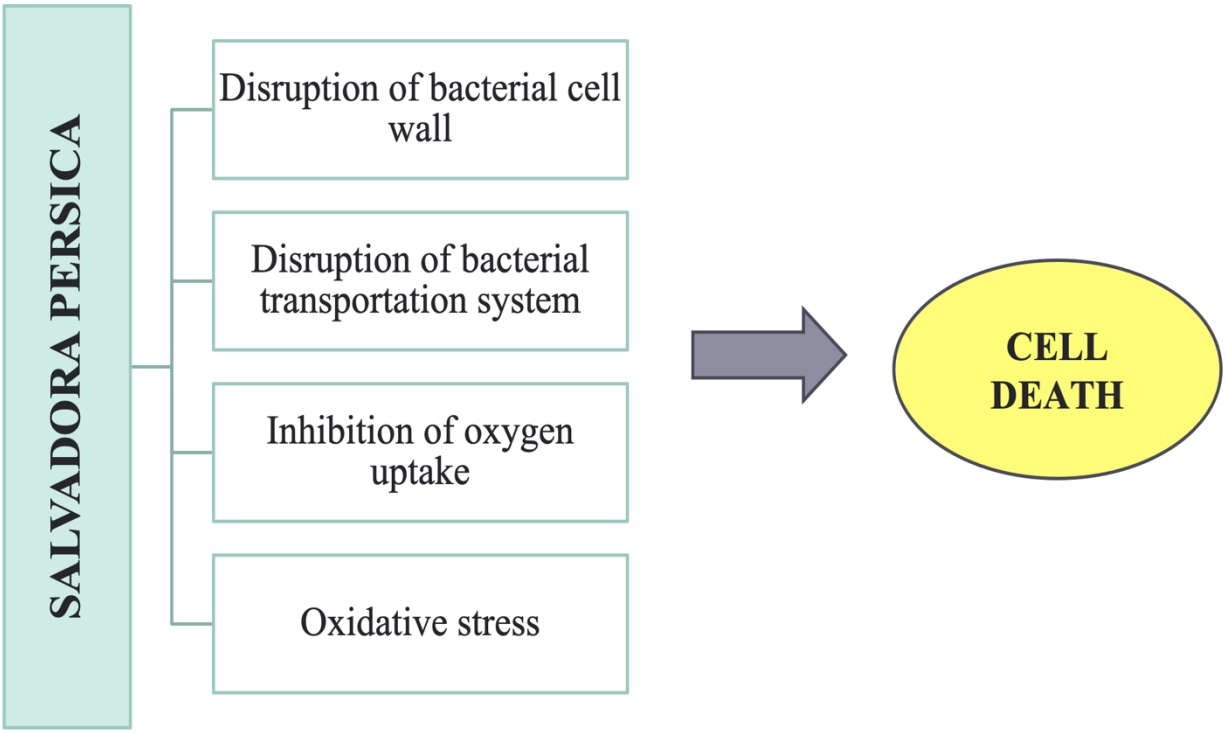


Figure No. 5: Figure illustrating mechanism of action of *Salvadora persica.*

Pertaining to determine the effectiveness of miswak against sodium fluoride, a randomized controlled triple blind trial was carried out to compare the antibacterial effect of 0.5% sodium fluoride impregnated miswak and plain miswak sticks on *S. mutans*. A sample size of 30 was taken comprising of healthy subjects who were randomly assigned in each group. Baseline sample of saliva was collected from each subject. They were then instructed to chew miswak sticks for 6 minutes in the morning before breakfast. Following the intervention, unstimulated salivary samples were again collected for microbiological assessment. *S. mutans* count was determined for both the samples. A statistically significant reduction in *S. mutans* count was observed and similar results were obtained for both the groups. This study concluded that plain miswak sticks are equally effective as fluoride impregnated miswak sticks in inhibiting the growth of *S. mutans*.[40]

In a similar context, a study was carried out to evaluate and compare the antimicrobial efficacy of dentifrices containing miswak, aloe vera and fluoride. A sample size of 60 was taken which included children aged between 6-12 years with DMF score 0. The participants were instructed to perform tooth brushing using the allotted dentifrices following a standard procedure for 1 month. Salivary samples were collected before intervention and after a period of 2 days, 15 days and 30 days. *S. mutans* count was done using colony counter machine. A reduction of 78.1% in *S. mutans* count was observed after a period of 15 days in miswak dentifrice group which was the maximum in all groups. After a period of 30 days, 83.5% reduction was observed. Similar results were obtained for fluoridated dentifrices group indicating the comparable antimicrobial efficacy of miswak and fluoride.[41]

The anticariogenic potential of miswak is also dependent on its role in protection of tooth surfaces against demineralization. Owing to this, an in vitro study was conducted to evaluate the effects of miswak on acid eroded enamel surface. Aqueous extract of miswak was prepared. Premolar teeth with macroscopically sound enamel surface were selected for the study and stored in artificial saliva. A stylus based inductive gauge was used to measure the surface roughness. The average surface roughness before treatment was found to be 3.58 μm. Enamel specimens were then treated with citric acid solution with pH of 3.3 for 120 seconds. The specimens were then rinsed with deionized water. Following the exposure, the surface roughness degraded and was found to be 3.92 μm. The specimens were then treated with aqueous extract of miswak for 120 seconds, following which the surface roughness improved and was found to be 2.97 μm. The surface topography of all samples was analyzed by Scanning Electron Microscopy. Samples treated with citric acid solution showed severe erosion with voids along with loss of material. On the other hand, treatment with

aqueous extract of miswak produced a smoother surface with flat areas. These results indicate that miswak acts as a protective agent against the effect of acidic solutions on enamel surface [Figure No. 6].[42]

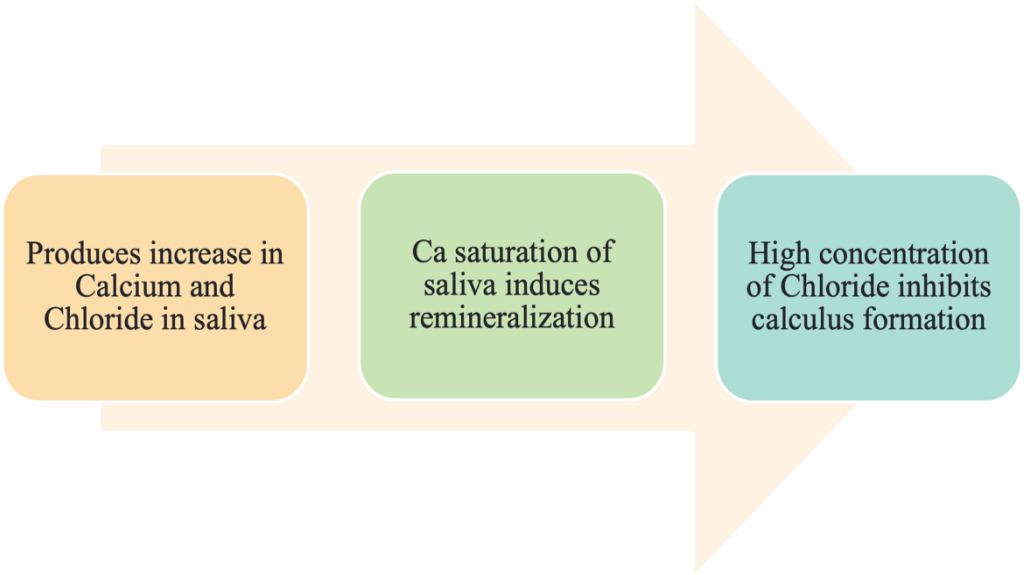


Figure No. 6: Figure illustrating mechanism of action of *Salvadora persica* in remineralization.

LITERATURE IN RELATION TO *CINNAMOMUM ZEYLANICUM*

Cinnamomum zeylanicum, belongs to the family *Lauraceae*. It is a tropical evergreen tree which is native to Sri Lanka and the Malabar Coast of India [Figure No. 7]. It is called differently in different languages such as dalchini in Hindi, cannelle in French, kaneel in German, canela in Spanish, and yook gway in Chinese.

Figure No. 7: Figure showing *Cinnamomum zeylanicum* tree with bark.

An in-vitro study aimed to determine the anti bacterial effect of *Cinnamomum zeylanicum* oil against oral pathogens like *Streptococcus mutans, Pophyromonas gingivalis, Treponema denticola* and *Enterococus faecalis.* Dried bark of cinnamon was grounded and essential oil was extracted using hydro-distillation technique. Gas chromatography was carried out to analyze the constituents of cinnamon oil. The oil comprised of 82% cinnamaldehyde and 1.4% alpha- pinene. Determination of Minimum Inhibitory Concentration was done using broth microdilution technique. The antibacterial activity was recorded using visual confirmation and reading of absorbance using a plate reader at 590 nm wavelength. The tests were carried out three times and mean MIC value was recorded. Cinnamon oil inhibited the activity of all pathogenic bacteria with a mean MIC value ranging between 0.21$\pm$ 0.04 and 0.63$\pm$0.23 µL/mL. Scanning Electron Microscopy was used to analyze the changes in bacterial membranes following 2-hour exposure to cinnamon oil. An increase in translucency of bacterial cell walls was observed in all the bacteria tested which indicated the changes that occurred in bacterial cell membrane. The study concluded that cinnamon oil and its constituents have a potential to prevent bacteria related oral diseases.[43]

Another in-vitro study was aimed to determine the antibacterial efficacy of five plant extracts namely, Cinnamon, Ginger, Turmeric, Clove and Black seed against *Streptococcus mutans* and *Lactobacillus acidophillus*. Methanolic extract was prepared for each plant extract. Antibacterial activities of methanolic extracts for all plants was determined using disc diffusion method in which diameter of zone of inhibition was measured. Methanolic extract of cinnamon showed maximum antibacterial efficacy with an inhibitory zone of 14 mm against *Streptococcus mutans*. An inhibitory zone of 16.67 mm was observed against *Lactobacillus acidophilus*. Furthermore, minimum inhibitory concentration and minimum bactericidal concentration was determined for

Cinnamon. MIC of 13.44 mg/ml and 5.18 mg/ml was obtained against *S. mutans* and *L. acidophilus* respectively. MBC of 23.60 mg/ml was obtained against *S. mutans* and 16.40 mg/ml against *L. acidophilus*. It was also observed that incorporating cinnamon extract into commercial varnish (5% Sodium fluoride varnish) led to an increase in the antibacterial activity as the combination was more effective in suppression of *S. mutans* and *L. acidophilus*. The study concluded that cinnamon extracts can be useful for the development of antibacterial agents against *S. mutans* and *L. acidophilus*. These antibacterial effects can be attributed to the constituents of cinnamon [Table No. 4].[44]

Table No. 4: Table showing phytoconstituents of *Cinnamomum zeylanicum* with their benefits.

Sl. No.	Phytoconstituents	Properties
1	Cinnamaldehyde	Antibacterial, Antioxidant, Anti inflammatory
2	Alpha – pinene	Antimicrobial, Anti inflammatory
3	Beta- Ocimene	Antioxidant, Anti inflammatory
4	Eugenol	Antibacterial

The antibacterial efficacy is also attributed to the procedure of extraction employed in the study. In context to this, a study was conducted to determine the antimicrobial efficacy of various extracts of *Cinnamomum zeylanicum* against caries causing organisms like *Streptococcus mutans*, *Staphylococcus aureus* and *Lactobacillus acidophilus*. Extracts were prepared using different solvents namely ethanol, methanol, acetone and aqueous. The antibacterial activity was assessed using agar well diffusion method and zone of inhibition was measured for each extract. Ethanolic extract showed equal antibacterial activity against *Streptococcus mutans* and

Staphylococcus aureus with a zone of inhibition of 14.95 mm and 14.65 mm respectively. Methanolic extract gave a 12.94 mm zone of inhibition against *Streptococcus mutans* followed by acetone extract which gave a 12.31 mm zone of inhibition. The aqueous extracts showed least antibacterial activity against all test organisms. *Lactobacillus acidophilus* was found to be resistant to all five extracts. This study concluded that ethanolic extract of cinnamon was the most potent of all five extracts against *Streptococcus mutans*.[45]

To further evaluate the antibacterial efficacy of ethanolic extract of *Cinnamomum zeylanicum,* a study was conducted wherein cinnamon bark was macerated followed by extraction in ethanol. The extract was found to be rich in alkaloids, flavonoids, saponins, and glycoside. *Streptococcus* samples were isolated from dental plaque of patients with dental caries. Antibacterial efficacy was determined by measuring the zone of inhibition. Cinnamon extract of 6.25%, 12.5% and 25% showed inhibitory zone of 6.78, 9, and 11.68 mm, respectively. The extract was further used to prepare mouthwash and it was found that there was a significant reduction in bacterial growth after rinsing with the prepared mouthwash.[46]

To further determine the effect of ethanolic extract of *Cinnamomum zeylanicum* on virulence factors of *Streptococcus mutans*, an in-vitro study was carried out to evaluate its glucosyltransferase adhesive ability, acid production suppression effect and antibacterial effect. *S. mutans* was cultured in Brain heart infusion medium in which ethanol was added to precipitate the protein and obtain crude glucosyltransferase. This was mixed with potassium phosphate buffer and cinnamon extract. The degree of cinnamon extracts adhesion-inhibition was calculated by measuring the absorbance at 550 nm with spectrophotometer. The glucosyltransferase adhesion-inhibition effect was 70% at 10 mg/ml followed by 56.6% at 8 mg/ml. These results confirmed that

cinnamon extract has adhesion-inhibition ability. The acid production suppression effect was evaluated by measuring the pH change in a solution containing bacterial suspension, glucose and cinnamon extract. The pH of the control group at 0 hour was 7.17. On addition of 2mg/mL of cinnamon extract, the pH was 7.41 and at 4mg/mL of cinnamon, the pH was 7.34. After 5 hours, the pH in control group dropped to 5.24. On the other hand, the pH at 2mg/mL of cinnamon extract was 7.67 and at 4mg/mL of cinnamon extract was 7.58. Thus, the cinnamon extract was efficient in inhibiting the acid production as compared to control group. The antibacterial activity of cinnamon extract was evaluated and 99% bactericidal action was achieved at a concentration of 4 mg/ml. Based on the study, cinnamon extract showed high effects on acids production inhibition and growth inhibition of bacteria. It also showed the ability to prevent the adhesion of glucosyltransferase [Figure No. 8].[47]

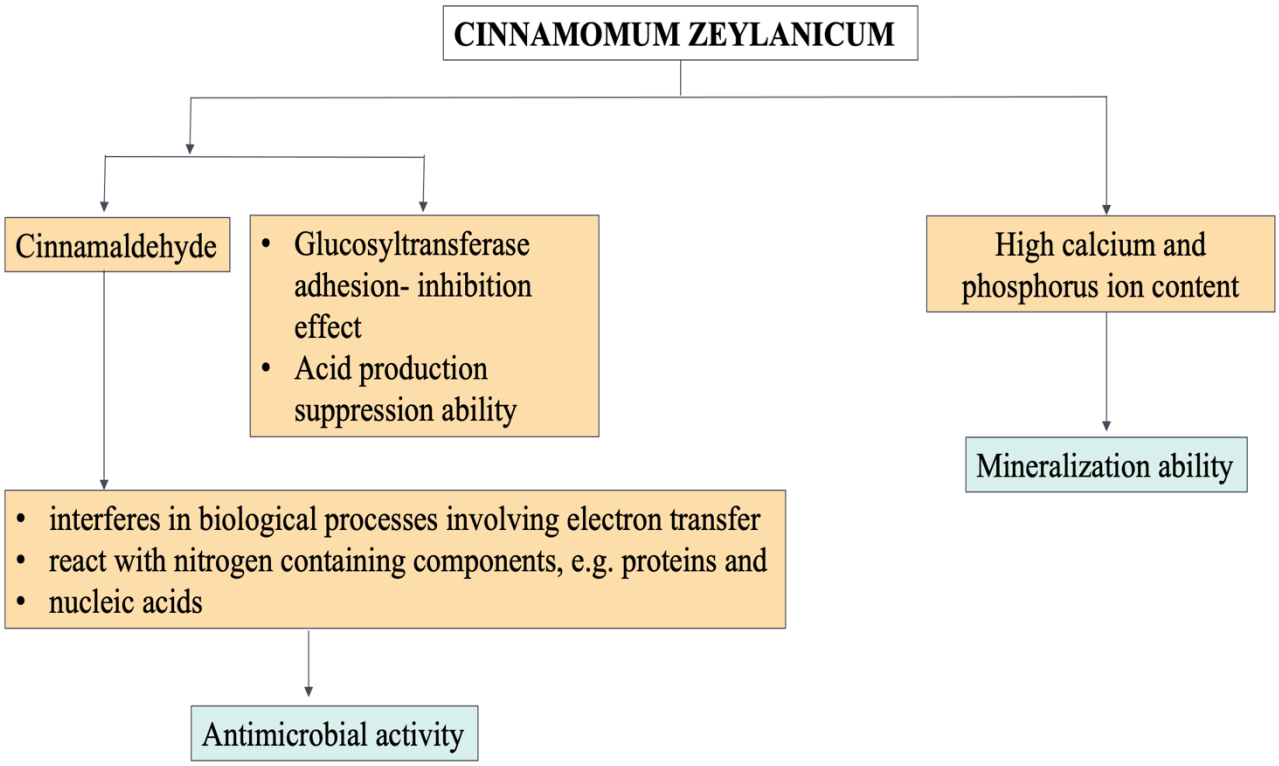


Figure No. 8: Figure illustrating mechanism of action of *Cinnamomum zeylanicum.*

Owing to its antibacterial effects, it is also important to determine the role of cinnamon as a surface protective agent. In context to this, an in-vitro study was carried out to determine the effect of cinnamon in protecting the enamel surface against acid attack in comparison to sodium fluoride. 20 extracted maxillary premolars were collected and cleaned and stored in 0.1% thymol solution. 5% and 10% aqueous cinnamon extracts were prepared. Teeth were immersed once daily for 2 minutes in 30 ml of test solutions and were then washed with deionized water. This procedure was carried out for 20 days. Following this treatment, tooth specimen were covered by sticky wax leaving a circular window of 3 mm. This window was left to perform etching which was done 2 times for five and ten seconds using 5 ml of HCl. The concentration of dissolved calcium ion was determined by flame atomic absorption spectrometer. Results of the study revealed that released Calcium ion concentration was minimum for 5% aqueous cinnamon extract for both etching times. Maximum dissolution of calcium was seen in 10% cinnamon extract which was an important finding of the study as higher amount of dissolved calcium was found when high concentration of extract was used. This study shows the effective nature of cinnamon in mineralization of the enamel surface.[48]

With the above stated objective, an invitro study aimed to assess the change in enamel surface roughness after immersion in cinnamon extract solution. 12 thoroughly cleaned extracted premolar teeth were taken and 100 μm of their surface was sanded off. Aqueous extract of cinnamon was prepared at 4% and 12.5% concentration. Tooth specimens were soaked in these solutions for 60, 120 and 180 minutes. pH and mineral content of the cinnamon extract solutions were measured. pH of 4% and 12.5% cinnamon extract solution was found to 5.38 and 5.45 respectively. A surface roughness tester was used to measure the enamel surface roughness. It was observed that 60-

minute immersion led to a slight increase in surface roughness whereas 120-minute and 180-minute immersion decreased the surface roughness of enamel specimen. However, the increase observed after 60 minutes was not significantly different from the initial surface roughness. It was also found that the change in surface roughness after 180-minute immersion was not significantly different in both the concentrations tested. This study concluded that cinnamon extract solution effected the surface roughness of enamel and it can be used to promote remineralization of enamel.[49]

To compare the effectiveness of aqueous extracts of cinnamon with commercially available stannous fluoride on the microhardness of artificially initiated carious lesions on enamel surface, an in vitro study was carried out using permanent premolars extracted from 12–14-year-old patients. Their buccal and lingual surfaces were coated with an acid resistant nail varnish leaving a circular window of 6 mm in diameter. The teeth were then subjected to pH cycling procedure by immersing them for 6 hours in demineralizing solution and 17 hours in remineralizing solution. This procedure was carried out for 10 days which resulted in induction of carious lesions. Aqueous cinnamon extracts were then prepared in concentrations of 1%, 5% and 10%. Stannous fluoride was taken in concentrations of 8% and 10%. Teeth specimens were treated by all test solutions for four minutes daily for one week. Vicker's microhardness device and optical microscope were used to measure the surface hardness. A highly significant reduction in enamel microhardness was seen after demineralization. Following the treatment of the specimens, a statistically highly significant increase in microhardness values was seen in all the test groups. 8% and 10% stannous fluoride produced maximum remineralization followed by 1% and 5% cinnamon extract. This study advocated the use of cinnamon for treatment of initial carious lesions.[50]

MATERIALS AND METHOD

The present in-vitro study was designed to evaluate and compare the antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel Containing *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum*.

The study was conducted in the Department of Pediatric and Preventive Dentistry at KLE Academy of Higher Education and Research (KAHER), KLE VK Institute of Dental Sciences, Belagavi in association with KAHER's Shri B.M.K Ayurvedic Mahavidyalaya, Belagavi; Department of Pharmaceutics, KAHER's College of Pharmacy, Belagavi; KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi and Department of Oral and Maxillofacial Pathology and Microbiology, KAHER's KLE VK Institute of Dental Sciences, Belagavi.

Outpatients with the inclusion criteria reporting to the Department of Pediatric & Preventive Dentistry and Department of Oral and Maxillofacial Surgery for the purpose of therapeutic extraction of premolar teeth in children undergoing orthodontic treatment at KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research, Belagavi were selected for the study. Ethical clearance certificate for the study was obtained from the Institutional review board (IRB) of KAHER's KLE VK Institute of Dental Sciences, Belagavi [Annexure I].

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. 9 and 10]**:**

- *Zingiber officinale* (Shri B.M.K Ayurvedic Mahavidyalaya, KLE Academy of Higher Education and Research, Belagavi)
- *Salvadora persica* (Trust Herbs Chittorgarh, Rajasthan)
- *Cinnamomum zeylanicum* (Shri B.M.K Ayurvedic Mahavidyalaya, KLE Academy of Higher Education and Research, Belagavi)
- Grinder (Rallison Appliances, Bengaluru)
- Pulverizer (Mill Power Industries, Ahemdabad, Gujarat)
- Weighing scale (Citizen CY220, Citizen Scale Pvt. Ltd., Malad, Mumbai)
- Waterbath (Labline Equipments Pvt. Ltd., Vadodara, Gujarat)
- Beaker (Borosil Glass Works Ltd., Mumbai)
- Ethanol (Antares Chemicals Pvt. Ltd., Mumbai)
- Conical flask (Borosil Glass Works Ltd., Mumbai)
- Whatman Qualitative filter paper (Sigma - Aldrich Co., USA)
- Parafilm (Bemis Company Inc.)

Figure No. 9: Photograph showing armamentarium for extract preparation of *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* in the study.

(a) (b)

Figure No. 10: Photograph showing (a) weighing scale and (b) water bath used for extract preparation of *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* in the study.

[ii] Armamentarium For Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal Concentration (MBC) [Figure No. 11 and 12]**:**

- Ethanolic extract of *Zingiber officinale*
- Ethanolic extract of *Salvadora persica*
- Ethanolic extract of *Cinnamomum zeylanicum*
- MTCC Strains of *Streptococcus mutans* and *Lactobacillus acidophilus*
- Weighing scale (Citizen CY220, Citizen Scale 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)

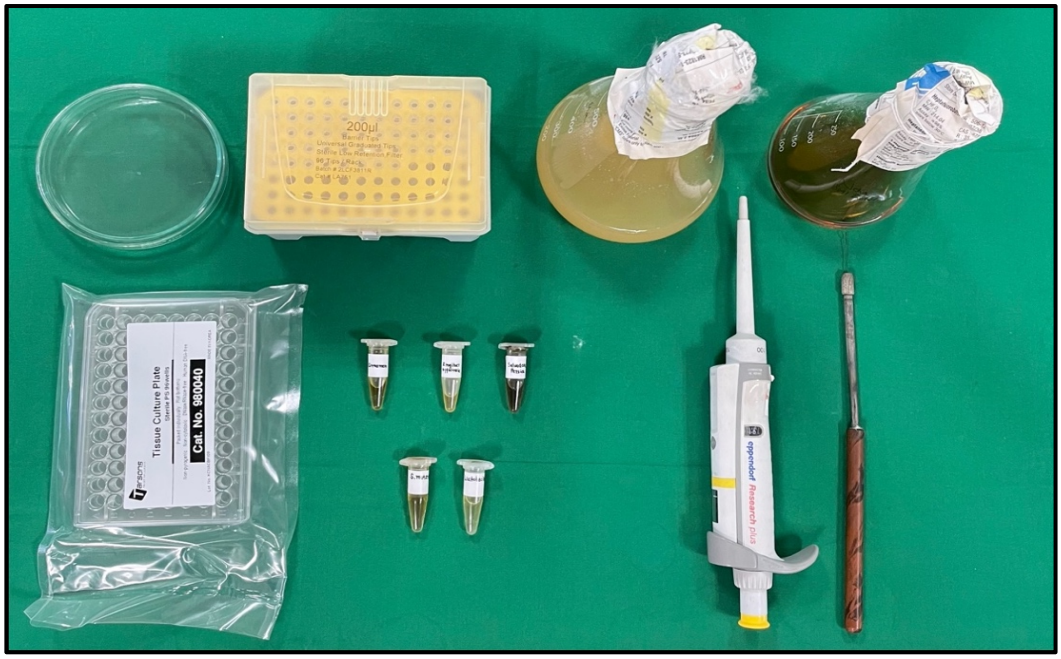


Figure No. 11: Photograph showing armamentarium for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in the study.

(a) (b)

Figure No. 12: Photograph showing (a) Vortex Mixer (b) Incubator used in the study.

[iii] Armamentarium For Herbal Dental gel Preparation [Figure No. 13 and 14]:

- Ethanolic extract of *Zingiber officinale*
- Ethanolic extract of *Salvadora persica*
- Ethanolic extract of *Cinnamomum zeylanicum*
- Carbapol 940 (HiMedia Laboratories Pvt. Ltd., Mumbai)
- 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)
- Trietholamine (SDFCL Sd Fine Chemicals Ltd., Chennai, Tamil Nadu)
- Weighing Scale (Citizen CY220, Citizen Scale Pvt. Ltd., Malad, Mumbai)
- Magnetic Stirrer (Remi Laboratory Instruments, Mumbai, Maharashtra)
- Beaker (Borosil Glass Works Ltd., Mumbai)
- Conical Flask (Borosil Glass Works Ltd., Mumbai)
- Distilled Water

Figure No. 13: Photograph showing armamentarium for Herbal Dental gel preparation in the study.

Figure No. 14: Photograph showing magnetic stirrer used for Herbal Dental gel preparation in the study.

[iv] Armamentarium For Cytotoxicity [Figure No. 15 and 16]**:**

- L929 Fibroblast (National Centre for Cell Science, Pune, Maharashtra)
- Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*
- Acidulated Phosphate Fluoride gel (Fluorovil Gel, Vishal Dentocare Pvt. Ltd., Ahemdabad, Gujarat)
- Buffering Solution: 0.5M Phosphate Buffer (pH 7)
- Dulbecco's Modified Eagle Medium (Genetix Biotech Asia Pvt. Ltd.)
- Dimethyl Sulfoxide (MERCK, Specialty Pvt. Ltd.)
- Pipette with micro pipette tips (Tarsons Products Pvt. Ltd., West Bengal)
- 96 well titre plate (SPL Life Sciences Co. Ltd., Korea)
- 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent) [HiMedia Laboratories Pvt. Ltd., Mumbai]
- 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)

Figure No. 15: Photograph showing armamentarium for cytotoxicity evaluation in the study.

Figure No. 16: Photograph showing microplate absorbance reader used for cytotoxicity evaluation in the study.

[v] Armamentarium For Agar Well Diffusion Method [Figure No. 17]**:**

- Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*
- Acidulated Phosphate Fluoride gel (Fluorovil Gel, Vishal Dentocare Pvt. Ltd., Ahemdabad, Gujarat)
- MTCC strains of *Streptococcus mutans* & *Lactobacillus acidophilus*
- Agar Plates (Borosil Glass Works Ltd., 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 Pvt. Ltd., Malad, Mumbai)
- Eppendorf tubes (Tarsons Products Pvt. Ltd., West Bengal)
- Parafilm (Bemis Company, Inc.)
- Conical Flask (Borosil Glass Works Ltd., Mumbai)

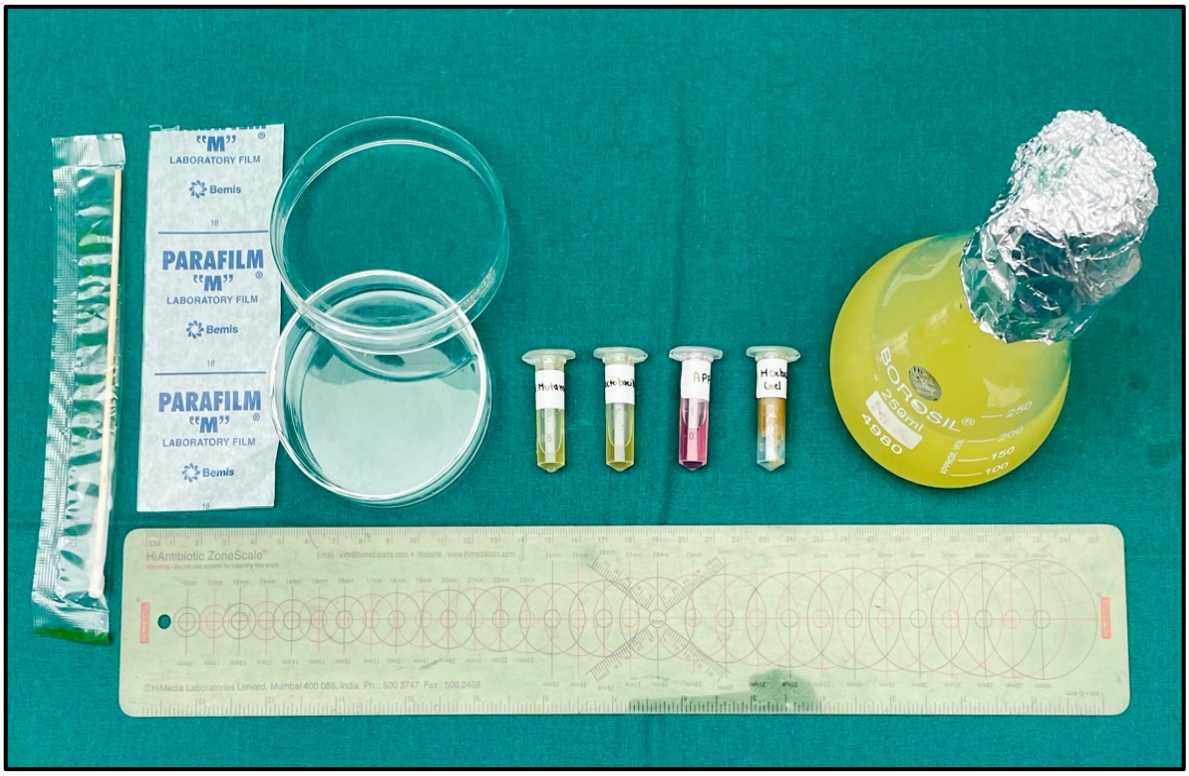


Figure No. 17: Photograph showing armamentarium for Agar Well Diffusion Method in the study.

[v] Armamentarium For Time Kill Assay [Figure No. 18 and 19]**:**

- Herbal Dental Gel of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*
- Acidulated Phosphate Fluoride gel (Fluorovil Gel, Vishal Dentocare Pvt. Ltd., Ahemdabad, Gujarat)
- 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)
- Vortex Mixer (IKA Industries, India)
- Absorbance Reader (Shimadzu Corporation, Kyoto, Japan)

Figure No. 18: Photograph showing armamentarium for Time Kill Assay in the study.

Figure No. 19: Photograph showing absorbance reader used for Time Kill Assay in the study.

[vi] Armamentarium For Evaluation Of Remineralization [Figure No. 20 and 21]**:**

- Herbal Dental Gel of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*
- Acidulated Phosphate Fluoride gel (Fluorovil Gel, Vishal Dentocare Pvt. Ltd., Ahemdabad, Gujarat)
- Extracted Premolar Teeth
- Formalin (ACS Chemicals, Ahmedabad, Gujarat)
- Diamond Disc (Addler Pvt. Ltd., Mumbai, Maharashtra)
- Acid Resistant Varnish (Elle 18, Hindustan Unilever, Mumbai)
- 37% Hypophosphoric Acid (Ivoclar Vivadent, Schaan, Liechtenstein)
- Stereomicroscope (Labomed Inc., Los Angeles)

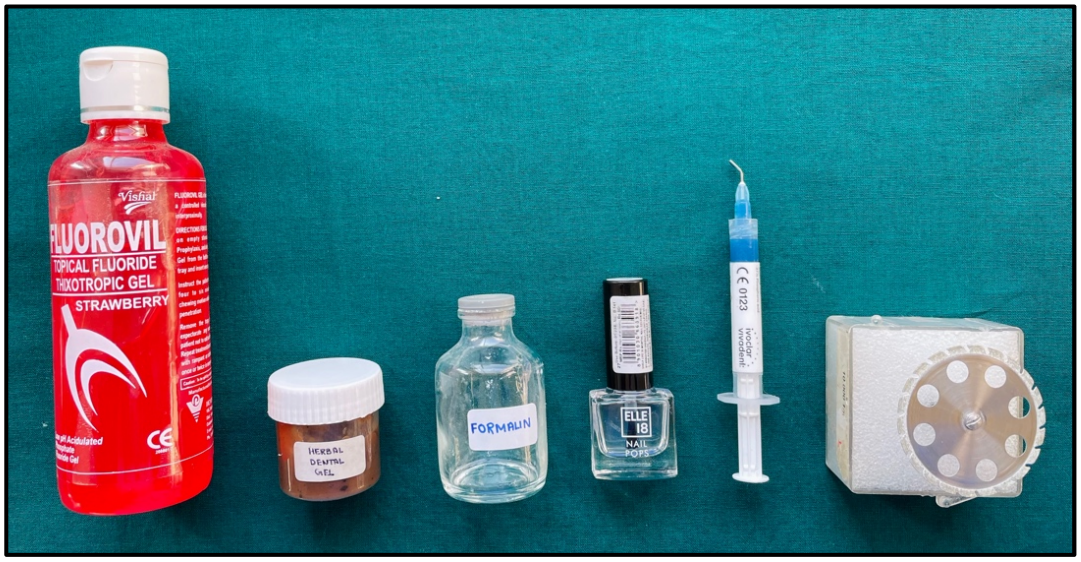


Figure No. 20: Photograph showing armamentarium used for evaluation of remineralization in the study.

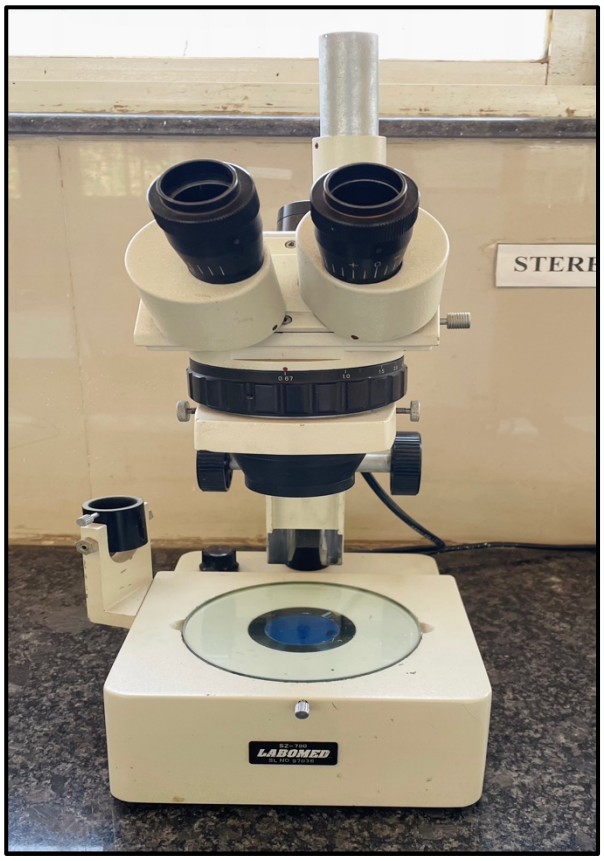


Figure No. 21: Photograph showing stereomicroscope used for evaluation of remineralization in the study.

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.

SELECTION OF SUBJECTS:

Subjects for the study were selected according to the following inclusion and exclusion criteria.

INCLUSION CRITERIA FOR THE STUDY:

- Premolar teeth indicated for therapeutic extraction in children undergoing orthodontic treatment with intact crown structure.

EXCLUSION CRITERIA FOR THE STUDY:

- Carious teeth.
- Hypoplastic teeth.
- Cracked areas in teeth.
- White spots present on teeth.
- Discoloured teeth.
- Fractured teeth.

SAMPLE SIZE:[51]

1. Evaluation of Antibacterial effectiveness

Sample size given was n=5,

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

2. The estimated sample size for remineralization was calculated according to the following formula where sample size of 30 was obtained for each group.

$$n = \frac{2S^2}{d^2}\left[z_\alpha + z_\beta\right]^2$$

Where:

- d = 1.54
- α error = 5%,
- Z α =1.96 (1.96 at 5% □ error)
- Z β = 1.037 (1.0370 at 15% □ error)
- S1 = 39.52 S2 = 38.70

(α = probability of type I error, Z = power of the study, S = standard deviation, d = mean difference.)

n=30 in each group.

A total of 60 samples of freshly extracted premolar teeth were taken for evaluation of remineralization potential.

STUDY GROUP:

The study groups were equally divided into two groups namely:

- **GROUP I (Control Group)**: Acidulated Phosphate Fluoride gel
- **GROUP II (Experimental Group)**: Herbal Dental gel Containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*

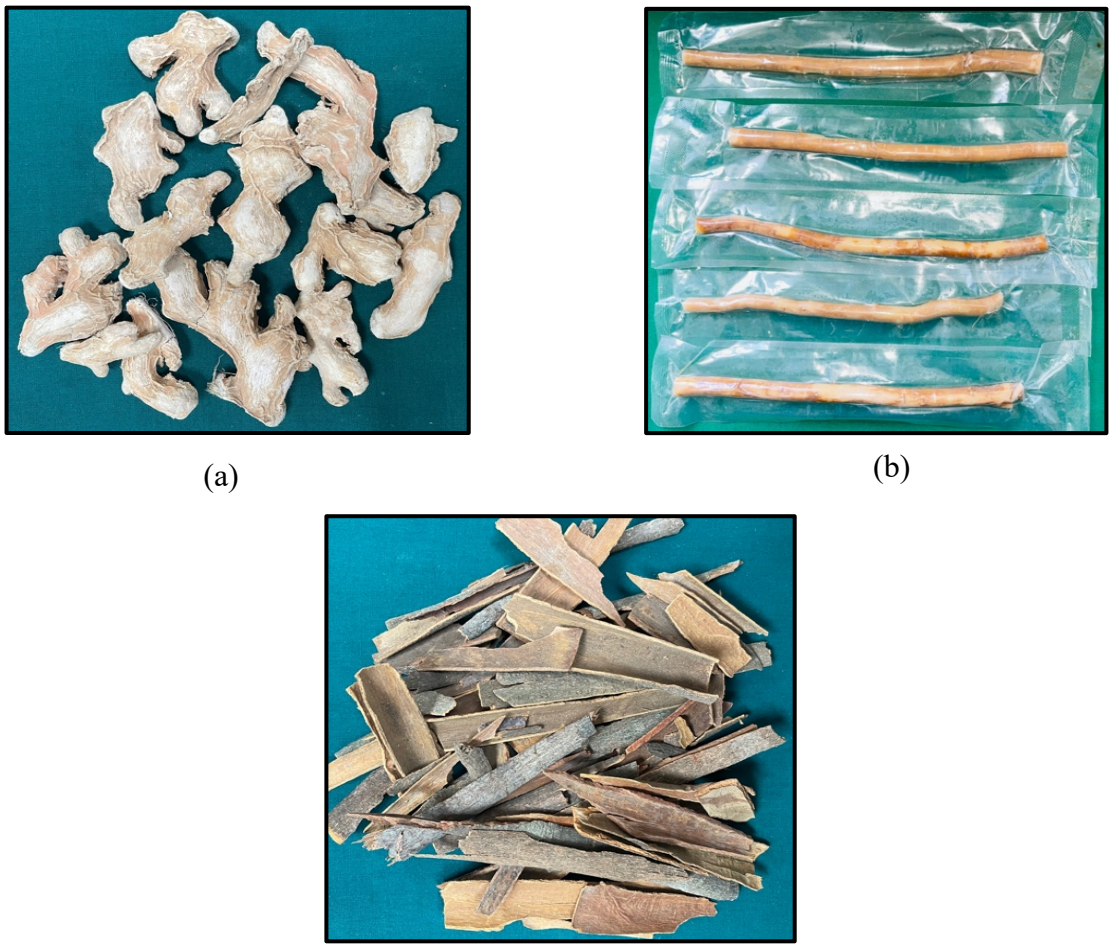
METHOD OF COLLECTION OF DATA

A) PREPARATION OF EXTRACT:

The extract was prepared in Central Research Facility of Shri. BMK Ayurveda Mahavidyalaya, KLE Academy of Higher Education and Research, Belagavi.

Preparation of Ethanolic Extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*:

The 150 gm of *Zingiber officinale* and *Cinnamomum zeylanicum* used in the study were obtained from Shri. BMK Ayurveda Mahavidyalaya, KLE Academy of Higher Education and Research, Belagavi. *Salvadora persica* sticks were obtained from Trust Herbs Chittorgarh, Rajasthan, India and verified from Shri. BMK Ayurveda Mahavidyalaya, Belagavi [Annexure II] [Figure No. 22]. They were grounded into fine powder using a pulverizer [Figure No. 23]. The powdered samples [Figure No. 24] were extracted with ethanol in ratio of 1:10 (v/v) for four days by keeping them inside rotary incubator [Figure No. 25 and 26]. The supernatant collected was filtered into clean sterile dried conical flask [Figure No. 27]. The yield obtained was weighed and stored in plastic containers at room temperature [52] [Figure No. 28].



(a) (b)

(c)

Figure No. 22: Photograph showing (a) Zingiber officinale, (b) *Salvadora persica* and (c) *Cinnamomum zeylanicum*.

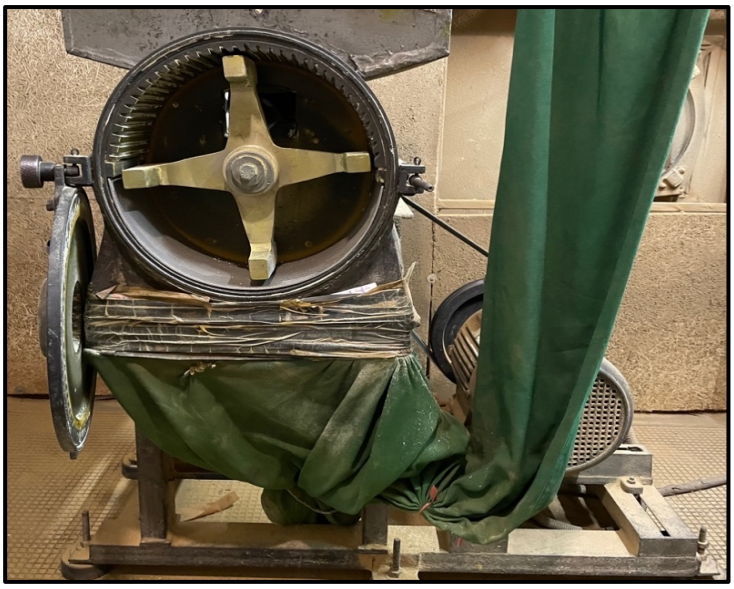
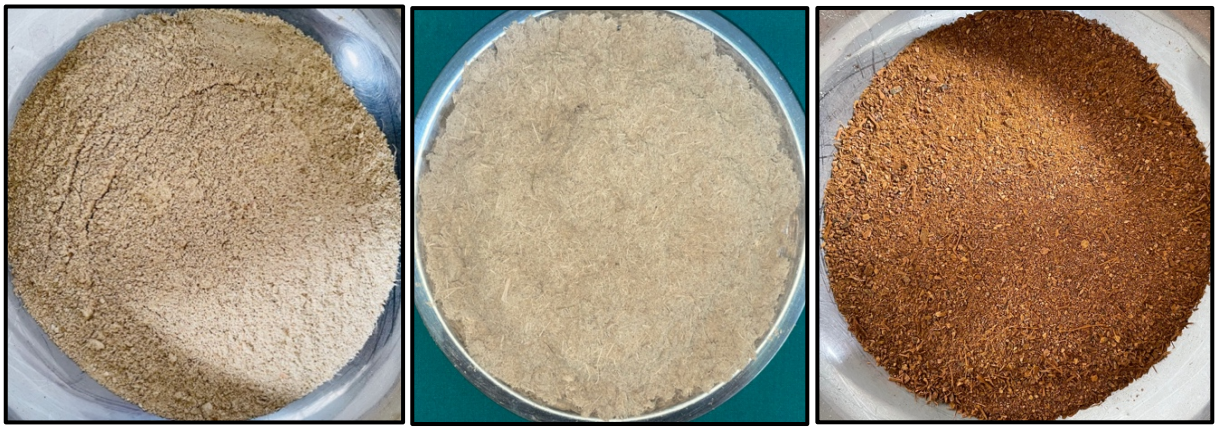
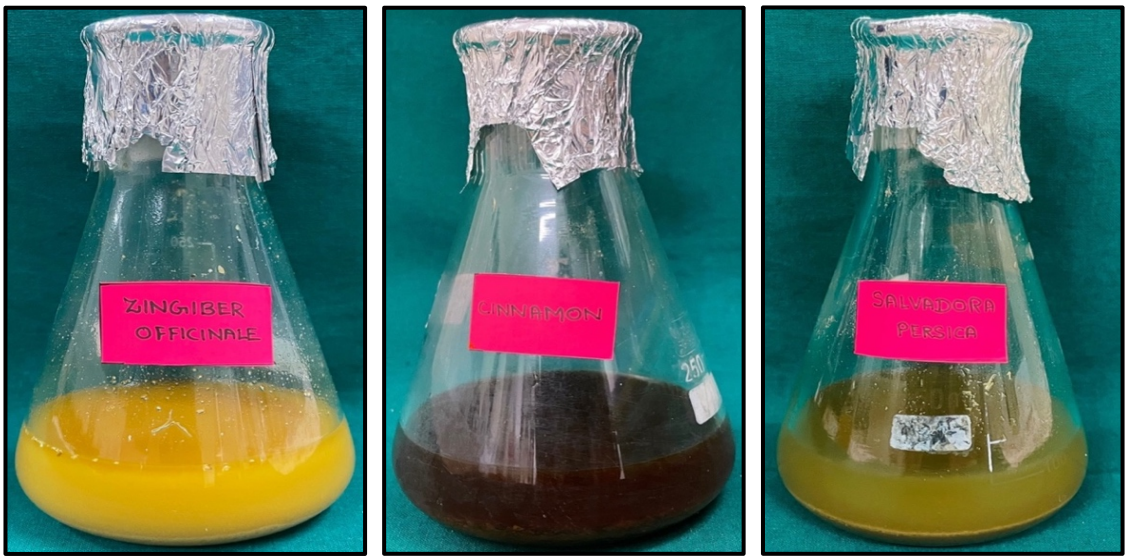


Figure No. 23: Photograph showing pulverizer used in the study.



(a) (b) (c)

Figure No. 24: Photograph showing grounded (a) *Zingiber officinale*, (b) *Salvadora persica* and (c) *Cinnamomum zeylanicum.*



(a) (b) (c)

Figure No. 25: Photograph showing extraction of ethanolic extract of (a) *Zingiber officinale*, (b) *Salvadora persica* and (c) *Cinnamomum zeylanicum*.

Figure No. 26: Photograph showing rotary incubator used in the study.

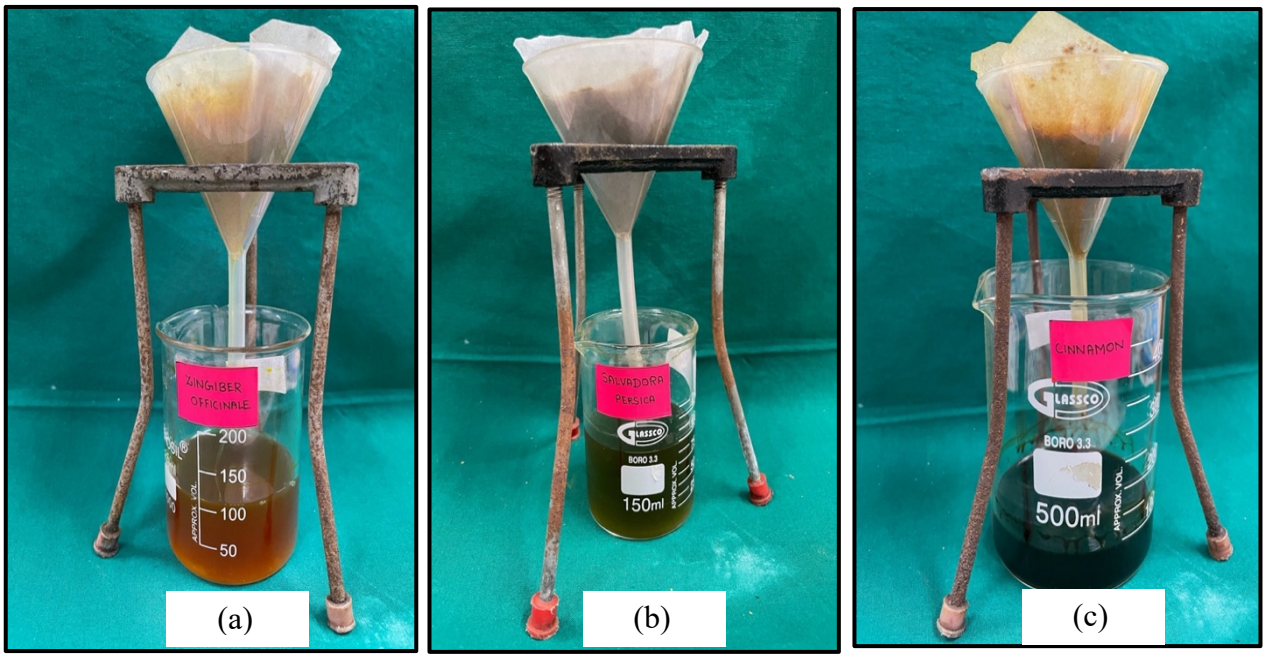
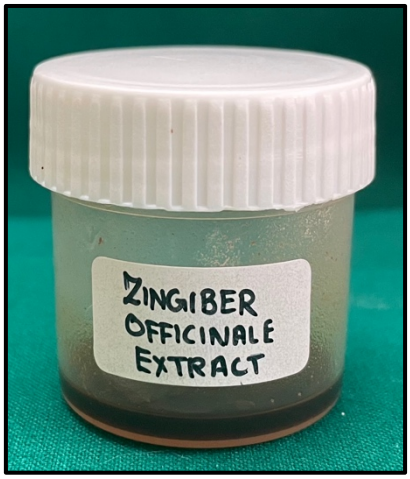


Figure No. 27: Photograph showing filtration of ethanolic extract of (a) *Zingiber officinale*, (b) *Salvadora persica* and *(c) Cinnamomum zeylanicum.*



(a)

(b)

(c)

Figure No. 28: Photograph showing stored ethanolic extract of (a) *Zingiber officinale*, (b) *Salvadora persica* and (c) *Cinnamomum zeylanicum.*

B) MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) DETERMINATION

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was carried out at KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration was assessed against Microbial Type Culture Collection (MTCC) strains of *Streptococcus mutans* and *Lactobacillus acidophilus.* Inoculum of standard strains of organisms was prepared as per 0.5 McFarland Standard.

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

Minimum Inhibitory Concentration 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.

Microbiological media was prepared by mixing 3.7 gm of Brain Heart Infusion (BHI) broth with 100 ml of distilled water in a conical flask. This mixture was autoclaved for 15-20 minutes. Working solution of test compounds was prepared by adding 100 µl of prepared ethanolic extracts of *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* into 1 ml of Dimethyl Sulfoxide (DMSO) respectively.

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 12th well. Excess diluted extract was pipetted and discarded from the 12th tube. 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 37^0 Celsius.

0.015% of resazurin was freshly prepared. 20 µl of this freshly prepared resazurin solution was added to each well. The 96 well plates were then incubated for 1-4 hours at 37^0 Celsius. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin [Figure No. 29]. The concentration at which resazurin was just reduced to resofurin by colour change from blue to slight pink was taken as Minimum Inhibitory Concentration. [53]

The Minimum Inhibitory Concentration for ethanolic extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* was carried out five times against both test organisms i.e. *Streptococcus mutans* [Figure No. 30] and *Lactobacilli acidophilus* [Figure No. 31] respectively.

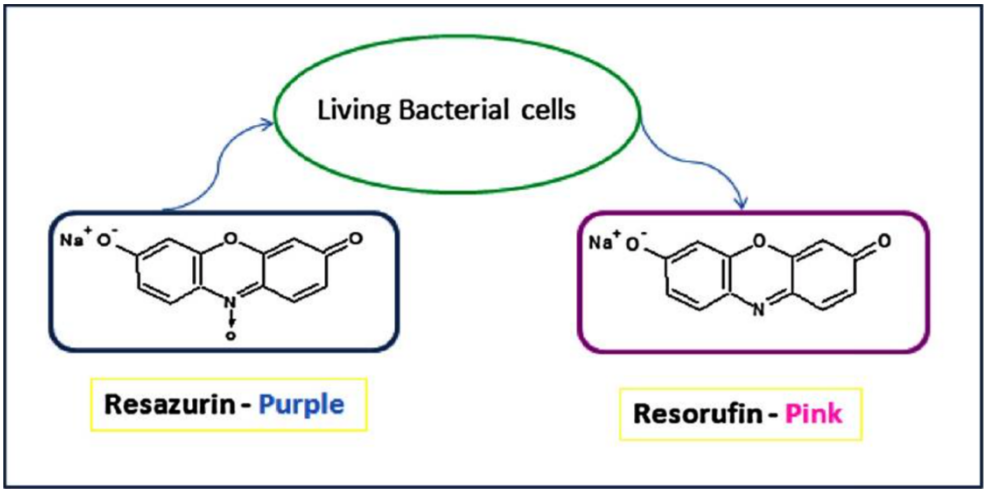


Figure No. 29: Photograph showing Resazurin method used for Minimum Inhibitory Concentration (MIC) determination.

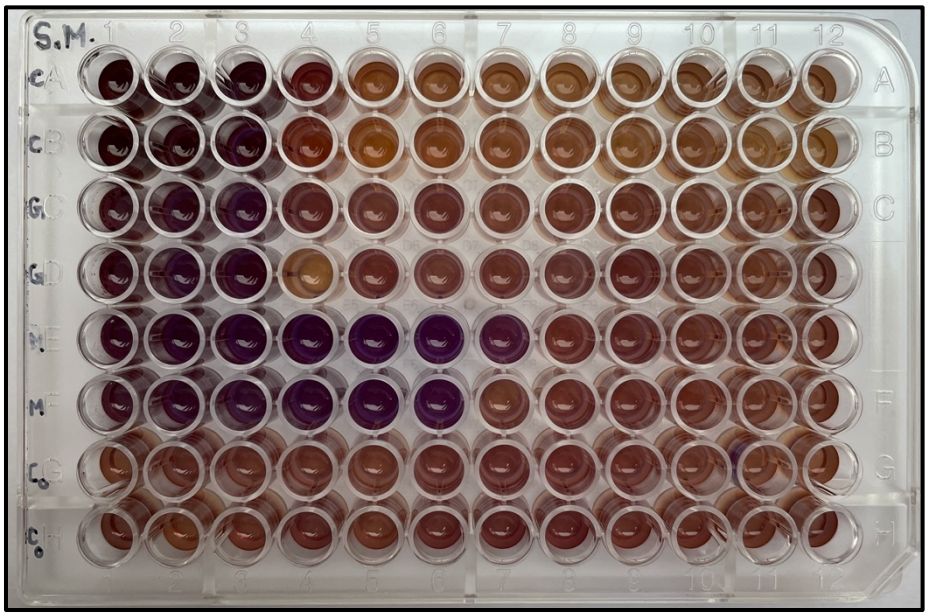


Figure No. 30: Photograph showing Minimum Inhibitory Concentration (MIC) for ethanolic extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans.*

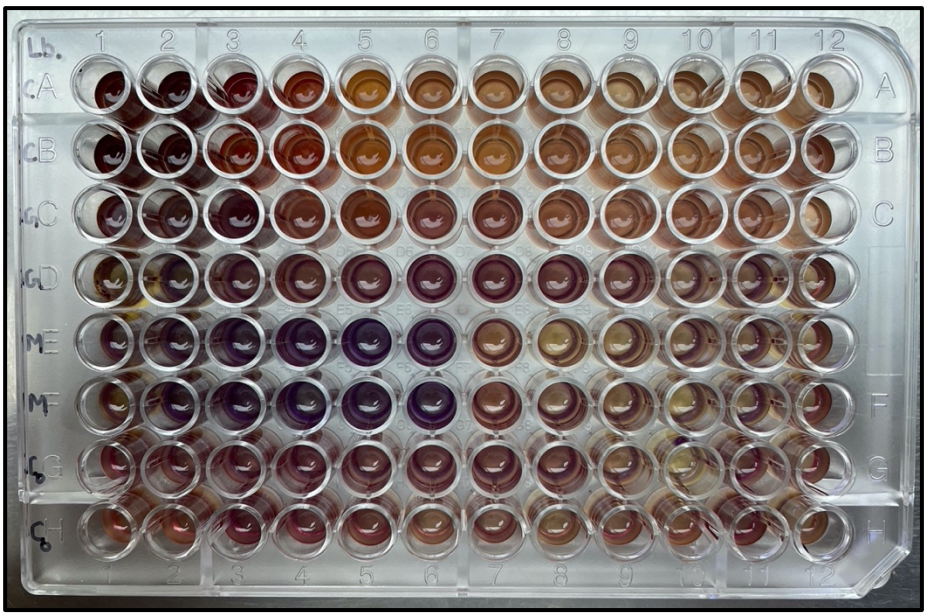


Figure No. 31: Photograph showing Minimum Inhibitory Concentration (MIC) for ethanolic extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Lactobacillus acidophilus.*

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

Minimum Bactericidal Concentration (MBC) 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 (BHI) agar into 100 ml of distilled water in a conical flask. This solution was autoclaved for 15-20 minutes at 121^0 Celsius 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. The 96 well plates used for MIC determination with bacterial growth were streaked on agar plate using Platinum Inoculation loops [Figure No. 32]. These agar plates were then incubated at 37^0 Celsius for 24 hours. Plates were observed for bacterial growth after 24 hours. The Minimum Bactericidal Concentration value was determined when there was no colony growth from the directly plated contents of the wells. [53]

The Minimum Bactericidal Concentration for ethanolic extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* was carried out five times against both test organisms i.e. *Streptococcus mutans* [Figure No. 33, 34, 35] and *Lactobacilli acidophilus* [Figure No. 36, 37, 38] respectively**.**

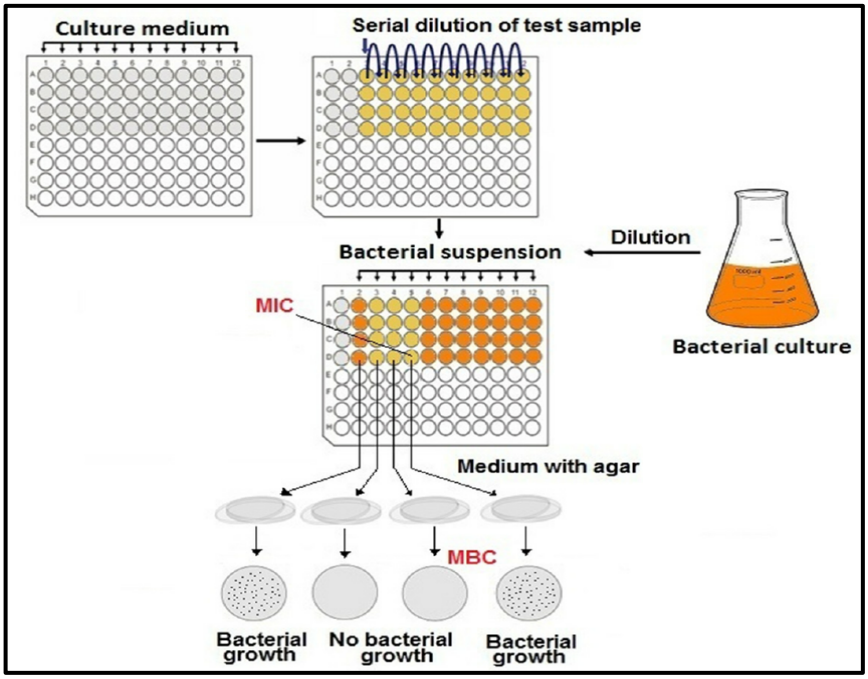


Figure No. 32: Photograph showing Agar Plate Streaking method used for Minimum Bactericidal Concentration (MBC) determination.

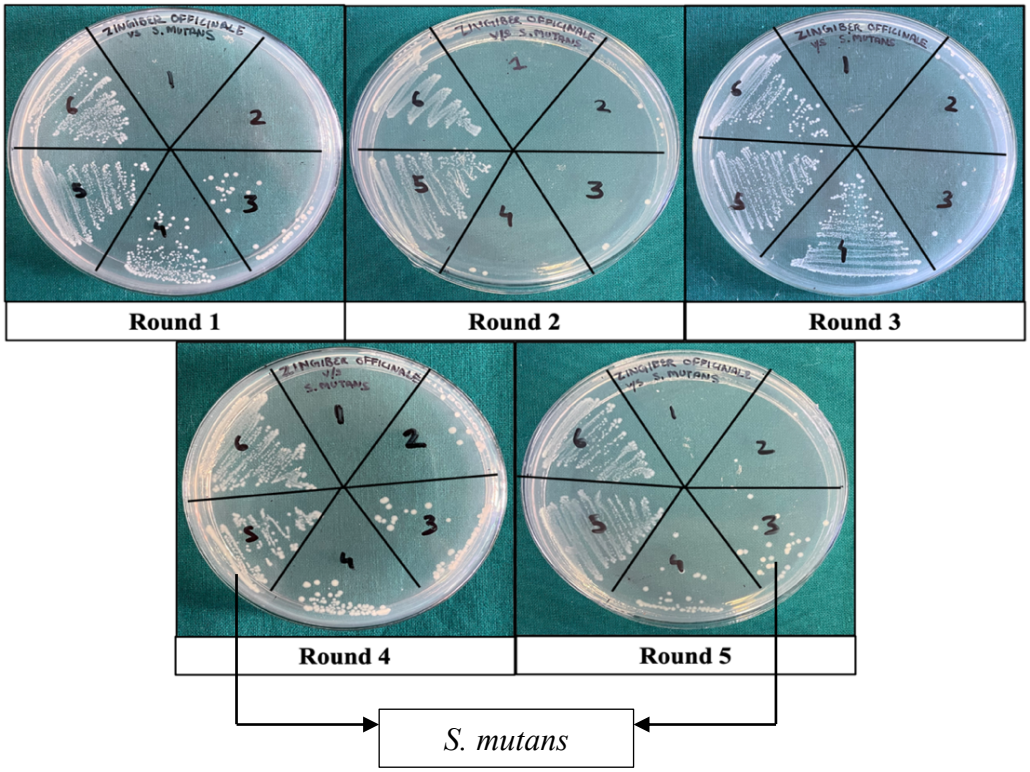


Figure No. 33: Photograph showing Minimum Bactericidal Concentration (MBC) for *Zingiber officinale* against *Streptococcus mutans.*

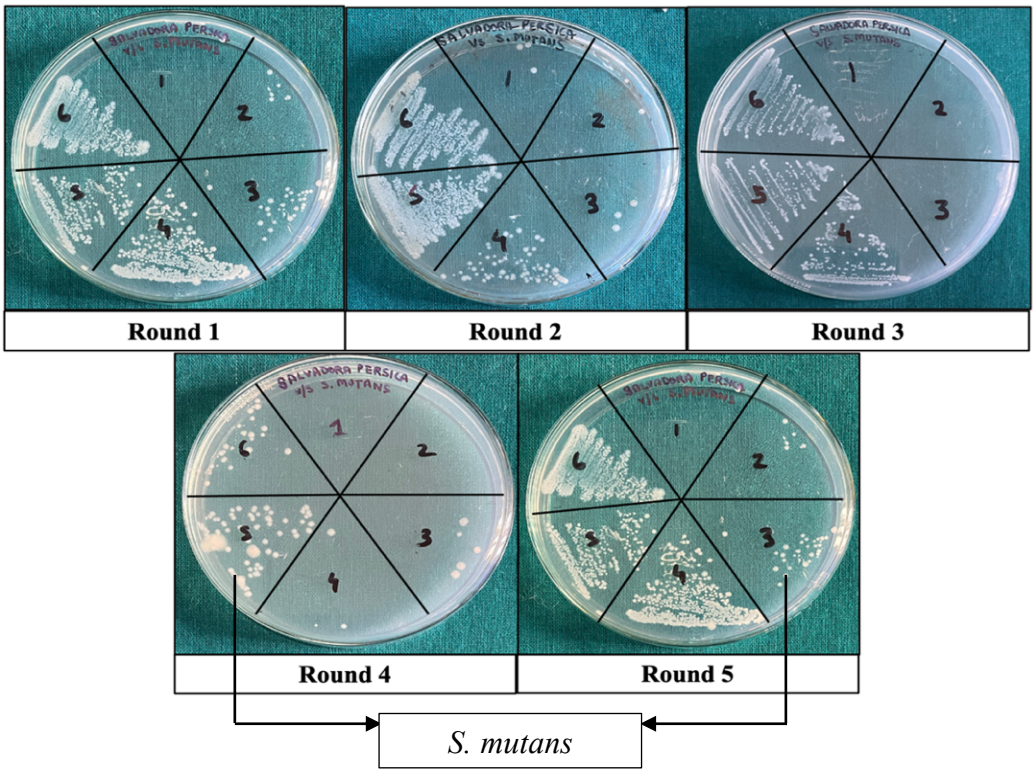


Figure No. 34: Photograph showing Minimum Bactericidal Concentration (MBC) for *Salvadora persica* against *Streptococcus mutans.*

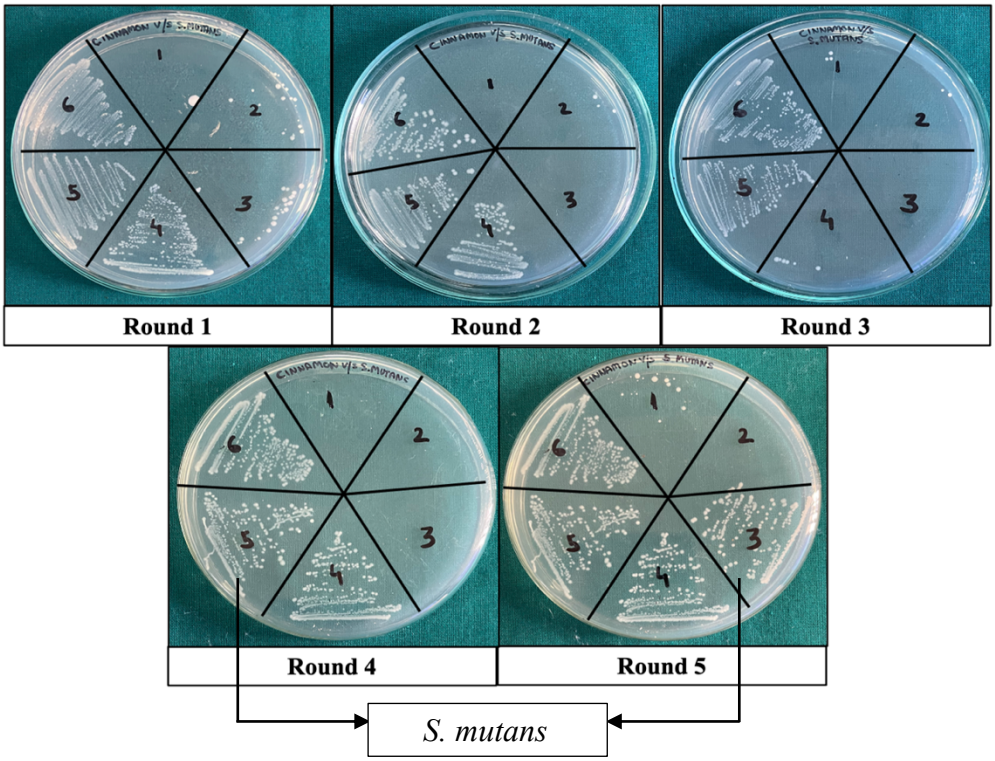


Figure No. 35: Photograph showing Minimum Bactericidal Concentration (MBC) for *Cinnamomum zeylanicum* against *Streptococcus mutans.*

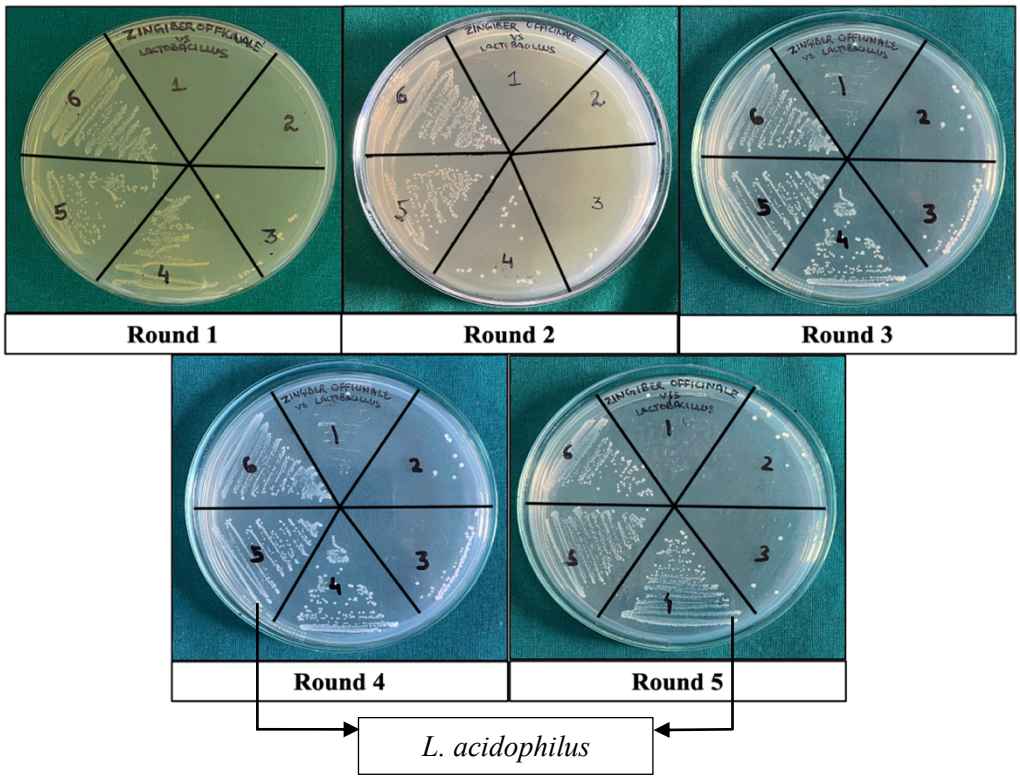


Figure No. 36: Photograph showing Minimum Bactericidal Concentration (MBC) for *Zingiber officinale* against *Lactobacillus acidophilus.*

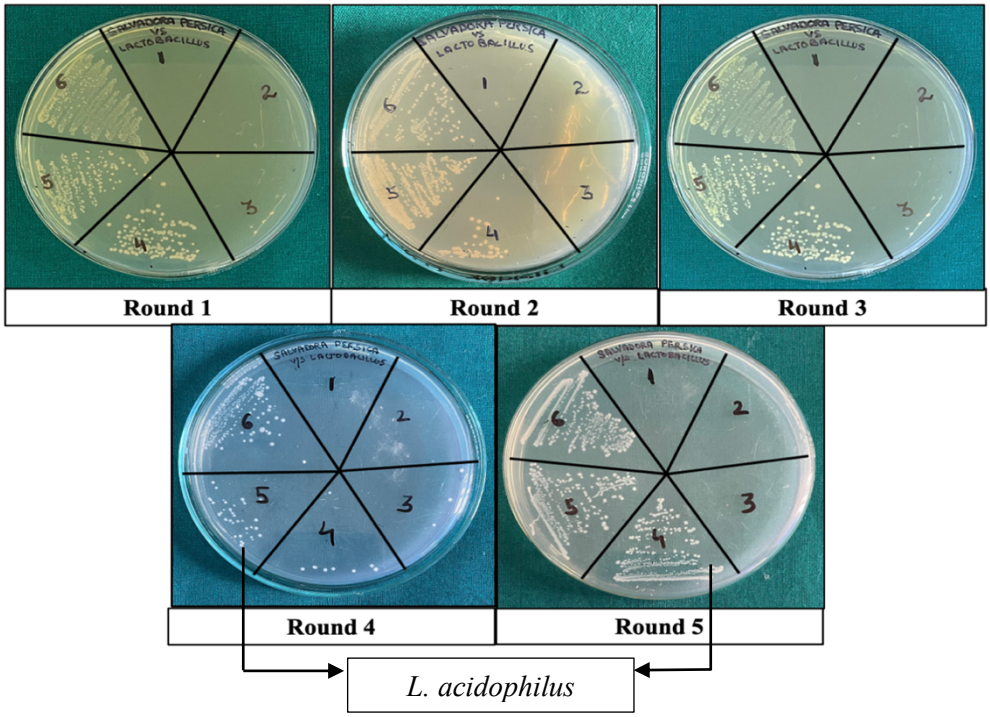


Figure No. 37: Photograph showing Minimum Bactericidal Concentration (MBC) for *Salvadora persica* against *Lactobacillus acidophilus.*

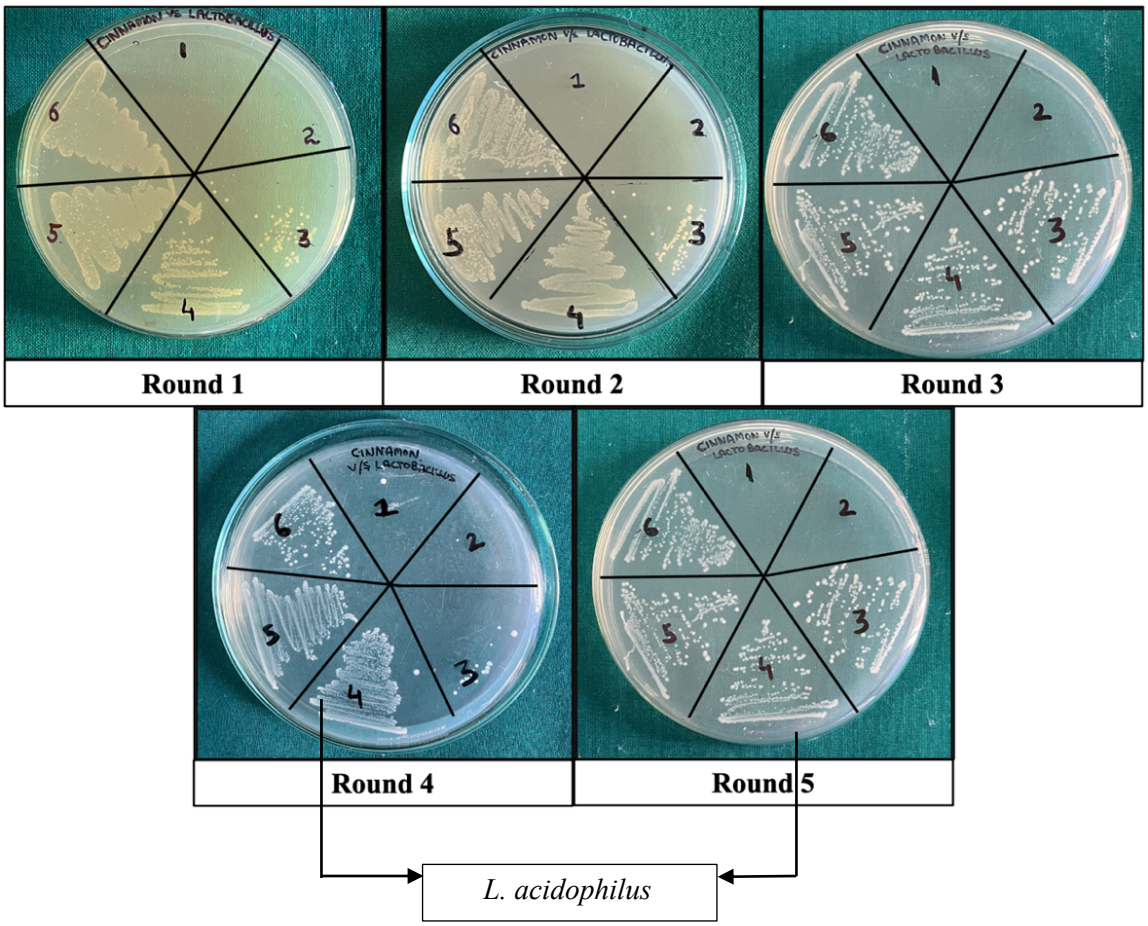


Figure No. 38: Photograph showing Minimum Bactericidal Concentration (MBC) for *Cinnamomum zeylanicum* against *Lactobacillus acidophilus.*

C) PREPARATION OF HERBAL DENTAL GEL:

Herbal Dental gel was prepared in the Department of Pharmaceutics, KAHER's College of Pharmacy, Belagavi. The values obtained as Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extracts were used to determine the composition of the formulation [Table No. 5].

Table No. 5: Table showing Composition of formulation of Herbal Dental gel containing *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum*

Sl. No.	Constituent	Function	Quantity
1.	*Zingiber officinale* ethanolic extract	Antibacterial and Remineralizing Agent	2 % w/w
2.	*Salvadora persica* ethanolic extract	Antibacterial and Remineralizing Agent	2 % w/w
3.	*Cinnamomum zeylanicum* ethanolic extract	Antibacterial and Remineralizing Agent	2 % w/w
4.	Carbopol 940	Gel Polymer	2 % w/w
5.	Glycerine	Humectant	5 % w/w
6.	Xylitol	Sweetener	5 % w/w
7.	Sodium benzoate	Anti-bacteriostatic	0.1 % w/w
8.	Methyl paraben	Anti-bactericidal	0.5 % w/w
9.	Propyl paraben	Anti-bactericidal	0.02 % w/w
10.	Distilled water	Solvent	-

The Preparation Was Carried Out In The Following Steps:

1. Weighed quantity of Carbopol 940 was dissolved in 50 ml of distilled water with slow stirring on a magnetic stirrer for 3 hours and kept for 24 hours for complete hydration. [Figure No. 39]

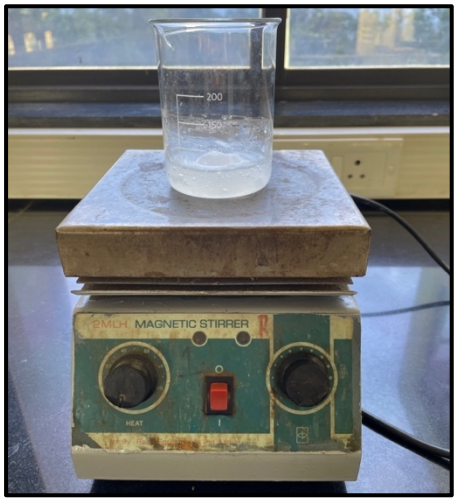


Figure No. 39: Photograph showing mixture of Carbapol 940 with water on a magnetic stirrer.

2. Ethanolic extract of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* were mixed with glycerine in a beaker. [Figure No. 40]

Figure No. 40: Photograph showing mixing of glycerine with ethanolic extracts of *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum.*

3. In 30 ml of distilled water, weighed quantities of xylitol, sodium benzoate, methyl paraben and propyl paraben were dissolved. [Figure No. 41]

Figure No. 41: Photograph showing weighed amount of xylitol, sodium benzoate, methyl paraben and propyl paraben.

4. Carbopol gel prepared in the first step was mixed with the mixture of the extracts prepared in second step on a magnetic stirrer at 500 rpm for half an hour to render uniform dispersion. [Figure No. 42]

Figure No. 42: Photograph showing mixing of Carbopol gel solution with the mixture of the extracts on a magnetic stirrer.

5. To the above mixture, the mixture containing preservatives prepared in the third step was added with constant stirring on magnetic stirrer with 100 rpm for 30 minutes.
6. The final weight of gel was adjusted to 100 gm with distilled water and pH of the gel was adjusted to 7.5 by adding Triethanolamine drop by drop.
7. Gel was transferred to the air-tight container kept at room temperature. [Figure No. 43]

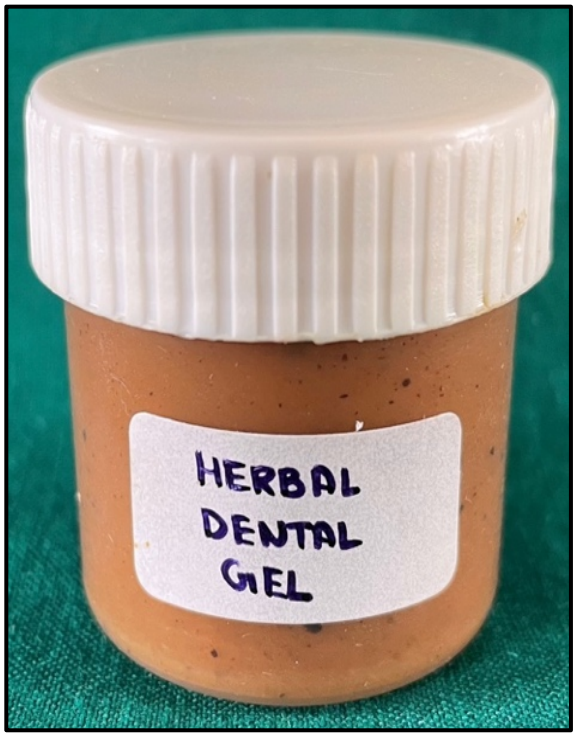


Figure No. 43: Photograph showing Herbal Dental gel containing *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* stored in a sterile container.

D) CYTOTOXICITY ASSAY

Cytotoxicity assay was carried out at KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi.

Cytotoxicity Assay Principle:

This is a colorimetric assay for assessing cell metabolic activity by measuring the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase [Figure No. 44]. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble formazan product which is dark purple in colour. The cells are then solubilized with an organic solvent and the released, solubilized formazan reagent is measured using 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. [54]

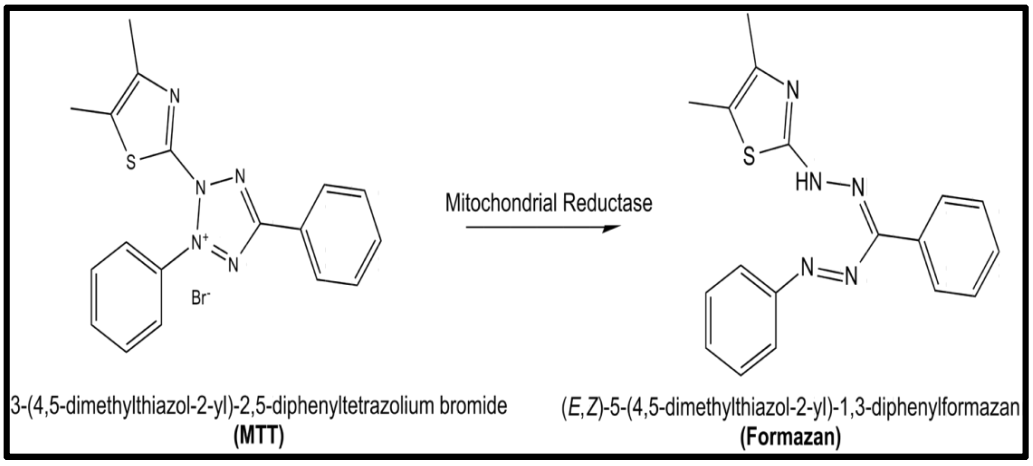


Figure No. 44: Photograph showing chemical structure of MTT reagent.

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. 45]. 5 ml of fresh media was added and mixed thoroughly.

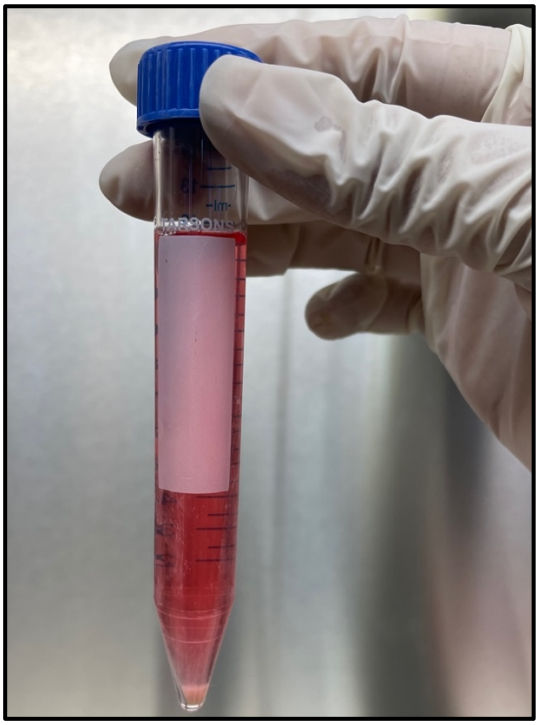


Figure No. 45: Photograph showing formation of pellet at bottom during manual detachment of cells.

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 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. 46].

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

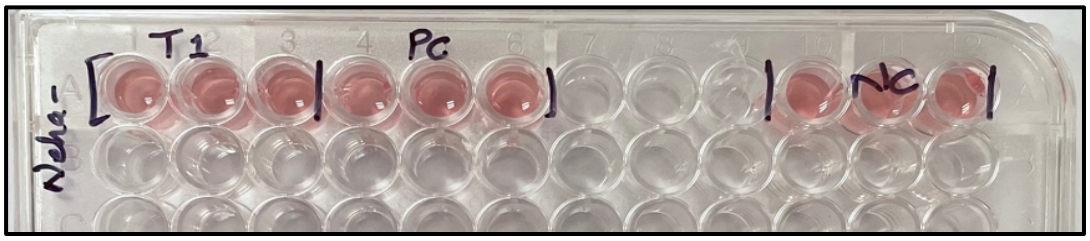


Figure No. 46: Photograph showing tissue culture plate with seeded fibroblast cells in DMEM media.

After 24 hours, 100 µl of test compounds were added to the wells and incubated for another 24 hours in the presence of 5 % CO_2, at 37^0 C into CO_2 incubator [Figure No. 47]. 20 µl of 5 mg/ ml MTT reagent was then added to each well [Figure No. 48]. The plate was kept for 4 hours incubation. The plate was covered with aluminum foil, since MTT reagent is photosensitive.

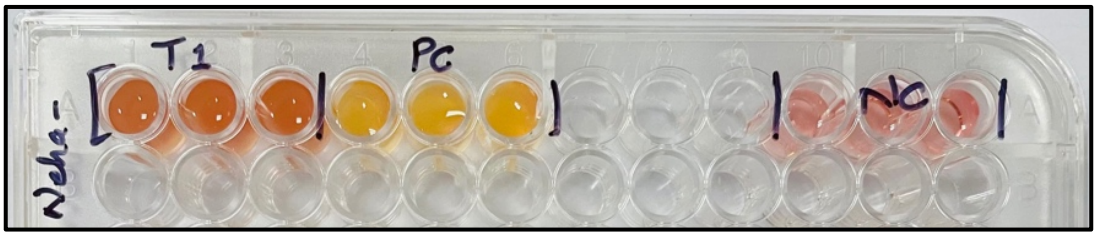


Figure No. 47: Photograph showing tissue culture plate with addition of test compounds.

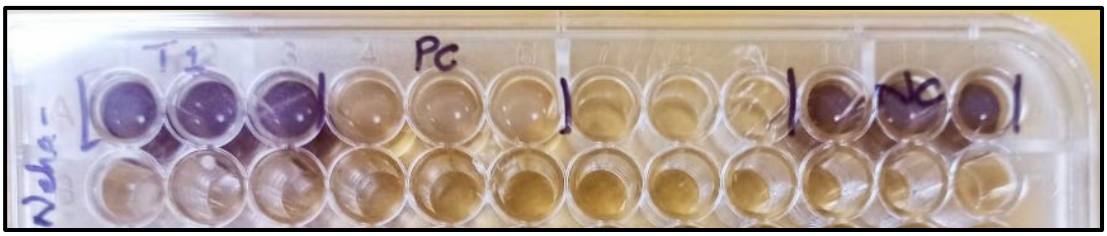


Figure No. 48: Photograph showing tissue culture plate after addition of MTT reagent.

The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100 µl of DMSO 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 492 nm. The surviving fibroblasts were also observed under microscope [Figure No. 49 and 50].

The percentage of surviving cells were measured with the help of following formula-

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of control (untreated cells)}} \times 100$$

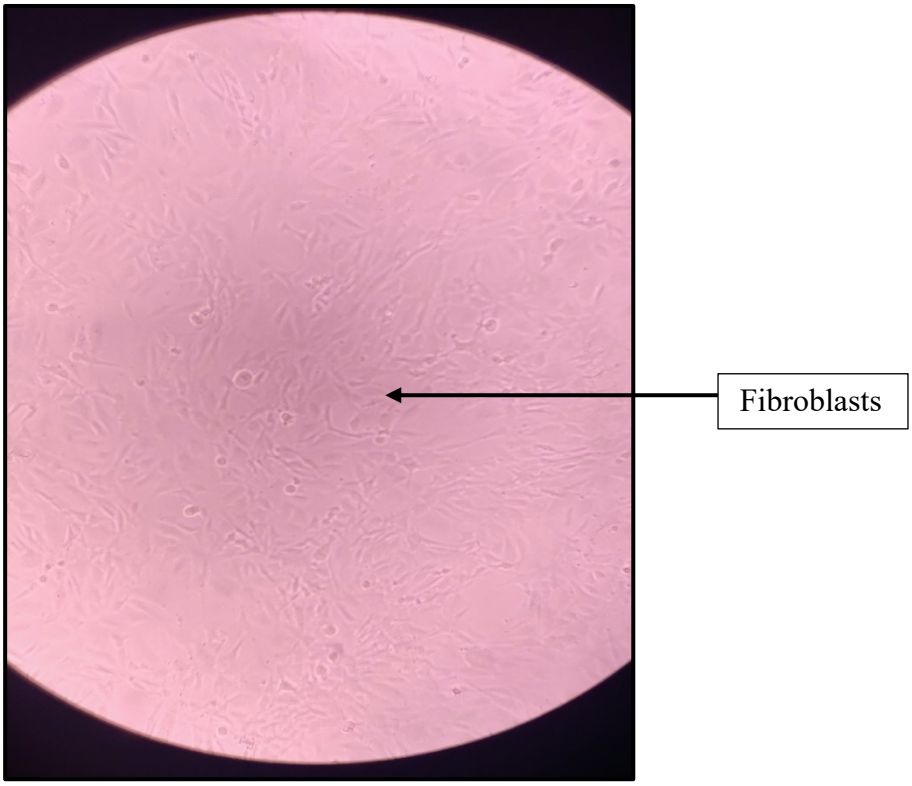


Figure No. 49: Photograph showing microscopic picture of the surviving fibroblasts with Acidulated Phosphate Fluoride gel (Group I).

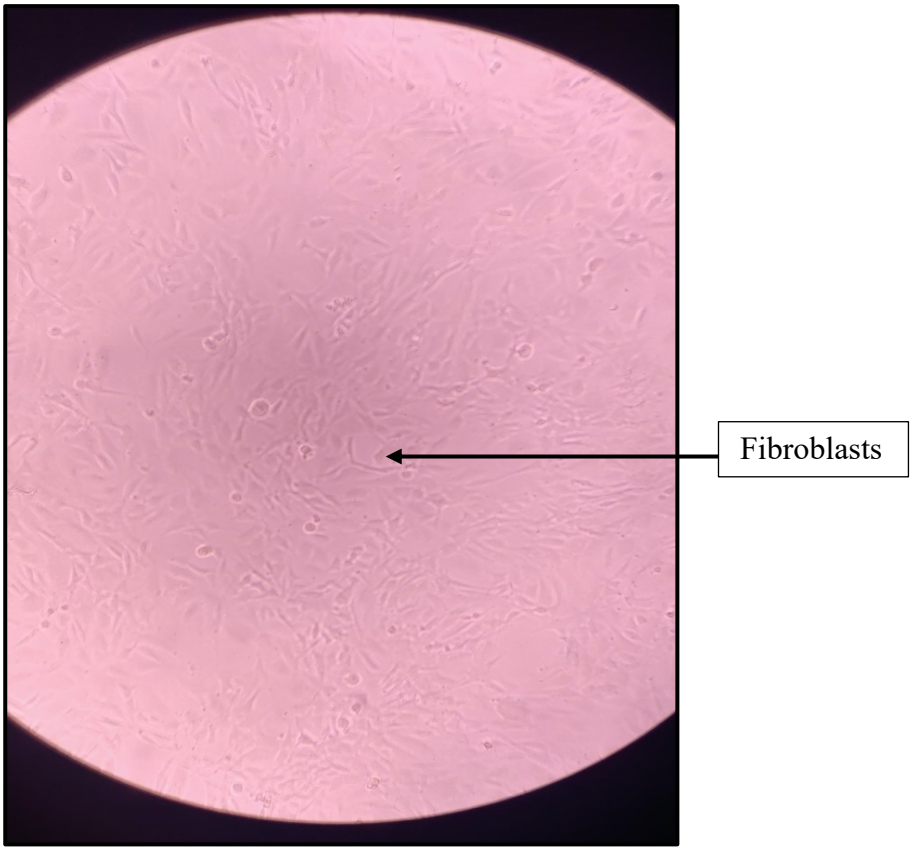


Figure No. 50: Photograph showing microscopic picture of the surviving fibroblasts with Herbal Dental gel (Group II).

E) ANTIBACTERIAL SUSCEPTIBILITY TESTING:

Antibacterial Susceptibility testing was carried out at KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi.

Antibacterial susceptibility for Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* (Group II) was tested against *Streptococcus mutans* and *Lactobacillus acidophilus* by Agar Well Diffusion Method and Time Kill Assay.

Procedure For Antimicrobial Susceptibility Using Agar Well Diffusion Method:

Microbiological media was prepared by adding 5.2 gm of Brain Heart Infusion (BHI) agar into 100 ml of distilled water in a conical flask. This solution was autoclaved for 15-20 minutes at 121^0 Celsius 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* and *Lactobacillus acidophilus*. Two wells with a diameter of 6 to 8 mm, equidistant from each other were punched aseptically following which the test compounds i.e. Acidulated Phosphate Fluoride gel and Herbal Dental gel 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 [55] [Figure No. 51 and 52].

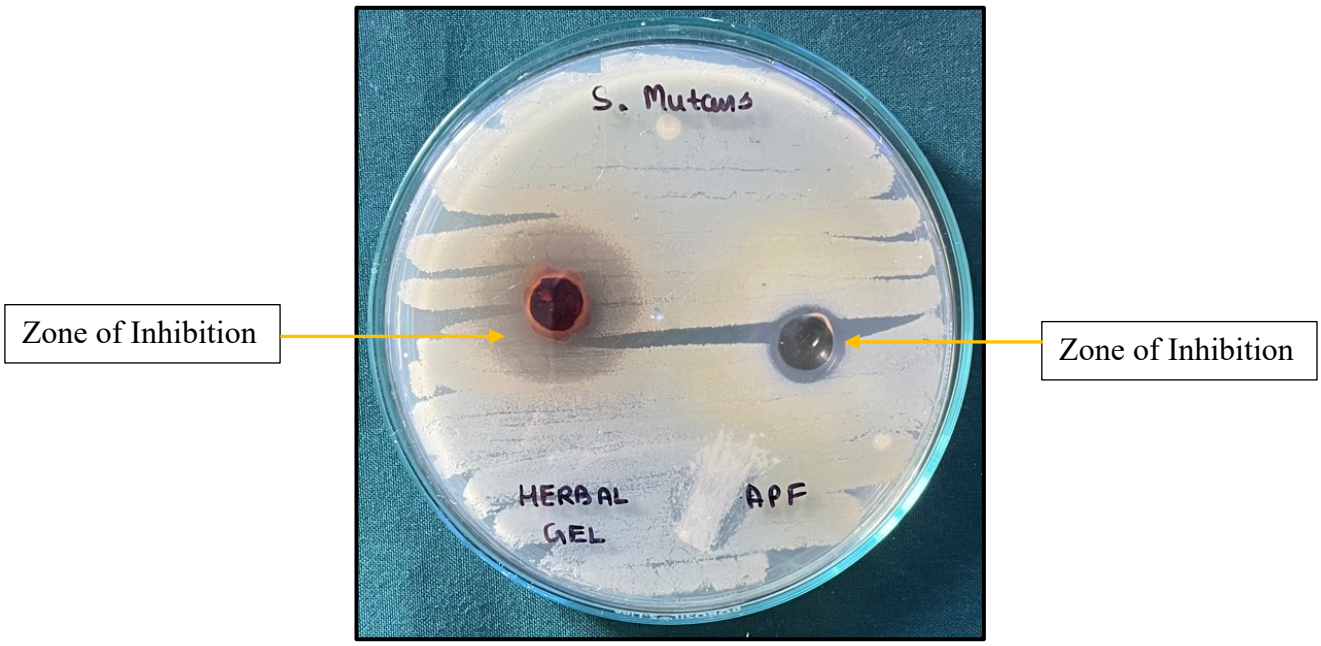


Figure No. 51: Photograph showing Zone of Inhibition of Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans*.

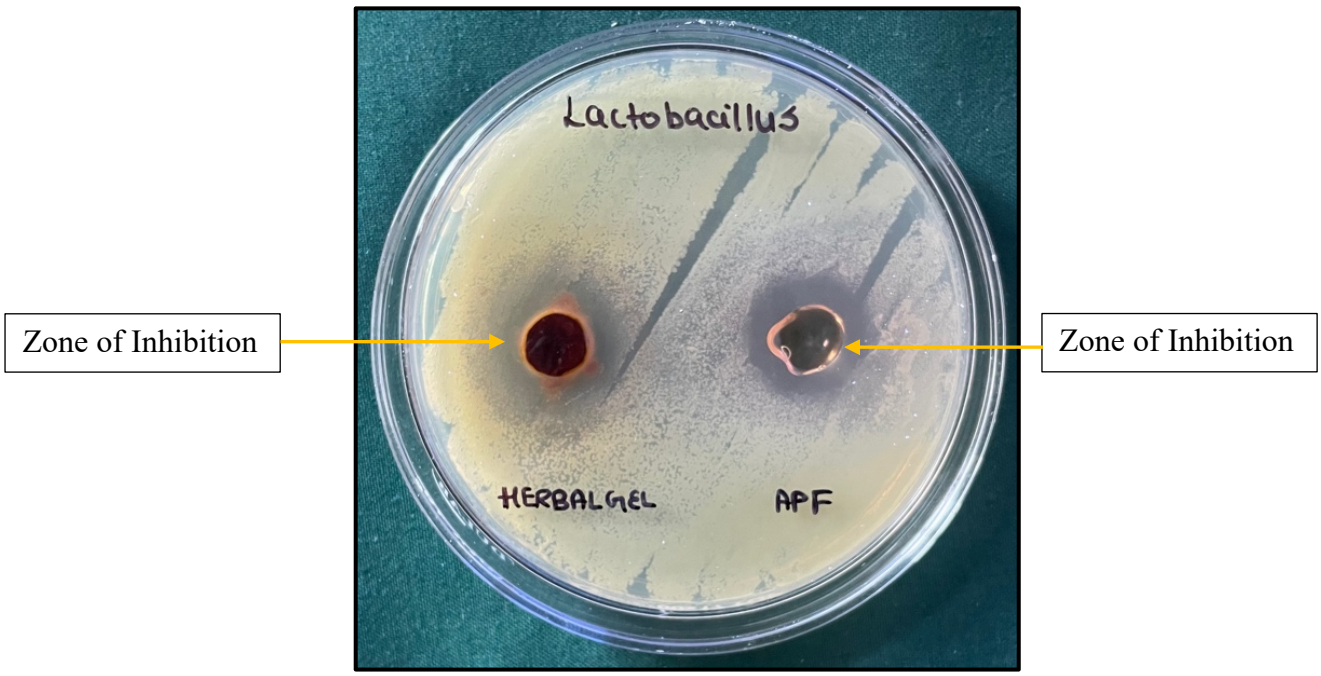


Figure No. 52: Photograph showing Zone of Inhibition of Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus*.

Procedure For Antimicrobial Susceptibility Using Time Kill Assay:

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 Acidulated Phosphate Fluoride gel and Herbal Dental gel respectively. 2 ml of aseptic broth was taken as negative control [Figure No. 53 and 54].

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. The bacterial growth rates were determined from changes in the density of viable bacteria whose readings were carried out using a spectrophotometer. [55]

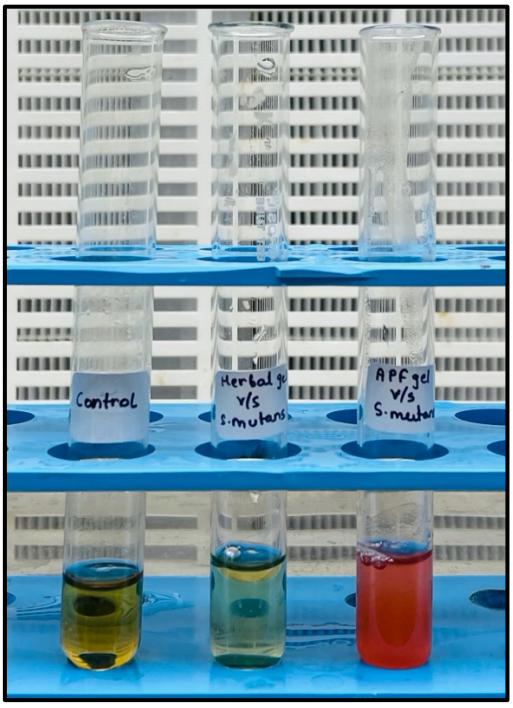


Figure No. 53: Photograph showing prepared stock solutions of Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans*.

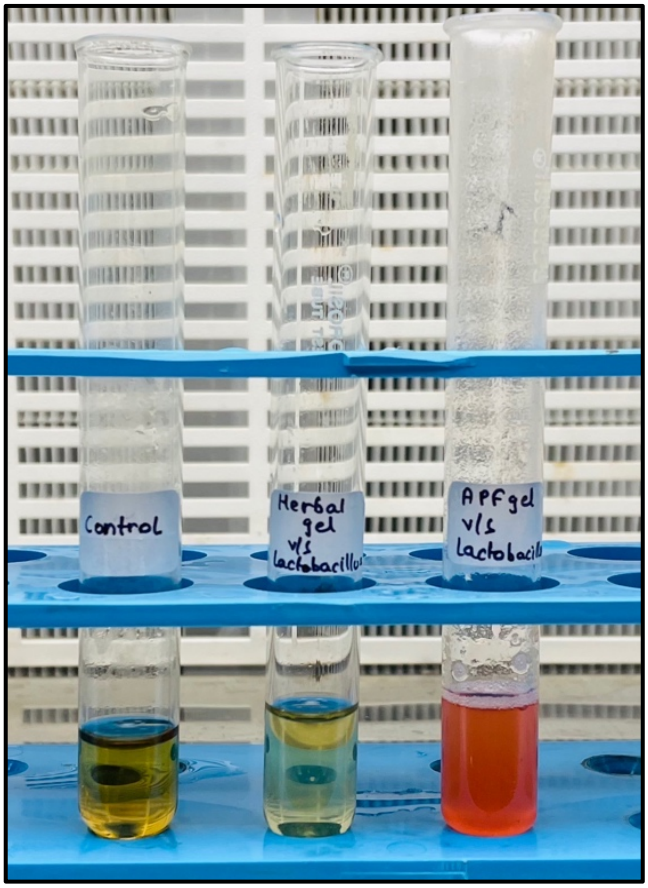


Figure No. 54: Photograph showing prepared stock solutions of Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus*.

F) EVALUATION OF REMINERALIZATION:

Evaluation of remineralization was carried out in the Department of Oral and Maxillofacial Pathology and Microbiology, KAHER's KLE VK Institute of Dental Sciences, Belagavi.

Extracted premolar teeth devoid of soft tissue debris were stored in 10% formalin. Stored extracted premolar teeth were dried and then longitudinally sectioned using a diamond disc in buccolingual direction [Figure No. 55 and 56]. The sectioned teeth were then coated with an acid resistant nail varnish, leaving a rectangular window 4 x 3 mm wide for demineralization, on the buccal surface [Figure No. 57]. An artificial demineralizing lesion was created using 37% phosphoric acid [Figure No. 58]. These demineralized sections were then studied under the stereomicroscope for evaluating the lesion depth [Figure No. 59 and 60]. The evaluated values were noted and tabulated. The demineralized sections in Group I were coated with Acidulated Phosphate Fluoride gel and Group II specimens were coated with Herbal Dental gel [Figure No. 61 and 62].

After 24, 48 and 72 hours, the remineralized sections in both the groups were observed under the stereomicroscope and the lesional depth was measured for each of the sections and recorded by using micrometer eyepiece as unit value.

The remineralized sections for Group I i.e. Acidulated Phosphate Fluoride gel group [Figure No. 63, 64, 65] and Group II i.e. Herbal Dental gel group [Figure No. 66, 67, 68] are depicted respectively.

The unit values were then converted to micrometre (μm) using the following formula:

$$\frac{\text{Number of Units}}{\text{Eye Piece Magnification}} \times \frac{1000}{\text{Zoom}} = \text{micrometer (μm)}$$

The evaluated values were noted and tabulated.

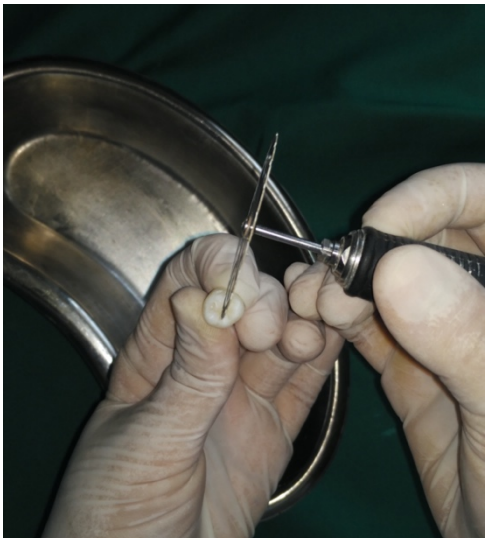


Figure No. 55 : Photograph showing sectioning of teeth using diamond disc.

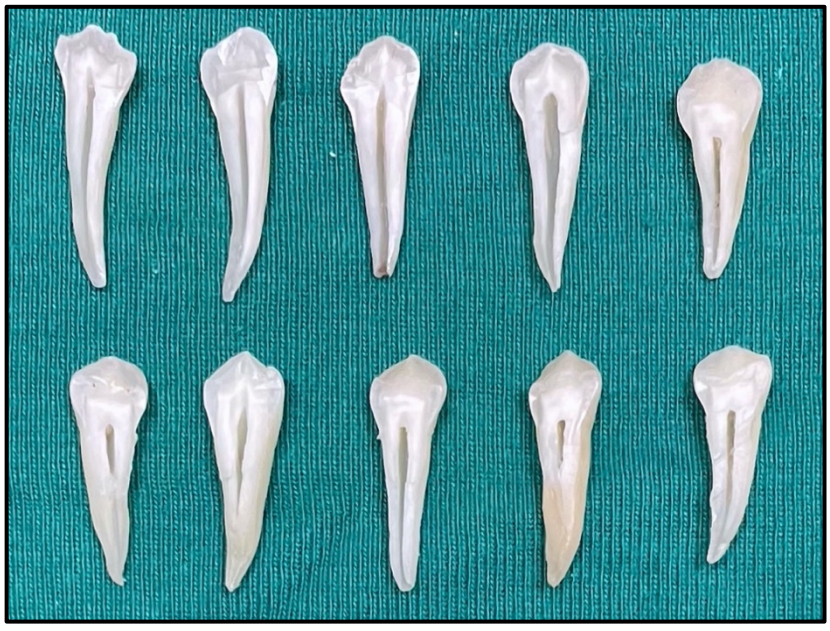


Figure No. 56 : Photograph showing sectioned premolar teeth.

Figure No. 57 : Photograph showing windows prepared by coating varnish.

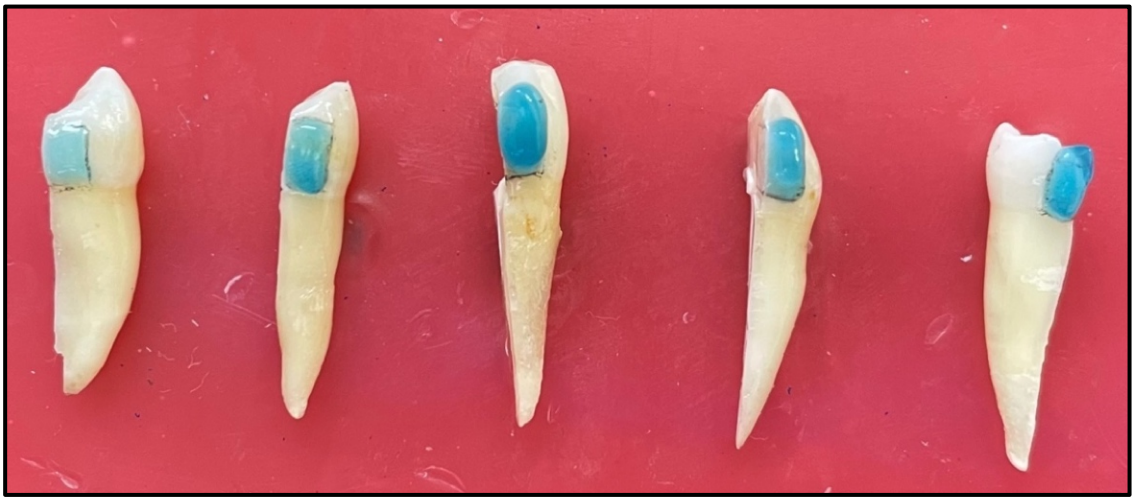


Figure No. 58 : Photograph showing application of 37% phosphoric acid for demineralizing the sectioned premolar teeth.

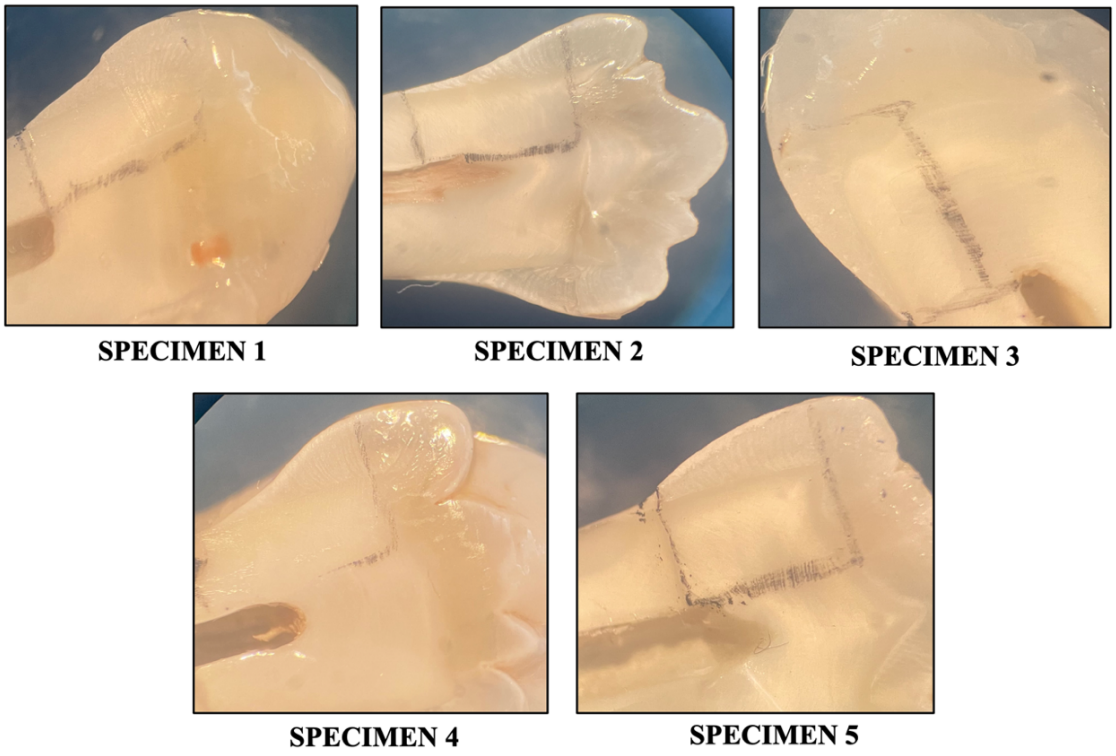


Figure No. 59: Photograph showing demineralization of specimens in Acidulated Phosphate Fluoride gel Group (Group I).

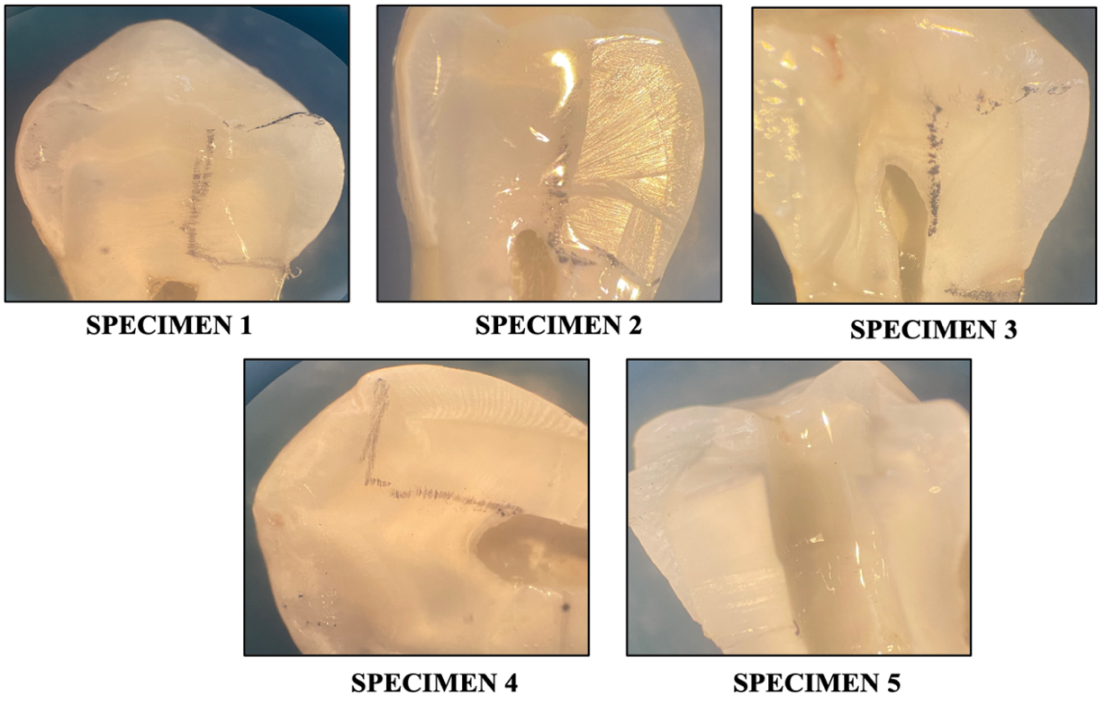


Figure No. 60: Photograph showing demineralization of specimens in Herbal Dental gel Group (Group II).

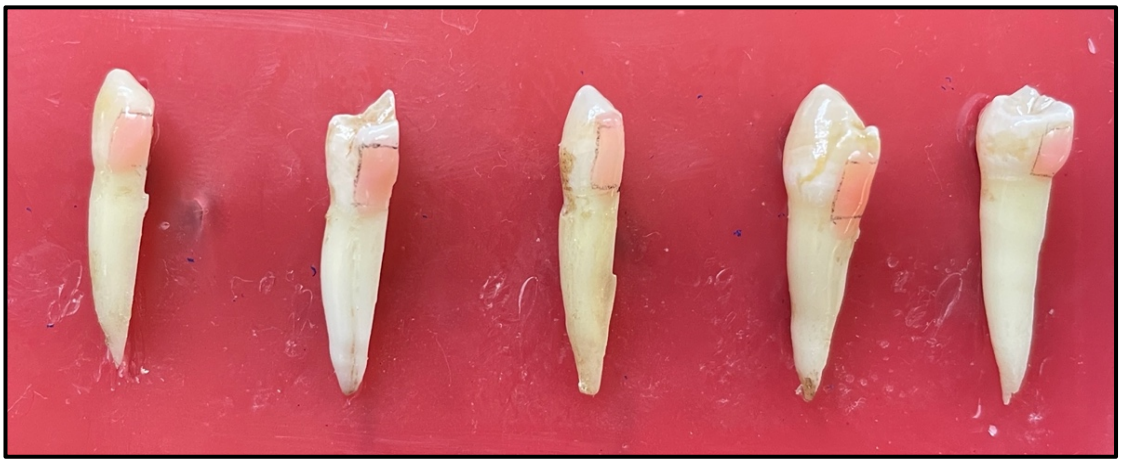


Figure No. 61 : Photograph showing application of Acidulated Phosphate Fluoride gel (Group I) to demineralised premolar teeth.

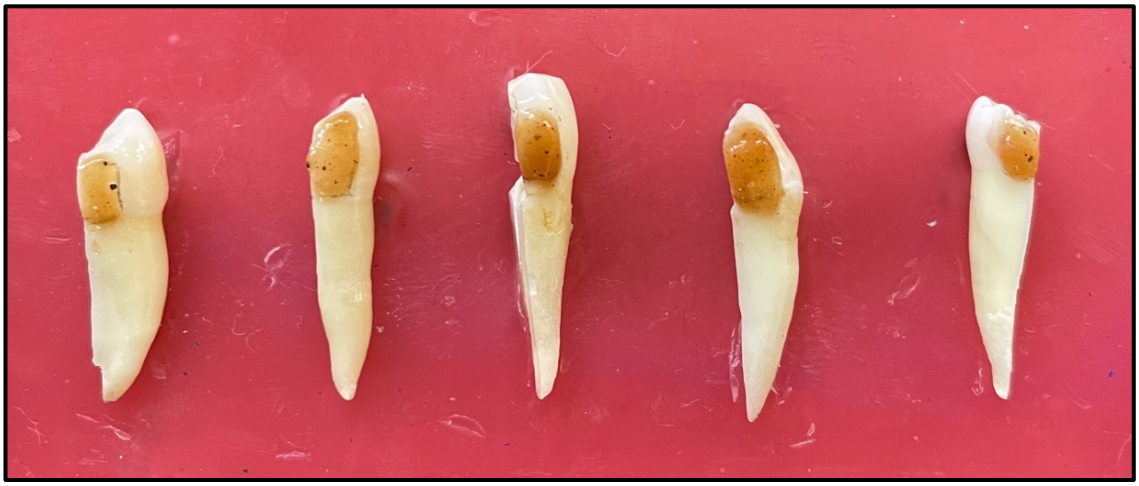


Figure No. 62 : Photograph showing application of Herbal Dental gel (Group II) to demineralised premolar teeth.

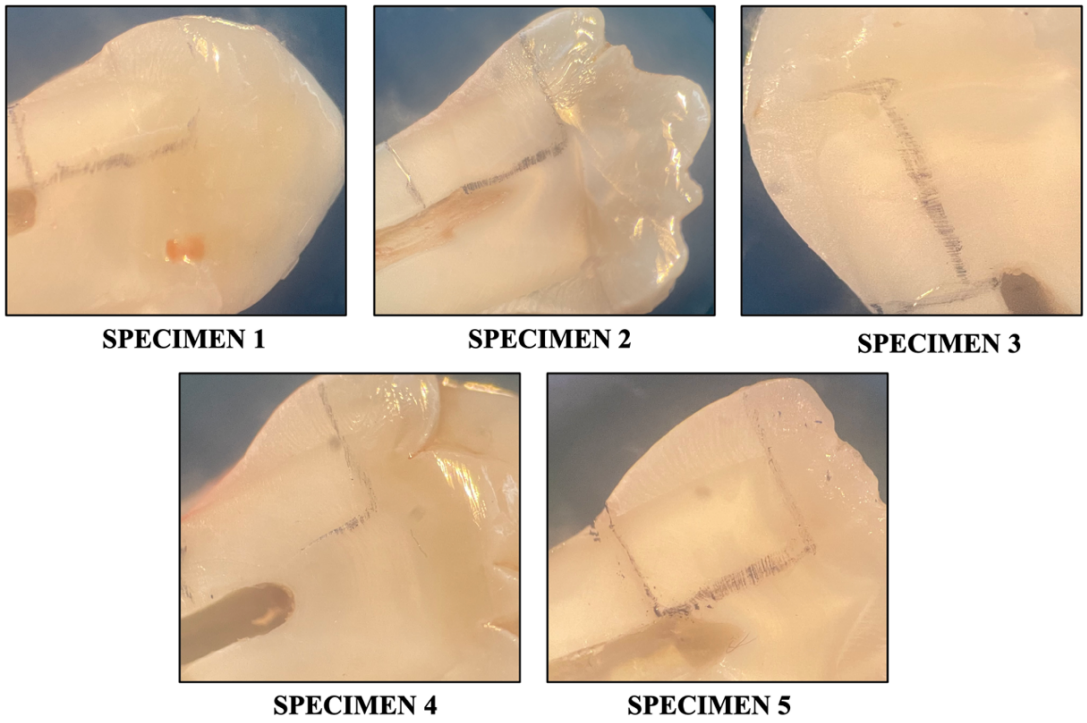


Figure No. 63: Photograph showing remineralization of specimens after 24 hours in Acidulated Phosphate Fluoride gel Group (Group I).

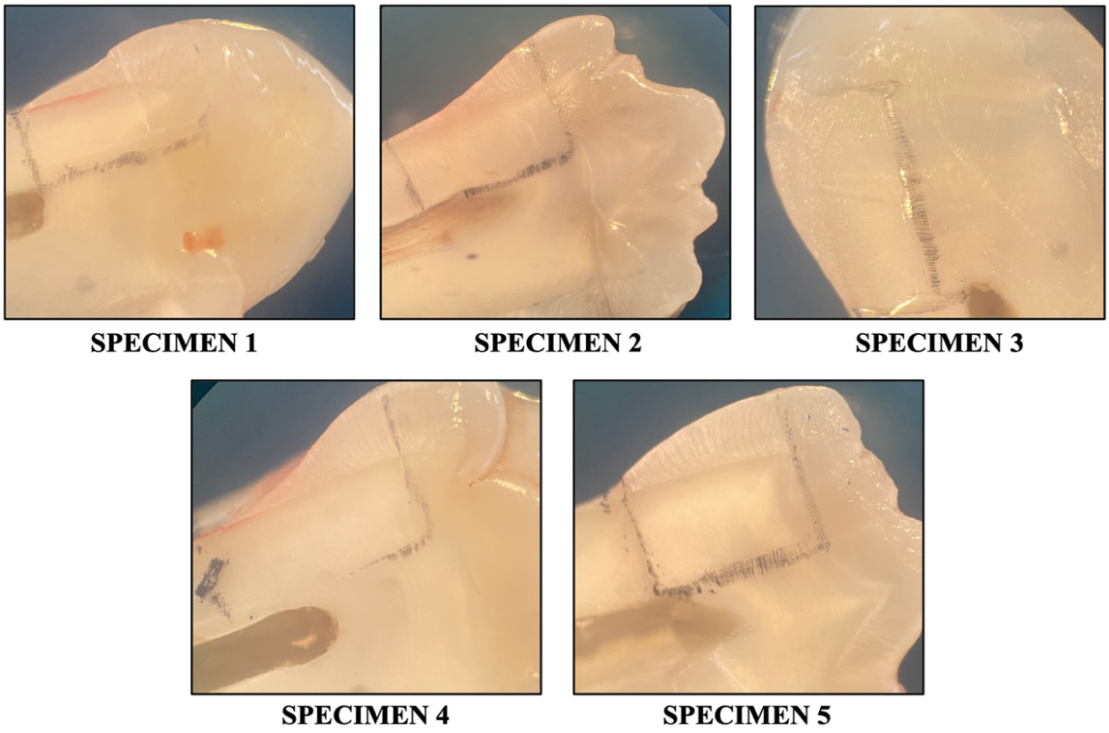


Figure No. 64: Photograph showing remineralization of specimens after 48 hours in Acidulated Phosphate Fluoride gel Group (Group I).

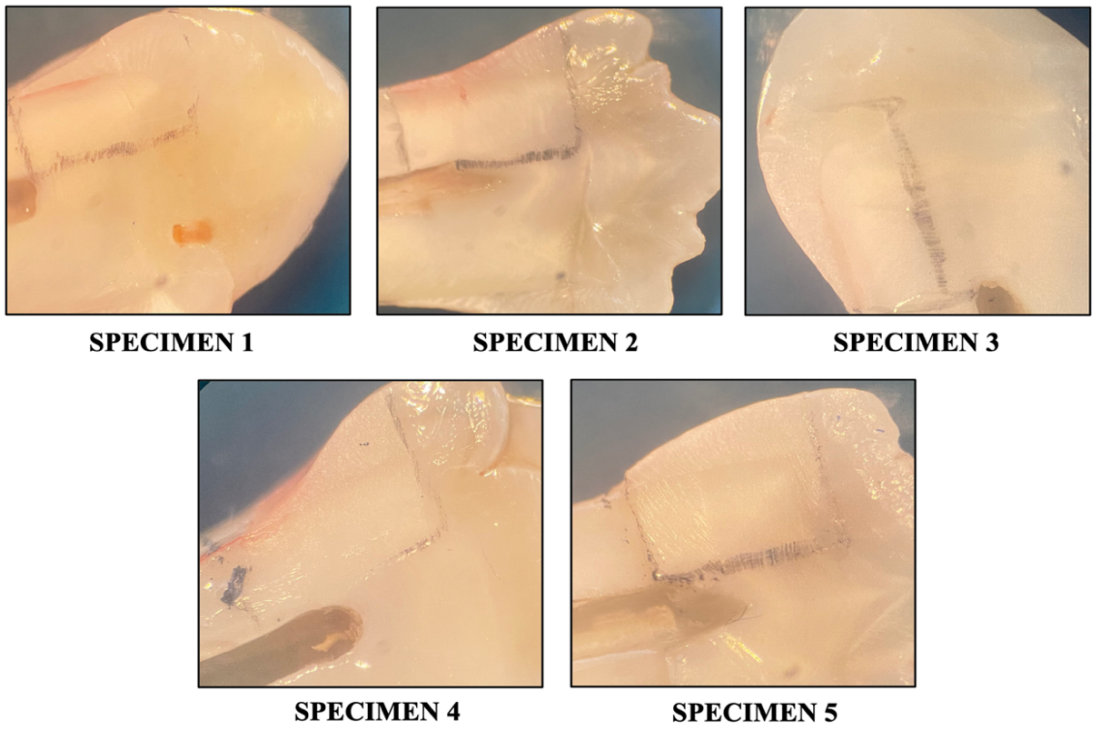


Figure No. 65: Photograph showing remineralization of specimens after 72 hours in Acidulated Phosphate Fluoride gel Group (Group I).

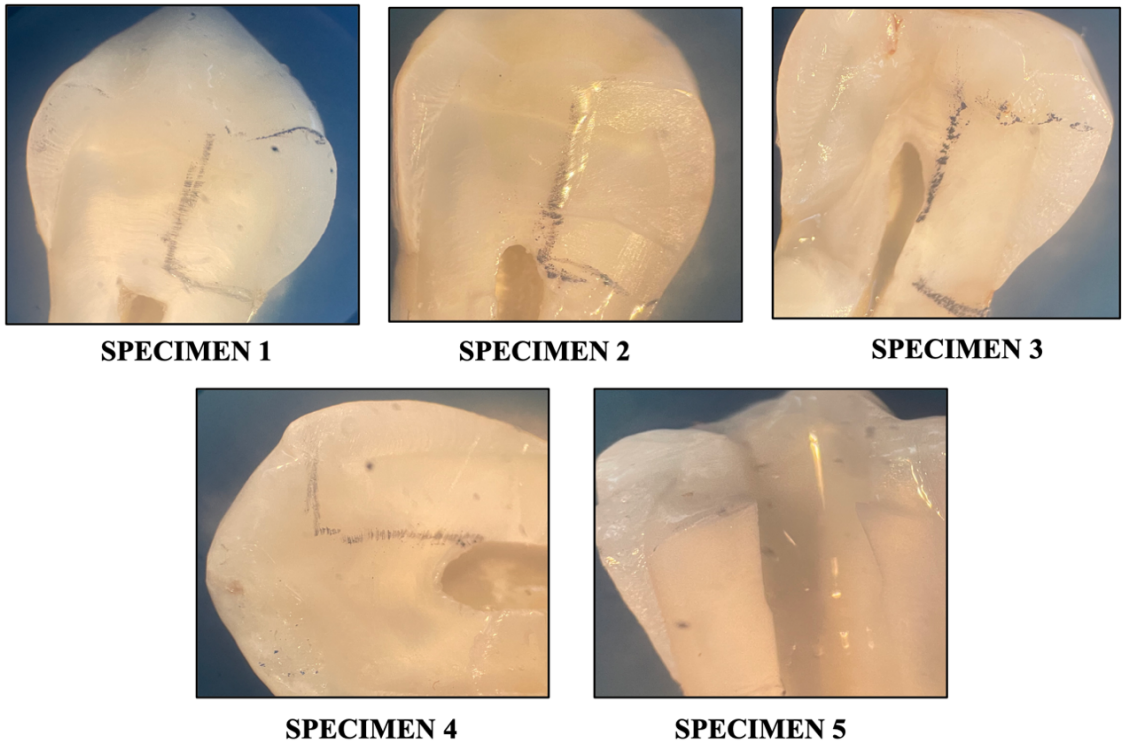


Figure No. 66: Photograph showing remineralization of specimens after 24 hours in Herbal Dental gel Group (Group II).

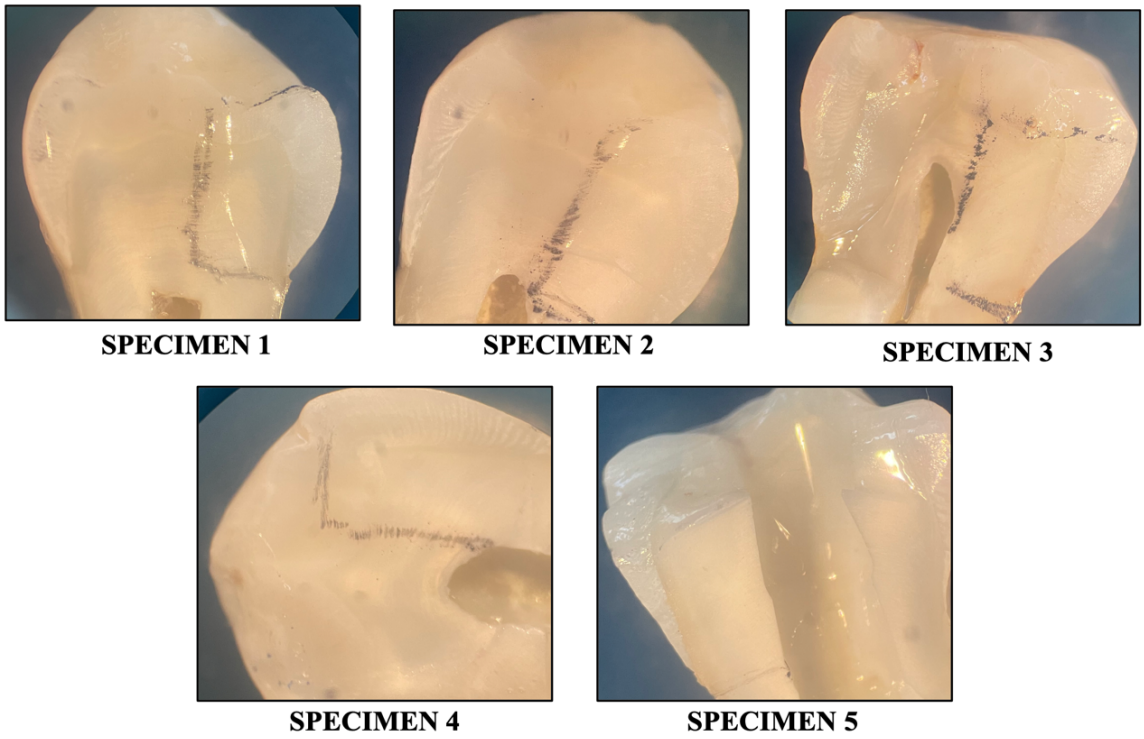


Figure No. 67: Photograph showing remineralization of specimens after 48 hours in Herbal Dental gel Group (Group II).

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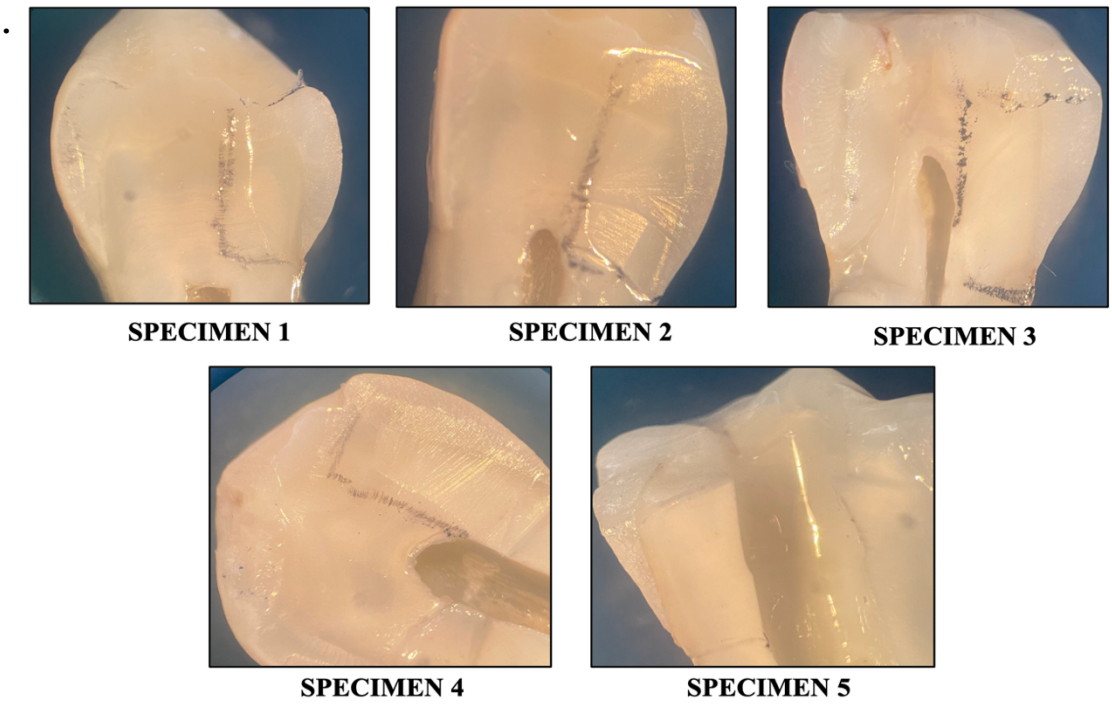

Figure No. 68: Photograph showing remineralization of specimens after 72 hours in Herbal Dental gel Group (Group II).

STATISTICAL ANALYSIS

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

Antibacterial tests were analysed using following Statistical analysis:

- Chi square test for comparison between three groups
- One Way ANOVA for intergroup comparison
- Kruskal Wallis One Way Analysis of Variance for cytotoxicity evaluation between two groups

Statistical analysis for evaluation of remineralization potential was done using:

- Descriptive analysis
- Dependent ‘t’ test for comparison within groups
- Independent ‘t’ test for comparison between groups

RESULTS

TABLES, GRAPHS AND OBSERVATIONS

The present in-vitro study was designed to evaluate and compare the antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel Containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations of ethanolic extracts of *Zingiber officinale* (ZO), *Salvadora persica* (SP) and *Cinnamomum zeylanicum* (CZ) were determined against *Streptococcus mutans* and *Lactobacillus acidophilus*. Based on the obtained values, preparation of Herbal Dental gel was carried out. The two study groups, namely, Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) were subjected to cytotoxicity test, antibacterial susceptibility tests and evaluation for remineralization potential.

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. Antibacterial tests were analyzed using Chi square test for comparison of three groups and One Way ANOVA for intergroup comparison. Cytotoxicity was statistically analyzed using Kruskal Wallis One Way Analysis of Variance. Statistical analysis for evaluation of remineralization potential was done using Dependent ‘t’ test for comparison within groups and Independent ‘t’ test for comparison between groups.

Table No. 6: Table showing Master Chart of Minimum Inhibitory Concentration (MIC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* at various concentrations.

Streptococcus mutans										
Minimum Inhibitory Concentration (MIC)										
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
ZO	S	S	S	R	R	R	R	R	R	R
ZO	S	S	S	R	R	R	R	R	R	R
ZO	S	S	S	S	R	R	R	R	R	R
ZO	S	S	S	R	R	R	R	R	R	R
ZO	S	S	S	R	R	R	R	R	R	R
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
SP	S	S	S	S	S	S	R	R	R	R
SP	S	S	S	S	S	R	R	R	R	R
SP	S	S	S	S	S	S	S	R	R	R
SP	S	S	S	S	S	S	S	R	R	R
SP	S	S	S	S	S	S	R	R	R	R
µg/ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	S	S	R	R	R	R	R	R

ZO= *Zingiber officinale*; SP= *Salvadora persica*; CZ= *Cinnamomum zeylanicum*; S= Sensitive; R=Resistance; µg/ml = microgram/ milliliter; µl/ml= microliter/ milliliter.

Table No. 7: Table showing Master Chart of Minimum Bactericidal Concentration (MBC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* at various concentrations.

Streptococcus mutans										
Minimum Bactericidal Concentration (MBC)										
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	NG	NG	G	G	G	G	G	G	G
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	G	G	G	G	G	G	G	G	G
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
SP	NG	NG	NG	G	G	G	G	G	G	G
SP	NG	NG	G	G	G	G	G	G	G	G
SP	NG	NG	NG	G	G	G	G	G	G	G
SP	NG	NG	NG	NG	G	G	G	G	G	G
SP	NG	NG	NG	G	G	G	G	G	G	G
µg/ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	NG	NG	G	G	G	G	G	G	G
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	G	G	G	G	G	G	G	G	G

ZO= *Zingiber officinale*; SP= *Salvadora persica*; CZ= *Cinnamomum zeylanicum*; G = Growth; NG = No Growth; µg/ml = microgram/ milliliter; µl/ml= microliter/ milliliter.

Table No. 8: Table showing Master Chart of Minimum Inhibitory Concentration (MIC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Lactobacillus acidophilus* at various concentrations.

Lactobacillus acidophilus										
Minimum Inhibitory Concentration (MIC)										
µl/ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
ZO	S	S	S	R	R	R	R	R	R	R
ZO	S	S	R	R	R	R	R	R	R	R
ZO	S	S	S	S	S	R	R	R	R	R
ZO	S	S	S	S	S	S	R	R	R	R
ZO	S	S	S	R	R	R	R	R	R	R
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
SP	S	S	S	S	S	R	R	R	R	R
SP	S	S	S	S	S	R	R	R	R	R
SP	S	S	S	S	S	S	R	R	R	R
SP	S	S	S	S	S	R	R	R	R	R
SP	S	S	S	S	S	S	R	R	R	R
µg/ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
CZ	S	S	R	R	R	R	R	R	R	R
CZ	S	S	R	R	R	R	R	R	R	R
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	S	R	R	R	R	R	R	R
CZ	S	S	R	R	R	R	R	R	R	R

ZO= *Zingiber officinale*; SP = *Salvadora persica*; CZ= *Cinnamomum zeylanicum*;
S= Sensitive; R=Resistance; µg/ml == microgram/ milliliter; µl/ml= microliter/ milliliter.

Table No. 9: Table showing Master Chart of Minimum Bactericidal Concentration (MBC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Lactobacillus acidophilus* at various concentrations.

Lactobacillus acidophilus										
Minimum Bactericidal Concentration (MBC)										
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	NG	NG	G	G	G	G	G	G	G
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	NG	G	G	G	G	G	G	G	G
ZO	NG	NG	G	G	G	G	G	G	G	G
µl /ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
SP	NG	NG	G	G	G	G	G	G	G	G
SP	NG	NG	NG	G	G	G	G	G	G	G
SP	NG	NG	G	G	G	G	G	G	G	G
SP	NG	NG	NG	G	G	G	G	G	G	G
SP	NG	NG	G	G	G	G	G	G	G	G
µg/ml	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.195
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	G	G	G	G	G	G	G	G	G
CZ	NG	NG	G	G	G	G	G	G	G	G
CZ	NG	G	G	G	G	G	G	G	G	G

ZO= *Zingiber officinale*; SP= *Salvadora persica*; CZ= *Cinnamomum zeylanicum*; G = Growth; NG = No Growth; µg/ml = microgram/ milliliter; µl/ml= microliter/ milliliter.

Table No. 10: Table showing Mean, Standard Deviation, Standard Error and Coefficient of Variation of Minimum Inhibitory Concentration (MIC) scores of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Organisms	Groups	Rounds					Mean	SD	SE	CV
		I	II	III	IV	V				
S. Mutans	ZO	12.5	12.5	6.25	12.5	12.5	11.25	2.80	1.25	24.85
	SP	1.562	3.125	0.781	0.781	1.562	1.56	0.96	0.43	61.26
	CZ	12.5	12.5	12.5	12.5	6.25	11.25	2.80	1.25	24.85
L. acidophilus	ZO	12.5	25	3.125	1.562	12.5	10.94	9.38	4.19	85.72
	SP	3.125	3.125	1.562	3.125	1.562	2.50	0.86	0.38	34.25
	CZ	25	25	12.5	12.5	25	20.00	6.85	3.06	34.23

All readings are in µl/ml.

ZO= *Zingiber officinale*; SP= *Salvadora persica*; CZ= *Cinnamomum zeylanicum*; SD= Standard Deviation, SE= Standard Error, CV= Coefficient of Variation.

Graph No. 1: Graphical representation of Mean scores of Minimum Inhibitory Concentration of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus.*

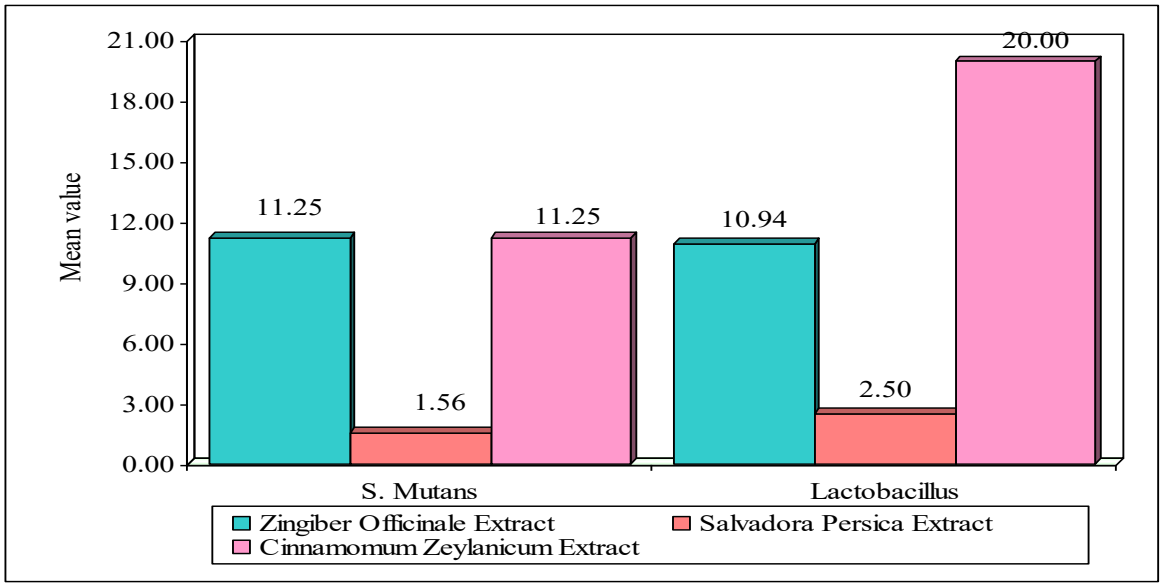

Table No. 11: Table showing Mean, Standard Deviation, Standard Error and Coefficient of Variation of Minimum Bactericidal Concentration (MBC) scores of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Organisms	Groups	Rounds					Mean	SD	SE	CV
		I	II	III	IV	V				
S. mutans	ZO	25	12.5	25	25	50	27.50	13.69	6.12	49.79
	SP	12.5	25	12.5	6.25	12.5	13.75	6.85	3.06	49.79
	CZ	25	25	12.5	25	50	27.50	13.69	6.12	49.79
L. acidophilus	ZO	25	12.5	25	25	25	22.50	5.59	2.50	24.85
	SP	25	12.5	25	12.5	25	20.00	6.85	3.06	34.23
	CZ	25	25	50	25	50	35.00	13.69	6.12	39.12

All readings are in µl/ml.

ZO= *Zingiber officinale*; SP= *Salvadora persica*; CZ= *Cinnamomum zeylanicum*; SD= Standard Deviation, SE= Standard Error, CV= Coefficient of Variation.

Graph No. 2: Graphical representation of Mean scores of Minimum Bactericidal Concentration (MBC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *S. mutans* and *L. acidophilus*.

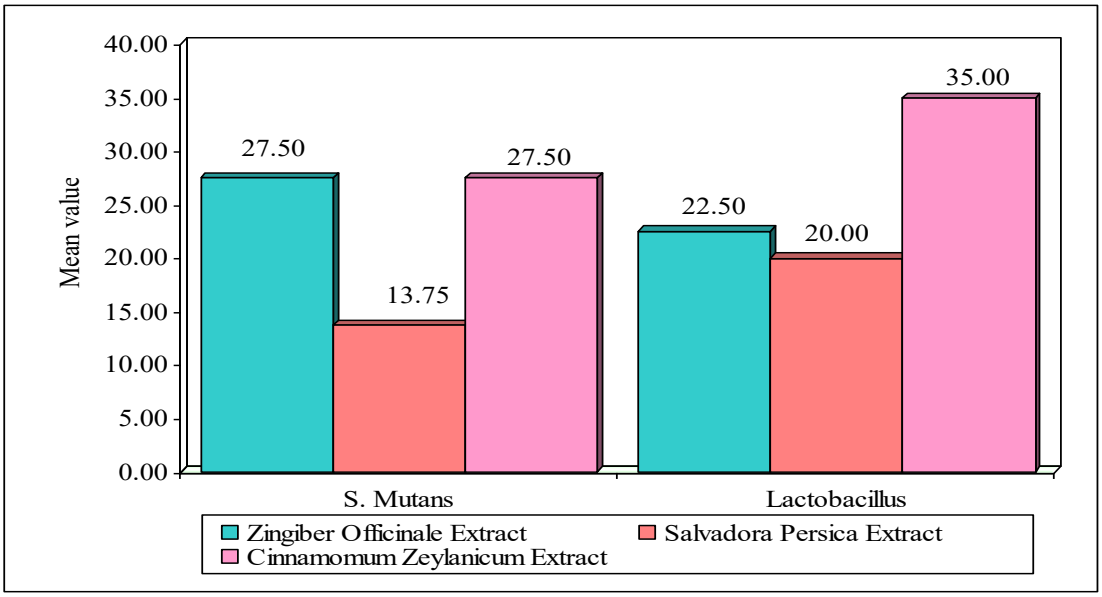

Table No. 6 and 7 represent the master chart of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* at various concentrations.

Table No. 8 and 9 represent the master chart of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Lactobacillus acidophilus* at various concentrations.

Table No. 10 and Graph No. 1 represent the Mean, Standard Deviation, Standard Error and Coefficient of Variation of Minimum Inhibitory Concentration (MIC) scores of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus*. The tests were carried out in quintuplicates and a mean value was calculated based on the readings obtained.

Minimum Inhibitory Concentration of *Zingiber officinale* was found to be 11.25 $\pm$ 2.80 µl/ml against *Streptococcus mutans* and 10.94 $\pm$ 9.38 µl/ml against *Lactobacillus acidophilus*. *Salvadora persica* had a Minimum Inhibitory Concentration of 1.56 $\pm$ 0.96 µl/ml against *Streptococcus mutans* and 2.50 $\pm$ 0.86 µl/ml against *Lactobacillus acidophilus*. Minimum Inhibitory Concentration of *Cinnamomum zeylanicum* was found to be 11.25 $\pm$ 2.80 µg/ml against *Streptococcus mutans* and 20.00 $\pm$ 6.85 µg/ml against *Lactobacillus acidophilus*.

Table No. 11 and Graph No. 2 represent the Mean, Standard Deviation, Standard Error and Coefficient of Variation of Minimum Bactericidal Concentration (MBC) scores of the three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against *Streptococcus mutans* and *Lactobacillus acidophilus*. The tests were carried out in quintuplicates and a mean value was calculated based on the readings obtained.

Minimum Bactericidal Concentration of *Zingiber officinale* was found to be 27.50 ± 13.69 µl/ml against *Streptococcus mutans* and 22.50 ± 5.59 µl/ml against *Lactobacillus acidophilus*. *Salvadora persica* had Minimum Bactericidal Concentration of 13.75 ± 6.85 µl/ml against *Streptococcus mutans* and 20.00 ± 6.85 µl/ml against *Lactobacillus acidophilus*. Minimum Bactericidal Concentration of *Cinnamomum zeylanicum* was found to be 27.50 ± 13.69 µg/ml against *Streptococcus mutans* and 35.00 ± 13.69 µg/ml against *Lactobacillus acidophilus*.

Table No. 12: Table showing comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MIC) of *Streptococcus mutans* and *Lactobacillus acidophilus* using Chi-square test.

	Zingiber officinale	%	*Salvadora persica*	%	*Cinnamomum zeylanicum*	%	Total	%
S. mutans								
Sensitive	3	30	6	60	3	30	12	40
Resistance	7	70	4	40	7	70	18	60
Chi-square=2.5000, p=0.2870								
L. acidophilus								
Sensitive	3	30	5	50	2	20	10	33.3
Resistance	7	70	5	50	8	80	20	66.7
Chi-square=2.1000, p=0.3500								
Total	10	100	10	100	10	100	30	100

Graph No. 3: Graphical representation of comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MIC) of *Streptococcus mutans* and *Lactobacillus acidophilus*.

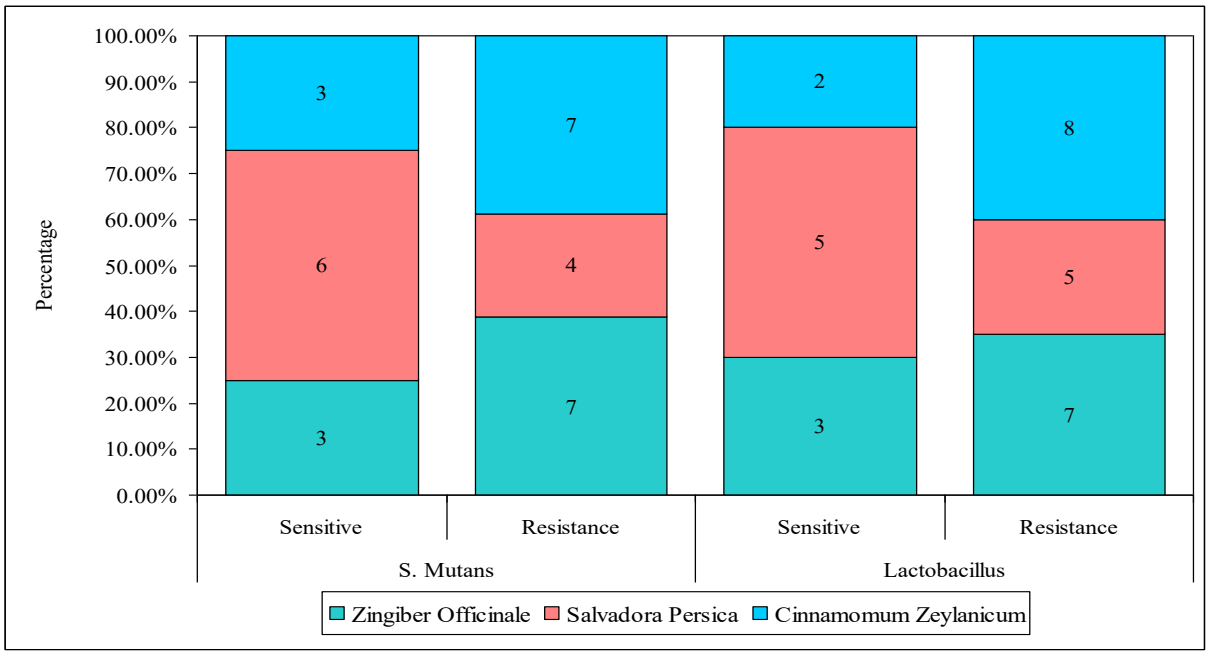

Table No. 13: Table showing comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with Minimum Inhibitory Concentration (MIC) scores of *Streptococcus mutans* and *Lactobacillus acidophilus* by One-way ANOVA.

	Sources of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F-value**	**p-value**
S. Mutans	Between groups	2	312.84	156.42	28.3704	0.0001*
	Within groups	12	66.16	5.51		
	Total	14	379.01			
L. acidophilus	Between groups	2	765.97	382.98	8.4793	0.0051*
	Within groups	12	542.00	45.17		
	Total	14	1307.97			

*Denotes statistically significant ($p<0.05$).

Table No. 12 and Graph No. 3 show comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MIC) of *Streptococcus mutans* and *Lactobacillus acidophilus* using Chi-square test.

When sensitivity patterns were assessed for three herbal extracts using Chi Square test, it was found that 30 percent of *S. mutans* organisms were sensitive to *Zingiber officinale* extract, 60 percent to *Salvadora persica* extract and 30 percent to *Cinnamomum zeylanicum* extract. However, on comparing the sensitivity patterns of these three extracts with each other, *Salvadora persica* extract showed better sensitivity but no statistically significant difference was found between them as indicated by 'p' value of 0.2870 ($p<0.05$) and Chi square value of 2.500.

When assessment of sensitivity patterns was carried out against *L. acidophilus*, 30 percent of *L. acidophilus* were found to be sensitive to *Zingiber officinale* extract, 50 percent to *Salvadora persica* extract and 20 percent to *Cinnamomum zeylanicum* extract. However, on comparing the sensitivity patterns of these three extracts with each other, *Salvadora persica* extract showed better sensitivity but there was no statistically significant difference between them as indicated by 'p' value of 0.3500 ($p<0.05$) and Chi square value of 2.100.

Table No. 13 show comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with respect to their Minimum Inhibitory Concentration (MIC) scores of *S. Mutans* and *L. acidophilus* by One-way ANOVA. When sensitivity patterns of these extracts were assessed against *S. Mutans* using One-way ANOVA, it was found that all three extracts showed good antibacterial effectiveness as indicated by 'F' value of 28.3704 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$). When sensitivity patterns of these extracts were assessed against *L. acidophilus*, all three extracts showed good antibacterial effectiveness as indicated by 'F' value of 8.4793 and a highly statistically significant 'p' value of 0.0051 ($p<0.05$).

Table No. 14: Table showing comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MBC) of *Streptococcus mutans* and *Lactobacillus acidophilus* using Chi-square test.

	Zingiber officinale	%	*Salvadora persica*	%	*Cinnamomum zeylanicum*	%	Total	%
S. Mutans								
Growth	8	80	7	70	8	80	23	76.67
No growth	2	20	3	30	2	20	7	23.33
Chi-square=0.3790, p=0.8300								
L. acidophilus								
Growth	8	80	8	80	8	80	24	80.00
No growth	2	20	2	20	2	20	6	20.00
Chi-square=0.0000, p=1.0000								
Total	10	100	10	100	10	100	30	100.00

Graph No. 4: Graphical representation of comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MBC) of *Streptococcus mutans* and *Lactobacillus acidophilus.*

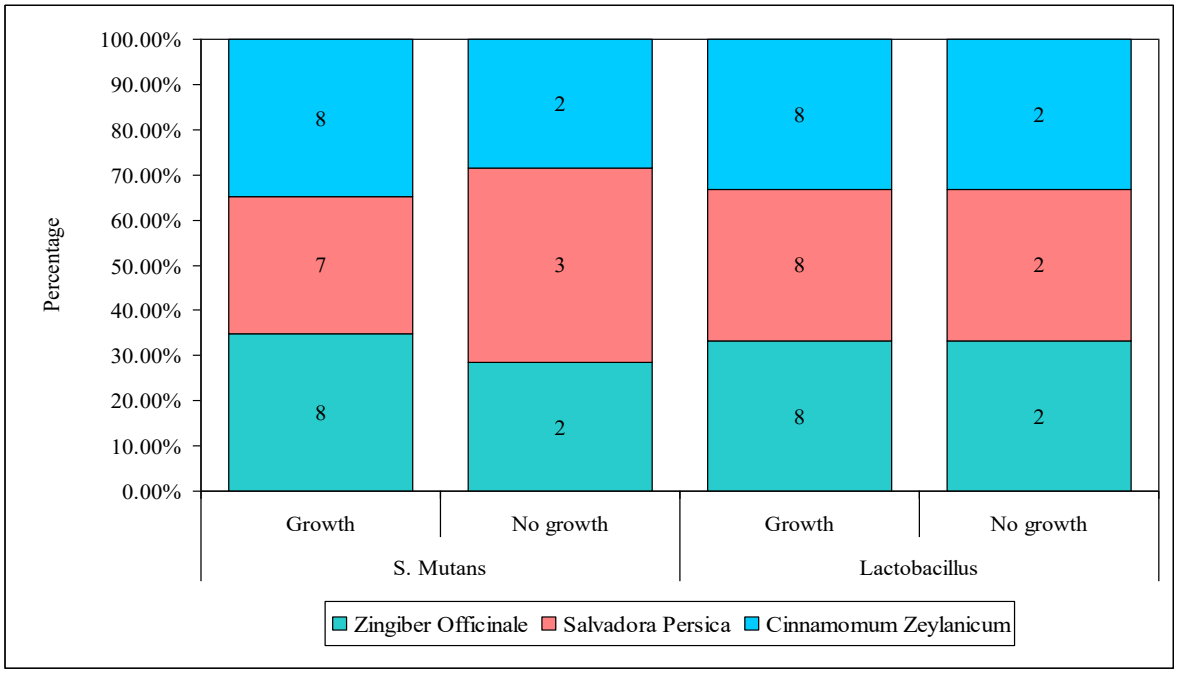

Table No. 15: Table showing comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with Minimum Bactericidal Concentration (MBC) scores of *Streptococcus mutans* and *Lactobacillus acidophilus* by One-way ANOVA.

	Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
S. Mutans	Between groups	2	630.21	315.10	2.2407	0.1490
	Within groups	12	1687.50	140.63		
	Total	14	2317.71			
L. acidophilus	Between groups	2	645.83	322.92	3.6471	0.0579
	Within groups	12	1062.50	88.54		
	Total	14	1708.33			

*Denotes statistically significant ($p<0.05$).

Table No. 14 and Graph No. 4 show comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with sensitivity pattern (MBC) of *Streptococcus mutans* and *Lactobacillus acidophilus* using Chi-square test.

When sensitivity patterns were assessed for three extracts using Chi Square test, it was observed that 20 percent of *S. Mutans* organisms showed no growth in presence of *Zingiber officinale* extract, 30 percent showed no growth in presence of *Salvadora persica* extract and 20 percent showed no growth in presence of *Cinnamomum zeylanicum* extract. The difference between the Minimum Bactericidal Concentrations between these groups was not statistically significant as denoted with 'p' value of 0.8300 ($p<0.05$) and Chi-square value of 0.3790.

While assessing the sensitivity patterns against *L. acidophilus,* 20 percent of *L. acidophilus* organism showed no growth in presence of all three extracts namely *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum.* There was no statistically significant difference in the Minimum Bactericidal Concentrations between these groups as denoted with 'p' value of 1.000 ($p<0.05$) and Chi square value of 0.000.

Table No. 15 show comparison of three extracts namely *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* with respect to their Minimum Bactericidal Concentration (MBC) scores against *S. Mutans* and *L. acidophilus* by One-way ANOVA. On carrying out the intergroup comparison of Minimum Bactericidal Concentration scores against *S. Mutans* using One Way ANOVA, no statistically significant difference was obtained as indicated by 'F' value of 2.2407 and 'p' value of 0.1490 ($p<0.05$). When intergroup comparison was carried out for all three extracts against *L. acidophilus*, no statistically significant difference was obtained as indicated by 'F' value of 3.6471 and 'p' value of 0.0579 ($p<0.05$). These results indicate that all three extracts showed similar antibacterial activity against *S. Mutans* and *L. acidophilus.*

Table No. 16: Table showing mean of optical densities of surviving cells under two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) while carrying out the cytotoxicity test using Kruskal Wallis One Way analysis of variance.

	Absorption at 570 nm	**Mean Optical Density**	**SD**	**Mean rank**	**CV**	**% of viability**	**Interpretation**
100% Acidulated Phosphate Fluoride Gel	0.291	0.42	0.11	5.67	26.66	100.82	No Cell Death
	0.489						
	0.481						
100% Herbal Dental Gel	0.500	0.40	0.11	5.00	28.31	103.53	No Cell Death
	0.277						
	0.421						
Negative Control	0.455	0.37	0.08	4.33	20.85	100.00	No Cell Death
	0.339						
	0.310						
H-value	0.3555						
p-value	0.8371						

SD= Standard Deviation, CV = Coefficient of Variation
*Denotes statistically significant ($p<0.05$).

Graph No. 5: Graphical representation of comparison of optical density scores in two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) while carrying out the cytotoxicity test.

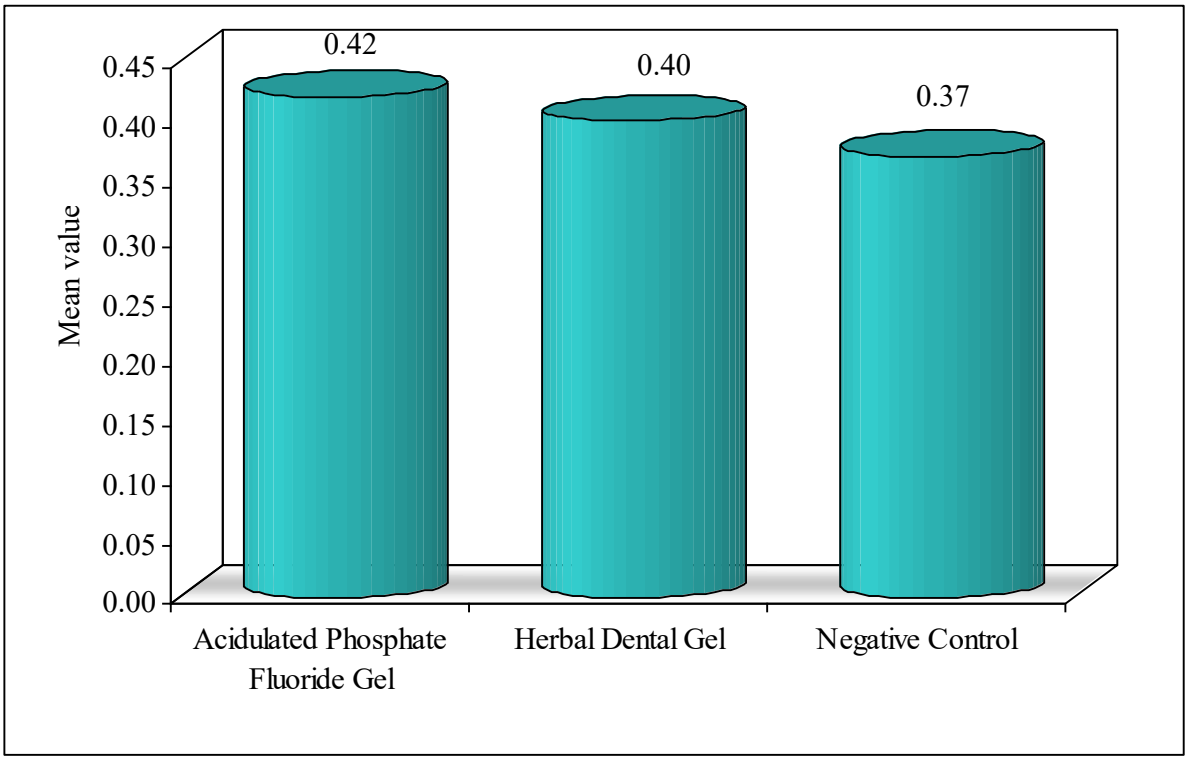

Table No. 16 and Graph No. 5 depict the mean optical densities (OD) of surviving cells under two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) while carrying out the cytotoxicity test using Kruskal Wallis One Way analysis of variance. The test was carried out using MTT assay against fibroblasts and readings were taken using a spectrophotometer at a wavelength of 570 nm.

The mean optical density in Acidulated Phosphate Fluoride gel (Group I) was found to be 0.42±0.11. In Herbal Dental gel (Group II), mean optical density was found to be 0.40±0.11. The mean optical density in the negative control group was found to be 0.37±0.08.

The fibroblasts showed 100% viability in presence of both the study groups at 100 percent concentration. There was no statistically significant difference found between the groups as indicated by 'H' value of 3.555 and 'p' value of 0.8371 ($p<0.05$).

Therefore, both the test compounds namely Acidulated Phosphate Fluoride gel and Herbal Dental gel appear to be non- toxic to the fibroblasts.

Table No. 17: Table showing the antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans.*

Groups	Zone of Inhibition		
	Round I (mm)	Round II (mm)	Mean (mm)
Acidulated Phosphate Fluoride Gel	11	12	11.50
Herbal Dental Gel	21	19	20.00

Graph No. 6: Graphical representation of antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans.*

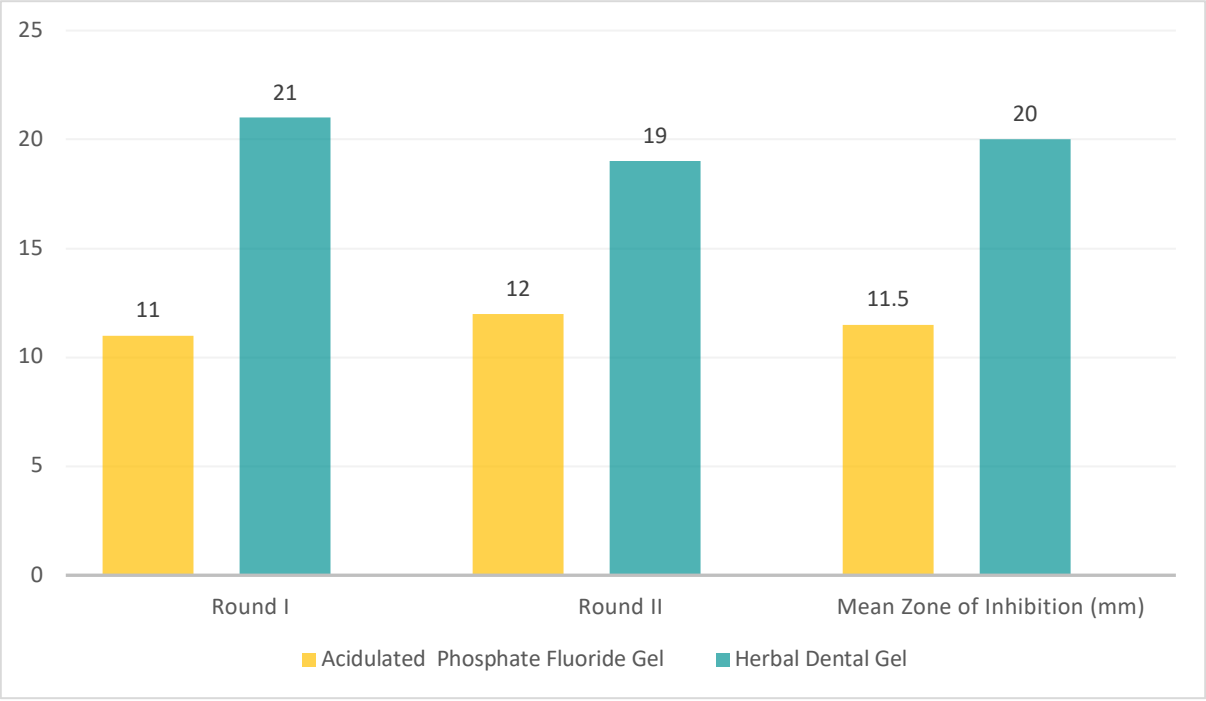

Table No. 18: Table showing the antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus.*

Groups	Zone of Inhibition		
	Round I (mm)	Round II (mm)	Mean (mm)
Acidulated Phosphate Fluoride Gel	17	16	16.50
Herbal Dental Gel	19	23	21.00

Graph No. 7: Graphical representation of antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus.*

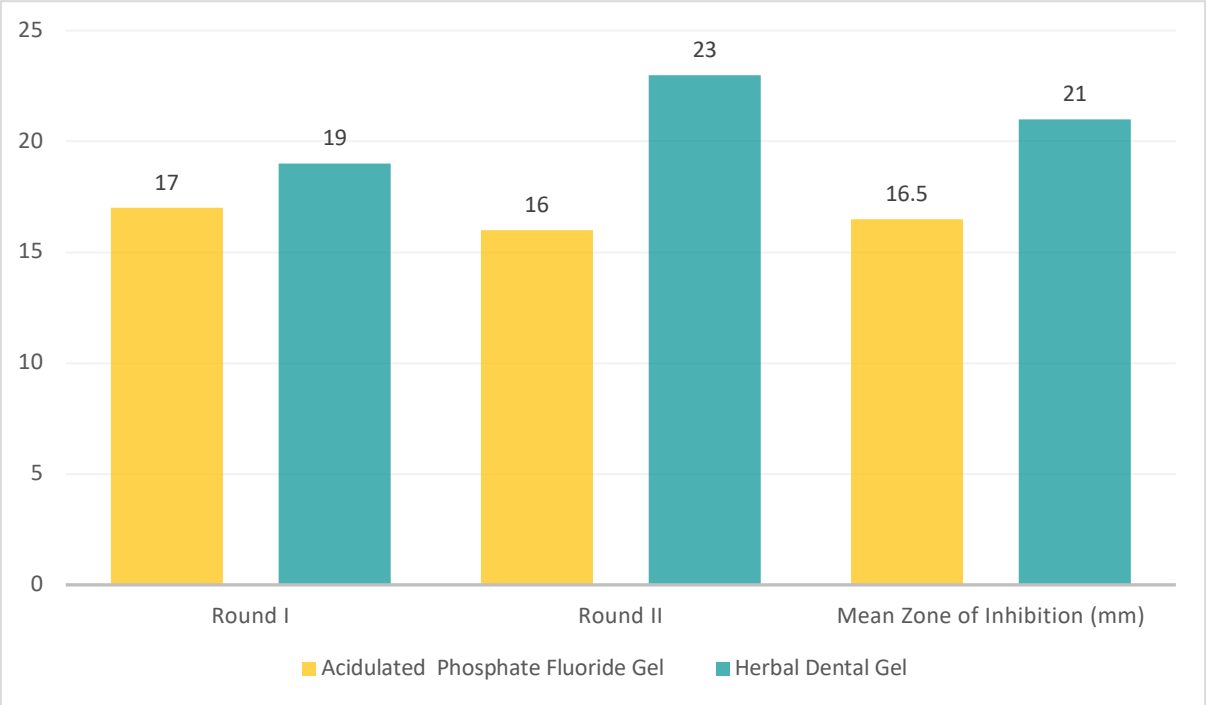

Table No. 17 and Graph No. 6 represent the mean of Zones of Inhibition obtained during antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans*. Mean Zone of Inhibition obtained by Acidulated Phosphate Fluoride gel (Group I) against *Streptococcus mutans* was 11.50 mm and Herbal Dental gel (Group II) was 20.00 mm indicating the superior antibacterial efficacy of Herbal Dental gel over Acidulate Phosphate Fluoride gel against *Streptococcus mutans*.

Table No. 18 and Graph No. 8 represent the mean of Zones of Inhibition obtained during antibacterial susceptibility using Agar Well Diffusion test in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus*. Mean Zone of Inhibition obtained by Acidulated Phosphate Fluoride gel (Group I) against *Lactobacillus acidophilus* was 16.50 mm and Herbal Dental gel (Group II) was 21.00 mm indicating the superior antibacterial efficacy of Herbal Dental gel over Acidulated Phosphate Fluoride gel against *Lactobacillus acidophilus*.

These results indicate that Herbal Dental gel (Group II) had better antibacterial efficacy as compared to Acidulate Phosphate Fluoride gel (Group I) against both organisms i.e. *Streptococcus mutans* and *Lactobacillus acidophilus.*

Table No. 19: Table showing the antibacterial susceptibility with respect to Time Kill Assay scores in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans* at various time intervals.

Groups	Antibacterial activity with Time Kill Assay scores					
	0 hr	2 hrs	4 hrs	6 hrs	8 hrs	24 hrs
Acidulated Phosphate Fluoride gel	0.278	0.200	0.168	0.118	0.118	0.008
Herbal Dental gel	0.162	0.155	0.152	0.152	0.121	0.009
Negative Control	0.108	0.153	0.212	0.310	0.310	0.310

Graph No. 8: Graphical Representation of antibacterial susceptibility with respect to Time Kill Assay scores in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *S. mutans* at various time intervals.

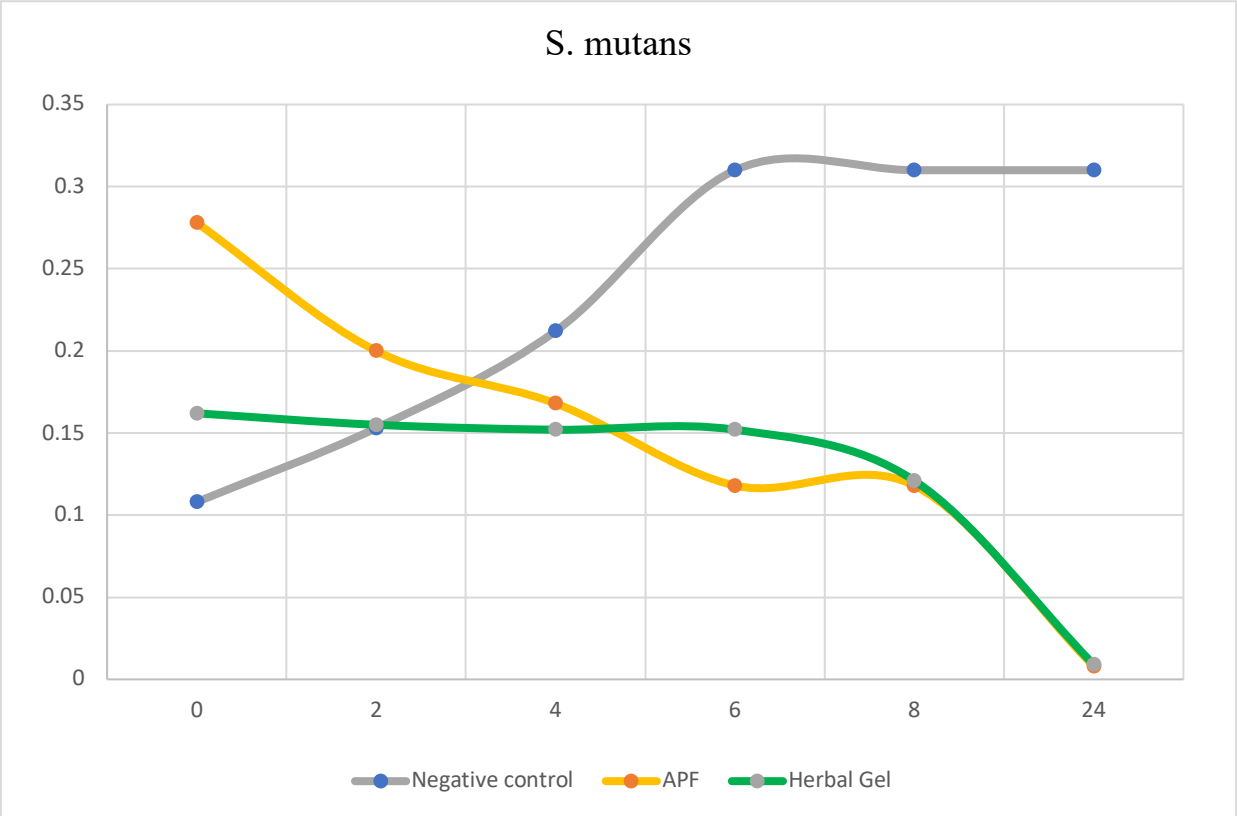

Table No. 20: Table showing the antibacterial susceptibility with respect to Time Kill Assay scores in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus* at various time interval.

Groups	Antibacterial activity with Time Kill Assay scores					
	0 hr	2 hrs	4 hrs	6 hrs	8 hrs	24 hrs
Acidulated Phosphate Fluoride gel	0.362	0.358	0.268	0.112	0.111	0.010
Herbal Dental gel	0.297	0.200	0.158	0.118	0.102	0.008
Negative Control	0.151	0.158	0.258	0.310	0.310	0.310

Graph No. 9: Graphical Representation of antibacterial susceptibility with respect to Time Kill Assay scores in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *L. acidophilus* at various time intervals.

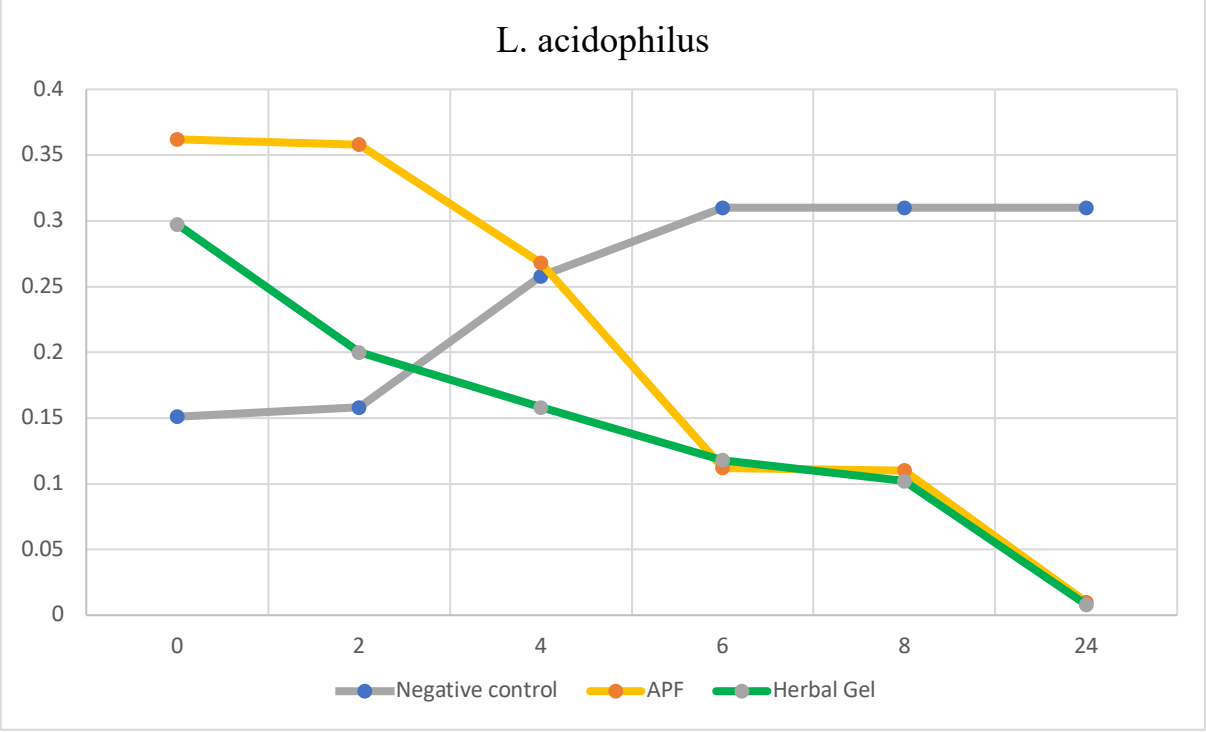

Table No. 19 and Graph No. 8 represent the antibacterial susceptibility with respect to Time Kill Assay scores of the two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Streptococcus mutans* at various time intervals. In Acidulated Phosphate Fluoride Gel group (Group I), the optical density value was 0.278 at baseline and gradually decreased to 0.200 at a time interval of 2 hours, 0.168 at a time interval of 4 hours, 0.118 at time interval of 6 and 8 hours, followed by 0.008 at a time interval of 24 hours. In Herbal Dental gel group (Group II), the optical density value at baseline was 0.162 which decreased to 0.155 after a time interval of 2 hours. The optical density values further decreased to 0.152 at 4 and 6 hours, 0.121 at 8 hours and 0.009 after a time interval of 24 hours.

This continuous decrease in optical density values is depicted in the graph wherein it can be seen that Acidulated Phosphate Fluoride gel (Group I) showed immediate reduction in *S. mutans* growth as apparent by the reduction in the number of viable cells. Herbal Dental gel (Group II) presented an initial lag phase where bacteria were metabolically active but not dividing. After an interval of 7 hours, both Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) showed equal efficacy in reduction of *S. mutans* growth as represented by the death phase characterized by an exponential decrease in the number of living cells. Both the test groups showed significant antibacterial activity against *S. mutans* as opposed to the control group wherein exponential bacterial growth was observed.

Table No. 20 and Graph No. 9 represent the antibacterial susceptibility with respect to Time Kill Assay scores of the two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus* at various time intervals. In Acidulated Phosphate Fluoride gel group (Group I), the optical density at baseline was found to be 0.362 which gradually decreased to 0.358 at an interval of 2 hours,

0.268 at a time interval of 4 hours, 0.112 at an interval of 6 hours, 0.111 at a time interval of 8 hours and 0.010 at a time interval of 24 hours. In Herbal Dental gel group (Group II), the optical density was found to be 0.297 at baseline, 0.200 at 2 hours, 0.158 at 4 hours, 0.118 at 6 hours, 0.102 at 8 hours and 0.008 at an interval of 24 hours.

This continuous decrease in optical density values represent a significant antibacterial activity of both Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) against *Lactobacillus acidophilus* as both showed immediate reduction in viable bacterial count which is depicted in the graph. An exponential decrease in the number of living cells was observed after an interval of 6 hours in both the groups which represents the death phase of the bacteria. Both the test groups showed significant antibacterial activity against *Lactobacillus acidophilus* as opposed to the control group wherein exponential bacterial growth was observed.

Table No. 21: Table showing Master Chart of remineralization rates at different time intervals in Acidulated Phosphate Fluoride gel group (Group I).

Sl. No.	Demineralization (units)	Remineralization (Units)		
		24 hours	48 hours	72 hours
1	7	6	7	7
2	11	4	6	10
3	11	4	5	9
4	17	4	5	15
5	13	5	5	12
6	6	5	6	6
7	10	4	5	10
8	16	5	7	10
9	10	4	8	10
10	7	4	4	6
11	12	4	4	5
12	10	5	7	8
13	20	7	8	14
14	28	5	9	15
15	15	5	7	14
16	13	5	10	13
17	6	5	5	6
18	14	4	10	14
19	13	5	7	10
20	8	6	6	8
21	14	5	6	10
22	5	2	5	5
23	11	4	10	10
24	15	2	5	5
25	15	1	3	5
26	13	4	10	12
27	8	5	7	8
28	10	3	7	10
29	15	10	12	13
30	10	5	9	9

Table No. 22: Table showing Master Chart of remineralization rates at different time intervals in Herbal Dental gel group (Group II).

Sl. No.	Demineralization (units)	Remineralization (Units)		
		24 hours	48 hours	72 hours
1	14	6	7	13
2	15	6	6	10
3	18	5	8	10
4	15	5	8	13
5	7	4	7	7
6	9	4	9	9
7	10	4	5	10
8	12	5	5	12
9	28	5	8	18
10	10	6	8	10
11	11	7	9	11
12	11	3	9	11
13	18	8	8	11
14	13	7	9	13
15	8	5	8	8
16	8	4	5	8
17	10	5	10	10
18	12	10	10	12
19	10	6	8	10
20	17	5	8	10
21	17	10	11	15
22	10	7	8	9
23	21	12	13	15
24	8	4	4	6
25	10	5	6	10
26	13	4	6	10
27	14	2	7	10
28	14	10	12	13
29	10	8	10	10
30	20	10	15	16

Table No. 21 and 22 represent the master chart of remineralization rates at different time intervals i.e., after 24 hours, 48 hours and 72 hours in Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) respectively.

Table No. 23: Table showing remineralization units at different time intervals in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) by dependent 't' test.

		Mean	SD	Mean Diff.	SD Diff.	% of change	t-value	p-value
Acidulated Phosphate Fluoride (APF) gel	Demineralization	12.10	4.68	7.53	4.72	62.26	8.7460	0.0001*
	24 hours	4.57	1.59					
	Demineralization	12.10	4.68	5.27	4.56	43.53	6.3321	0.0001*
	48 hours	6.83	2.17					
	Demineralization	12.10	4.68	2.47	3.50	20.39	3.8590	0.0006*
	72 hours	9.63	3.17					
	24 hours	4.57	1.59	-2.27	1.86	-49.64	-6.690	0.0001*
	48 hours	6.83	2.17					
	24 hours	4.57	1.59	-5.07	2.88	-110.9	-9.649	0.0001*
	72 hours	9.63	3.17					
	48 hours	6.83	2.17	-2.80	2.52	-40.98	-6.075	0.0001*
	72 hours	9.63	3.17					

Herbal Dental gel	Demineralization	13.10	4.66	7.53	4.72	62.26	8.7460	0.0001*
	24 hours	6.07	2.41					
	Demineralization	13.10	4.66	5.27	4.56	43.53	6.3321	0.0001*
	48 hours	8.23	2.43					
	Demineralization	13.10	4.66	2.47	3.50	20.39	3.8590	0.0001*
	72 hours	11.00	2.64					
	24 hours	6.07	2.41	-2.16	0.02	-49.64	-6.690	0.0001*
	48 hours	8.23	2.43					
	24 hours	6.07	2.41	-4.93	0.03	-110.9	-9.649	0.0001*
	72 hours	11.00	2.64					
	48 hours	8.23	2.43	-2.77	0.21	-40.98	-6.075	0.0001*
	72 hours	11.00	2.64					

SD = Standard Deviation, MD= Mean Difference

*Denotes statistically significant ($p<0.05$).

Graph No. 10: Graphical Representation of remineralization units at different time intervals in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II).

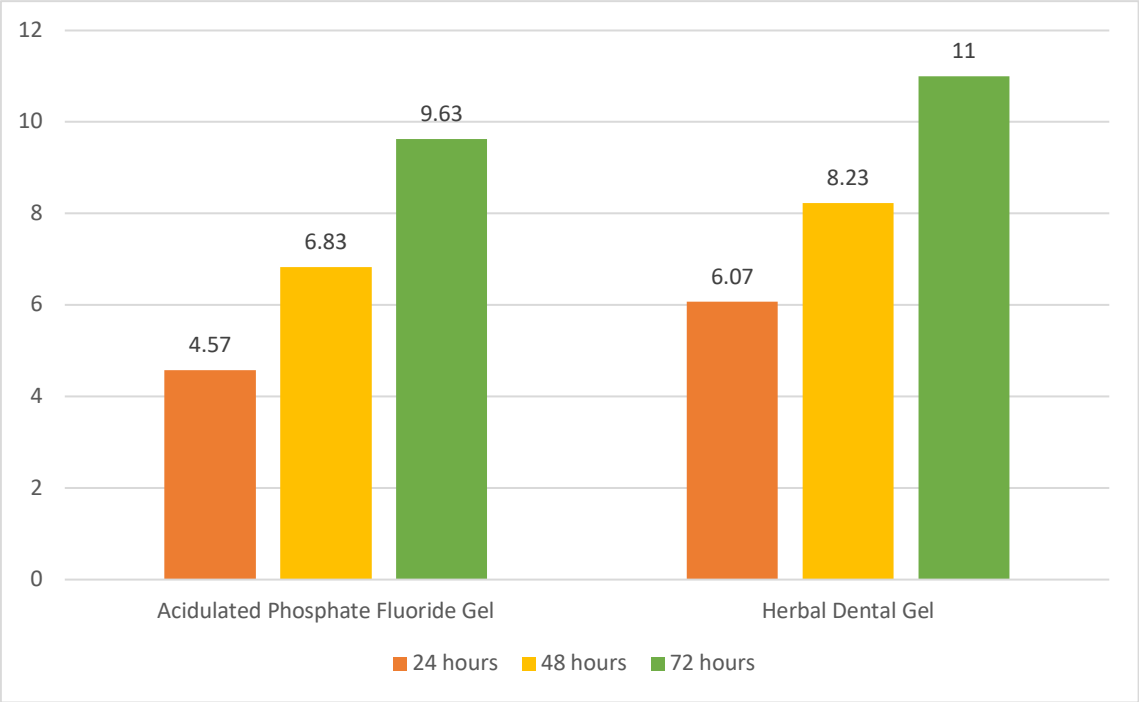

Table No. 23 and Graph No. 10 represent the units of remineralization at different time intervals i.e., after 24 hours, 48 hours and 72 hours in two study groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II).

In Acidulated Phosphate Fluoride gel group (Group I), mean demineralization was 12.10 $\pm$ 4.68. When demineralized tooth specimens were treated with Acidulated Phosphate Fluoride gel for 24 hours, 4.57 $\pm$ 1.59 units of remineralization was observed with a 't' value of 8.7460 and a very highly statistically significant 'p' value of 0.0001 ($p<0.05$). After 48 hours, the mean remineralization was found to be 6.83 $\pm$ 2.17 units. These values gave a 't' value of 6.3321 and a very highly statistically significant 'p' value of 0.0001 ($p<0.05$). After a time interval of 72 hours, mean remineralization was 9.63 $\pm$ 3.17 units with a 't' value of 3.8590 and a highly statistically significant 'p' value of 0.0006 ($p<0.05$).

When dependent 't' test was used to compare the remineralization rates in Acidulated Phosphate Fluoride gel group at various time intervals, i.e., 24 hours and 48 hours, the mean difference was found to be -2.27$\pm$1.86 and a 't' value of -6.690 was obtained. These results were also backed by a very highly statistically significant 'p' value of 0.0001 ($p<0.05$). When remineralization units at the end of 24 hours were compared with remineralization units at the end of 72 hours, a mean difference of -5.07$\pm$ was obtained with a 't' value of -9.649 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$). When comparison of remineralization units was carried out at the end of 48 hours and 72 hours, mean difference was found to be -2.80$\pm$2.52 with a 't' value of -6.075 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$).

In Herbal Dental gel group (Group II), mean demineralization was 13.10 $\pm$ 4.68. After application of Herbal Dental gel for 24 hours, 6.07 $\pm$ 2.41 units of remineralization was observed with a 't' value of 8.7460 and a very highly statistically significant 'p' value of

0.0001 ($p<0.05$). After 48 hours, the mean remineralization was found to be 8.23 $\pm$ 2.43 units and a 't' value of 6.3321 was obtained. These values also gave a very highly statistically significant 'p' value of 0.0001 ($p<0.05$). After an interval of 72 hours, mean remineralization was 11.00 $\pm$ 2.64 units with a 't' value of 3.8590 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$).

When dependent 't' test was used to compare the remineralization rates in Herbal Dental gel group at various time intervals, i.e., 24 hours and 48 hours, the mean difference was found to be -2.16$\pm$0.02 and a 't' value of -6.690 was obtained. These results were also backed by a very highly statistically significant 'p' value of 0.0001 ($p<0.05$). When remineralization units at the end of 24 hours were compared with remineralization units at the end of 72 hours, a mean difference of -4.93$\pm$0.03 was obtained with a 't' value of -9.649 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$). When comparison of remineralization units was carried out at the end of 48 hours and 72 hours, mean difference was found to be -2.77$\pm$0.21 with a 't' value of -6.075 and a highly statistically significant 'p' value of 0.0001 ($p<0.05$).

These highly statistically significant results represent that both the study groups i.e., Acidulated Phosphate Fluoride gel group (Group I) and Herbal Dental gel group (Group II) show good remineralization potential.

Table No. 24: Table showing the comparison of remineralization rates in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) by independent 't' test.

Treatment times	Acidulated phosphate fluoride gel		Herbal dental gel		t-value	p-value
	Mean	SD	Mean	SD		
24 hours after Remineralization	4.57	1.59	6.07	2.41	-2.848	0.0061*
48 hours after Remineralization	6.83	2.17	8.23	2.43	-2.354	0.0219*
72 hours after Remineralization	9.63	3.17	11.00	2.64	-1.815	0.0746

*Denotes statistically significant ($p<0.05$).

Graph No. 11: Graphical Representation of comparison of remineralization rates in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) by independent 't' test.

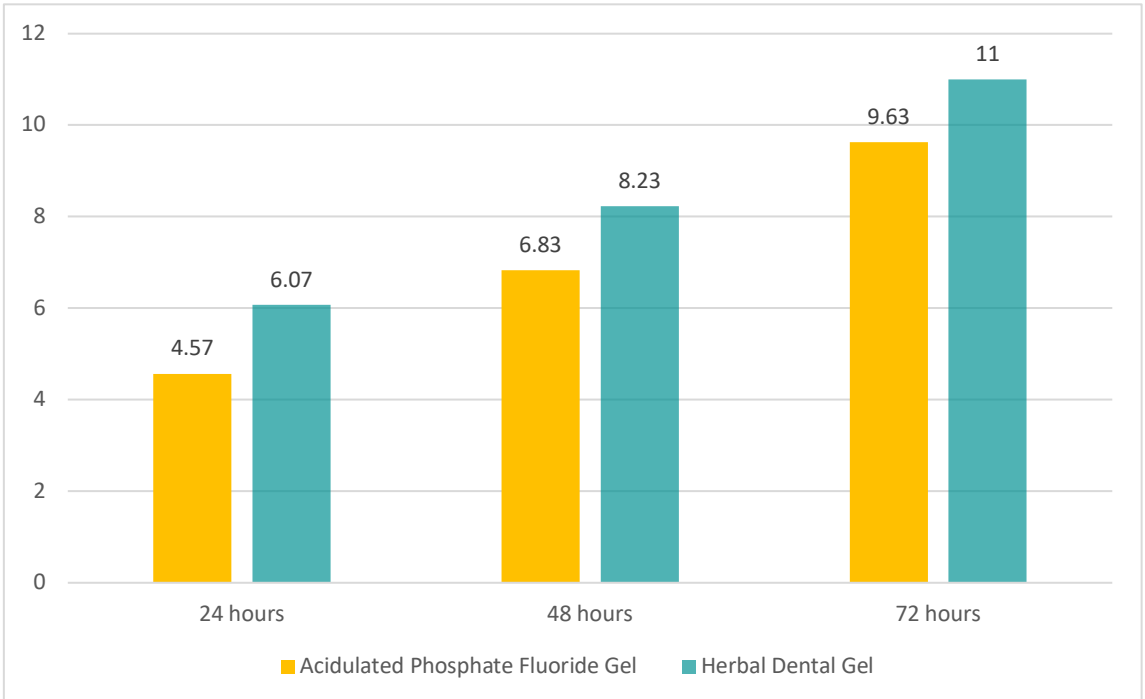

Table No. 24 and Graph No. 11 depict the comparison of remineralization rates in two groups namely Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel (Group II) by independent 't' test.

Demineralization units before the application of test compounds were similar in Acidulated Phosphate Fluoride gel (Group I) and Herbal Dental gel group (Group II) indicating the standardization between both the groups.

Mean remineralization after a time interval of 24 hours was 4.57 units in Acidulated Phosphate Fluoride gel group (Group I) and 6.07 in Herbal Dental gel group (Group II). These values indicate that remineralization potential at the end of 24 hours was more in Herbal Dental gel group as compared to Acidulated Phosphate Fluoride gel group with a statistically significant difference as indicated by 'p' value of 0.0061 ($p<0.05$).

After a time interval of 48 hours, mean remineralization in Acidulated Phosphate Fluoride gel group was 6.83 units as compared to 8.23 units in Herbal Dental gel group indicating the superior remineralization potential of Herbal Dental gel. These values are also supported by a statistically significant 'p' value of 0.0219 ($p<0.05$).

After application of test compounds for 72 hours, mean remineralization in Acidulated Phosphate Fluoride gel group was 9.63 units as compared to 11.00 units in Herbal Dental gel group. Although Herbal Dental gel showed more remineralizing potential than Acidulated Phosphate Fluoride gel after an interval of 72 hours, there was no statistically significant difference was observed as indicated by a 'p' value of 0.0746 ($p<0.05$). These results indicate that both the groups showed remineralization potential but among both the groups Herbal Dental gel (Group II) showed superior remineralization potential over Acidulated Phosphate Fluoride gel (Group I).

DISCUSSION

"Nature is the source of all true knowledge. She has her own logic, her own laws, she has no effect without cause nor invention without necessity."

- Leonardo da Vinci

Dental caries being the most prevalent disease in children has become an international public health challenge. It has numerous consequences, ranging from morphological to functional ones, affecting the growth and well-being of millions of young children. It is the primary cause of oral pain and tooth loss affecting the masticatory function of the child. It deleteriously effects the child's nutrition as various studies have reported that children with more decayed teeth have less than 80% of average weight, which they are expected to have for their age.[56] Moreover, loss of tooth affects the child physiologically, emotionally and socially.

However, to intercept dental caries it is essential to comprehend its etiology. With years of in-depth research, the newest concept explaining the cause of dental caries as a consequence of disruption of "Caries Balance" was put forward by Dr. John Featherstone in 2002.[57] This theory states that the destruction of the enamel surface results due to the ecological imbalance in the physiological equilibrium between demineralization and remineralization.

Endogenous bacteria, predominantly *Streptococcus mutans* and *Lactobacillus acidophilus* produce weak organic acids as a by-product of metabolism of fermentable carbohydrates. As a result, local pH values fall below a critical value resulting in demineralization of dental hard tissues. This diffusion of minerals such as calcium,

phosphate, and carbonate out of the tooth structure, over a long period of time leads to cavitation. Remineralisation occurs when the pH of the biofilm is restored by saliva, which acts as a buffer. It occurs through uptake of calcium, phosphate, and fluoride. With time, this process either leads to cavitation of the tooth structure or repair and reversal of the lesion, or maintenance of the status quo [Figure No. 69].

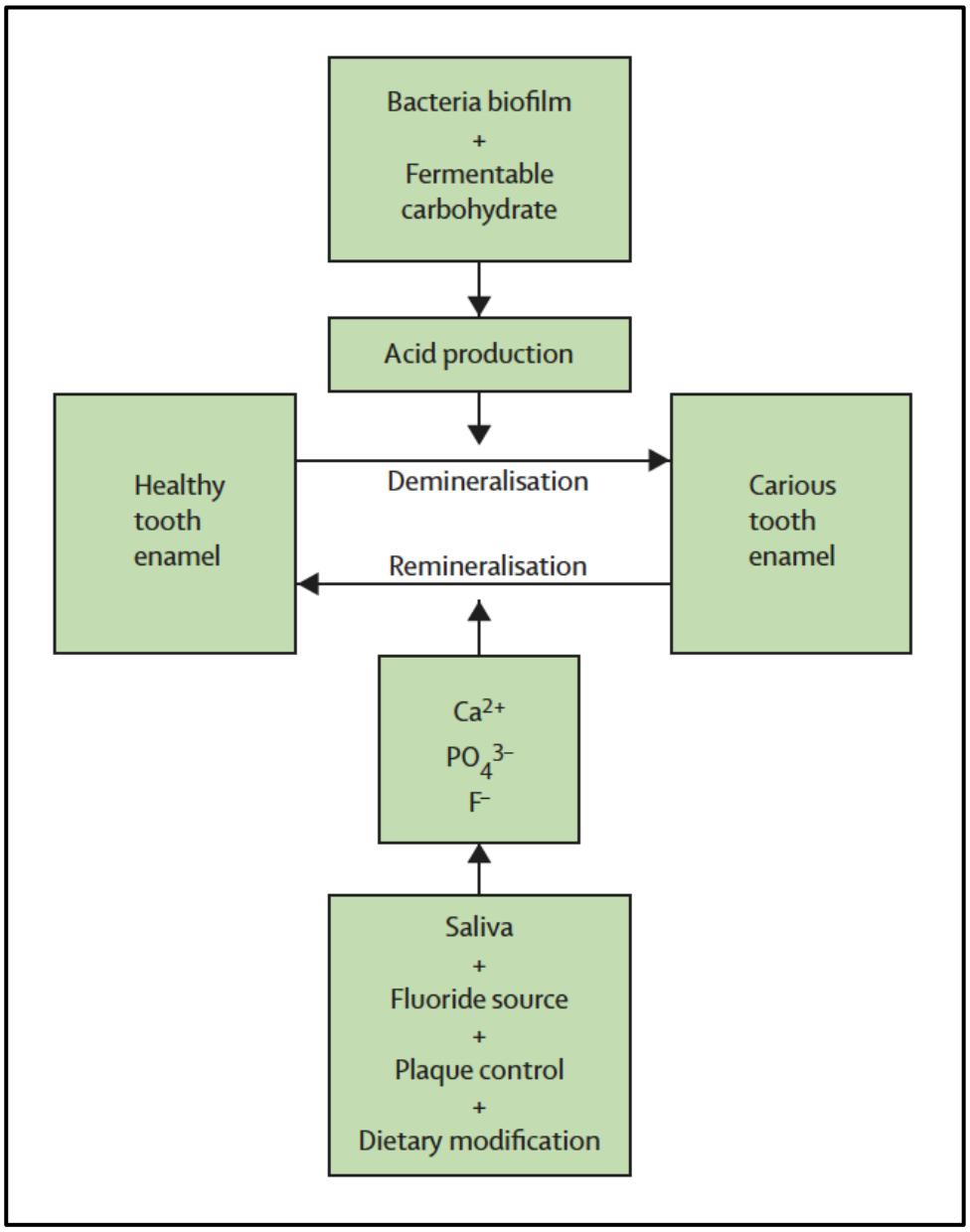

Figure No. 69: Figure showing an illustration of the caries process as regular flux of demineralisation and remineralisation.

The process that will dominate is dependent on the proportions of the factors that constitute the "Caries Balance", i.e. protective and pathological factors [Figure No. 70]. Pathological factors include acid-producing bacteria, frequent consumption of fermentable carbohydrates, sub-normal salivary flow and function. Domination of these factors increase the risk of dental caries. On the other hand, protective factors include optimum salivary flow and its components, consumption of non-cariogenic sweeteners, fluoride, calcium, phosphate and antibacterials. This indicates that the disease is initially reversible and can be halted by enhancing the resistance.

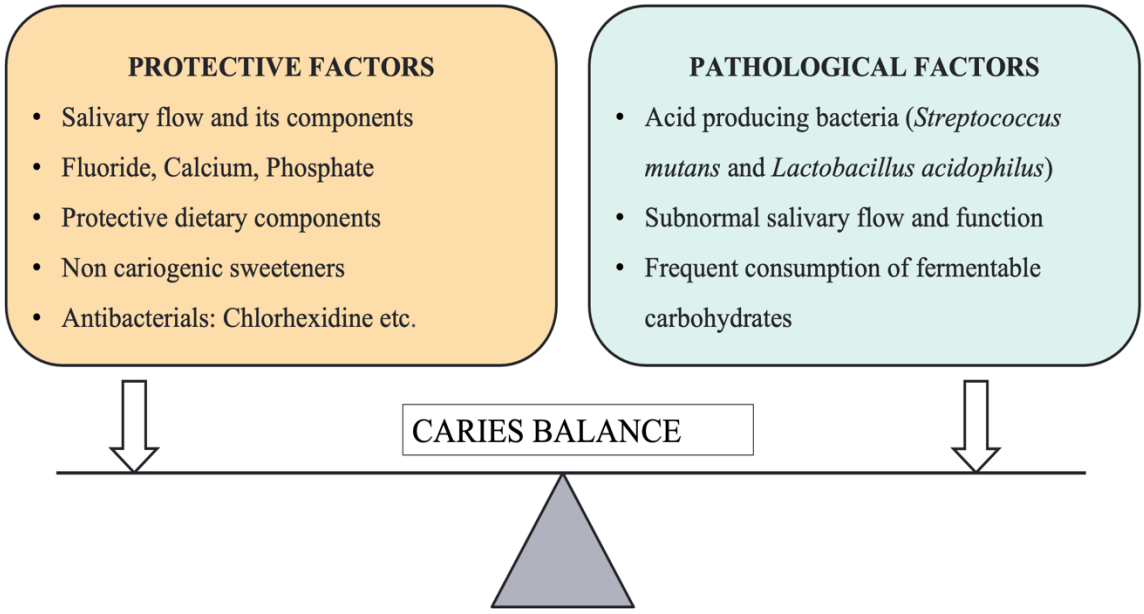

Figure No. 70: Figure showing the protective and pathological factors involved in "Caries Balance".

Enhancement of resistance can be achieved through use of fluorides, sugar substitutes, antimicrobials and mechanical barriers such as pit-and-fissure sealants. With the advancements in the field of preventive dentistry, newer materials are being continuously developed. Materials like casein phosphopeptides, amorphous calcium phosphate, sodium calcium phosphosilicate (bioactive glass) and dicalcium phosphate dehydrate are being explored for their anticariogenic potential.

Recent advances have also led to the development of nanoparticles for remineralization. These include calcium fluoride nanoparticles, calcium phosphate-based nanomaterials, nano hydroxyapatite particles, amorphous calcium phosphate nanoparticles and nano bioactive glass materials. These agents are being researched upon and many of them are being used clinically, with significantly predictable positive results. [17]

Prevention of caries with the aid of administration of fluoride in various forms has been the gold standard and is being extensively used since the past decades. It can either be delivered systematically or topically. Systemic modes of fluoridation are those that are ingested and become incorporated into forming tooth structures. These modalities include water fluoridation, milk fluoridation or salt fluoridation. It can also be administered in the forms of dietary supplements such as tablets, lozenges or liquid suspensions. Topical modes of fluoride administration include fluoride rinses, fluoridated toothpastes, topical fluoride gels, solutions and fluoride varnishes. Hence, fluoride has both systemic and topical modes of action, but the topical effects are generally considered to be the dominant factor in prevention of caries.

However, even after the widespread use of fluoride at both community level and individual level, and despite of dental caries being a completely preventable disease, it still remains the most widespread disease worldwide. Various studies have also reported that fluoride has a potential to cause major adverse health problems, while having only a meek dental caries prevention effect.[20] This fact gives us a clear understanding of the ineffectiveness of fluoride in eradication of dental caries. Hence, the need of the hour is to bring a change in the ideological approach of using fluoride for prevention of dental caries in the global public health community and high priority should be given to efforts laid on developing safer alternatives to address the disease.

Herbal medicine, today, symbolizes safety. The concept of "natural is better than synthetic" has led to the evolution of "neoherbalism". With herbal renaissance happening all over the world, they are now widely being used as an alternative to treat a plethora of health conditions. Herbal medicine are currently being primarily used as a form of complementary and alternative medicine (CAM) in both developed and developing countries and is considered to be an effective and comparatively affordable form of health care.

This shift in the interest towards the plant derived formulations has led to the screening of many herbal plants for their antimicrobial activity which can potentially be clinically used as an alternative to synthetic formulations used conventionally. India, being a major hub in traditional medicine and having a pluralistic healthcare system, offers an unfettered platform for the quest for exploration of various health benefits of traditional medicinal plants.

The field of dentistry has also begun to explore the properties of medicinal plants. In recent years, in the search for a novel agent with a good anti-cariogenic effect,

there has been a tremendous increase in studies using natural plant extracts. Herbal extracts of *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Azadirachta indica*, *Salvadora persica*, *Camellia sinensis*, *Mentha piperita*, *Eucalyptus globulus*, *Nigella sativa* and *Zingiber officinale* have shown remarkable antibacterial effect against caries causing microorganisms.[10,26]

However, on analysing the existing literature, there were no studies which have explored the synergistic effect of these extracts not just as an antibacterial agent, but also as a potential remineralizing agent. Hence, this study was planned to evaluate the antibacterial efficacy and remineralization potential of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* by combining them as a Herbal Dental gel and comparing it with commercially available Acidulated Phosphate Fluoride gel.

The initial step is extraction of the herb using a solvent in which its compounds are dissolvable. It involves the separation of medicinally active compounds of the herb from the inert components by using selective solvents in standard extraction procedures. Ethanol was used for the extraction procedure as it is an excellent solvent and it also contributes to the stability of the formulations. It is also considered as one of the safest solvents for extraction of herbal compounds.[58] It is also known to enhance the antimicrobial action of the prepared extracts. The ethanolic extracts were prepared for *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* by the process of maceration. The reason for carrying out extraction using maceration was its simplicity as it does not requires any complicated armamentarium and is easy to perform.

Following the preparation of extracts, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of these extracts were determined against

Streptococcus mutans and *Lactobacillus acidophilus* to evaluate their individual potency against these microorganisms. In our study, Microbial Type Tissue Culture strains of *Streptococcus mutans* and *Lactobacillus acidophilus* were used based on the observation made by various authors which cite these microorganisms as the basic etiological agent of dental caries.[59] A systematic review also revealed that the strongest risk factor for occurrence of dental caries is the presence of *Streptococcus mutans* and *Lactobacillus acidophilus* in the oral cavity.[16] Hence, the antibacterial efficacy of the formulation was carried out against these organisms.

Resazurin method was used to determine the Minimum Inhibitory Concentration as it is known to have a high level of accuracy and reproducibility, while being a simple and reliable method. The colour change is also easily appreciable to the naked eyes making this method highly feasible for MIC determination.[53] For the determination of Minimum Bactericidal Concentration, agar plate streaking method was used. This is the most widely used method because of its simplicity, low cost, and the ease of interpretation of results.[55] These tests were carried out in quintuplicates to ensure standardisation of the results.

In our study*, Zingiber officinale* showed a Minimum Inhibitory Concentration of 11.25 $\pm$ 2.80 µl/ml and 10.94 $\pm$ 9.38 µl/ml against S*treptococcus mutans* and *Lactobacillus acidophilus* respectively. Its Minimum Bactericidal Concentration was 27.50 $\pm$ 13.69 µl/ml and 22.50 $\pm$ 5.59 µl/ml against S*treptococcus mutans* and *Lactobacillus acidophilus* respectively. The results of our study showed slightly lower values as studies done by Da Silva et al and Babaeekhou et al depicted MIC in the range of 25-80 µg/ml and MBC in the range of 20-50 µg/ml against S*treptococcus mutans*.[27,29] However, we found no studies to compare the MIC and MBC values of

Zingiber officinale against *Lactobacillus acidophilus* as we found no such studies in our literature search.

Salvadora persica had a Minimum Inhibitory Concentration of 1.56 ± 0.96 µl/ml and 2.50 ± 0.86 µl/ml against S*treptococcus mutans* and *Lactobacillus acidophilus* respectively. Its Minimum Bactericidal Concentration was 13.75 ± 6.85 µl/ml and 20.00 ± 6.85 µl/ml against S*treptococcus mutans* and *Lactobacillus acidophilus* respectively. We found our values to be lower than study conducted by Balto et al wherein they evaluated the antibacterial activity of *Salvadora persica* and obtained values in the range of 4 mg/ml - 8 mg/ml.[37]

On determination of antibacterial activity of *Cinnamomum zeylanicum*, Minimum Inhibitory Concentration of 11.25 ± 2.80 µg/ml and 20.00 ± 6.85 µg/ml was obtained against S*treptococcus mutans* and *Lactobacillus acidophilus* respectively. The Minimum Bactericidal Concentration was 27.50 ± 13.69 µg/ml against S*treptococcus mutans* and 35.00 ± 13.69 µg/ml against *Lactobacillus acidophilus*. The results of our study have shown a correlation with the existing literature wherein *Cinnamomum zeylanicum* extracts showed MIC of 13.44 µg/ml and MBC of 23.60 µg/ml against S*treptococcus mutans.* However, the MIC and MBC values obtained against *Lactobacillus acidophilus* were slightly higher. In a study carried out by Elgamily et al, the MIC of *Cinnamomum zeylanicum* extract was found to be 5.18 µg/ml and MBC was found to be 16.40 µg/ml against *Lactobacillus acidophilus*.[44]

All three extracts, namely *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* showed good antibacterial effectiveness against the tested microorganisms, i.e., S*treptococcus mutans* and *Lactobacillus acidophilus*. The values obtained as Minimum Inhibitory Concentration and Minimum Bactericidal

Concentration were then used to determine their concentration during herbal gel preparation.

Based on the values obtained, the formulated gel consisted of 2% w/w of each extract. These extracts were mixed together using glycerin as a vehicle which also acted as a humectant. Carbopol 940, which is an acrylic polymer was used as a gelling agent which yielded a high viscosity to the gel. It was the agent of choice as it is non-toxic and non-irritable and forms a bio-adhesive gel.[60] Furthermore, xylitol was added as a natural sweetener. Being a herbal formulation, antimicrobial preservatives like sodium benzoate, methyl paraben and propyl paraben were added in trace amounts to prevent its degradation. Distilled water was used as a solvent to adjust the final weight of the gel to 100 gm and pH was adjusted to 7.5 using Triethanolamine.

A vital step in preparation of novel formulations is assessing its biocompatibility. Various studies have been conducted in the past which have evaluated the cytotoxicity of extracts of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* against human fibroblast cells individually, but there were no studies which tested their cytotoxicity in the form of a formulation.[27,37,47]

In the present study, we assessed the effect of formulated herbal dental gel on fibroblast cells by carrying out the cytotoxicity assay using 3-(4, 5dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) which is considered as a gold standard. It is easy and rapid to perform, and its advantages include reproducibility of the results and observed clinical correlation between in vitro and in vivo testing. Due to these reasons, it is the most widely used assay for assessing cytotoxicity.[61]

In the present study, cytotoxicity of Acidulated Phosphate Fluoride gel and Herbal Dental gel was assessed against fibroblast cells. The gels were tested at 100% concentration and it was found that the fibroblasts showed 100% viability in presence

of both the study groups indicating their non-toxic effect. As safety is a fundamental principle in the formulation of novel preparations, the Herbal Dental gel was found to be safe and biocompatible making it an alternative to synthetic preparations.

A vital prerequisite for a potent anticariogenic agent is its antibacterial effect. With an aim to evaluate the efficacy of the prepared formulation against caries causing microorganisms i.e., *Streptococcus mutans* and *Lactobacillus acidophilus*, the antibacterial susceptibility for Herbal Dental gel was assessed and compared with the conventionally used Acidulated Phosphate Fluoride gel using Agar Well Diffusion Method and Time Kill Assay.

Agar Well Diffusion method was employed in the study as it is easy to perform, does not require extensive armamentarium and the results are easily interpreted.[55] The test agents diffuse into the agar and inhibit the growth of the microorganisms and then the diameters of inhibitory growth zones are measured using standard parameters.

While testing the antibacterial susceptibility against *Streptococcus mutans*, the mean zone of inhibition obtained by Acidulated Phosphate Fluoride gel was 11.50 mm and Herbal Dental gel was 20.00 mm. In case of *Lactobacillus acidophilus,* the mean zone of inhibition achieved by Acidulated Phosphate Fluoride gel was 16.50 mm and Herbal Dental gel was 21.00 mm. These results indicated the superior antibacterial efficacy of Herbal Dental gel over Acidulate Phosphate Fluoride gel against both the tested organisms i.e., *Streptococcus mutans* and *Lactobacillus acidophilus*.

However, the agar well diffusion method only assessed the inhibition of bacterial growth, but not the bacterial death. Hence, this method did not distinguish between the bactericidal and bacteriostatic effects of the study groups. In order to determine these effects, Time Kill Assay was performed which revealed a time-dependent bactericidal and bacteriostatic activity.

While carrying out the Time Kill Assay against *Streptococcus mutans*, it was observed that Acidulated Phosphate Fluoride gel showed immediate reduction in bacterial growth and Herbal Dental gel presented a lag phase initially where bacteria were metabolically active but not dividing. This represented the bacteriostatic action of both the study groups. After an interval of 7 hours, both Acidulated Phosphate Fluoride gel and Herbal Dental gel showed equal efficacy in reduction of *Streptococcus mutans* growth which was represented by the death phase characterized by an exponential decrease in the number of living cells. This represented the bactericidal effect of the study groups. Hence, it can be concluded that both the study groups started showing bactericidal effect on *Streptococcus mutans* after a time period of 7 hours.

When the Time Kill Assay was performed against *Lactobacillus acidophilus,* both Acidulated Phosphate Fluoride gel and Herbal Dental gel showed similar results. There was an immediate reduction in the viable bacterial count in both the groups representing their bacteriostatic action on the microorganisms. After an interval of 6 hours, an exponential decrease in the number of viable cells occurred signifying the death of the bacteria. This represented the bactericidal action of both the study groups on *Lactobacillus acidophilus.*

These findings obtained in Time Kill Assay suggest that both Acidulated Phosphate Fluoride gel and Herbal Dental gel have a similar time dependent bactericidal action on *Streptococcus mutans* and *Lactobacillus acidophilus*. However, as indicated by the results obtained in agar well diffusion method, it can be implied that Herbal Dental gel has a superior antibacterial effect as compared to Acidulated Phosphate Fluoride gel pertaining to the greater zones of inhibition obtained against both the test organisms, i.e., *Streptococcus mutans* and *Lactobacillus acidophilus.*

The results obtained in both the antibacterial susceptibility tests signify the superior antibacterial effect of Herbal Dental gel over Acidulated Phosphate Fluoride gel. This can be attributed to the constituents present in the Herbal Dental gel which show a synergistic action in inhibiting the growth of the microorganisms. This can be deduced from the fact that the individual efficacy of these extracts was found to be less as determined in the existing literature.

Zingiber officinale and *Salvadora persica* have depicted an inhibitory zone of 8 mm against *Streptococcus mutans* in studies conducted by Giriraju et al and Balteo et al.[37,62] In a study conducted by Elgamily et al, *Cinnamomum zeylanicum* showed an inhibitory zone of 14 mm against *Streptococcus mutans.*[44] However, as per results obtained in our study, a combination of these extracts in the form of Herbal Dental gel has shown an inhibitory zone of 20 mm against *Streptococcus mutans*.

In a similar context, *Zingiber officinale* has shown an inhibitory zone ranging from 10 mm to 18 mm against *Lactobacillus acidophilus*.[28] In a study conducted by Siddeeqh et al, *Salvadora persica* extract showed an inhibitory zone of 10.67 mm against *Lactobacillus acidophilus*.[38] *Cinnamomum zeylanicum* has depicted an inhibitory zone of 16 mm against *Lactobacillus acidophilus* as per the results obtained in a study conducted by Elgamily et al.[44] However, the Herbal Dental gel showed an inhibitory zone of 21 mm against *Lactobacillus acidophilus* which is superior to the individual antibacterial efficacy of these extracts.

These antibacterial properties can be attributed to the specific phytoconstituents present in the formulation. Phytonutrients are the active components of the herbal extracts which include phenols, quinones, flavonoids, terpenoids etc. All three extracts, namely, *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* used in

the preparation of Herbal Dental gel are considered to be a rich source of these phytonutrients. The antibacterial action obtained by combining these extracts in the form of Herbal Dental gel is due to the synergistic effect of these phytonutrients.

Phenolic compounds cause inhibition of enzymes due to their interaction with sulfhydryl groups present in microbes. Quinones act by depriving the microorganisms of substrates. They also have an inhibitory effect on adhesins, cell wall polypeptides and membrane bound enzymes present in these microorganisms. Flavonoids exhibit antimicrobial activity by forming a complex with bacterial proteins and cell walls. Terpenoids cause disruption of cell membrane causing a disturbance in the membrane potential. Lectins and polypeptides form ion channels in the microbial membrane resulting in its altered permeability. They also inhibit the adhesion of microbial proteins to host polysaccharide receptors.[38,63] Thus, these bioactive compounds have several targets that lead to inhibition of microbial growth and a rich combination of these phytonutrients is responsible for the synergistic action of the Herbal Dental gel.

These extracts also have a specific mechanism of action by which they target the microorganisms. The antibacterial efficacy of *Zingiber officinale* can be attributed to the presence of gingerol and shagelol whose target is the bacterial cell wall. These phytoconstituents alter the permeability and fluidity of bacterial cell membrane. *Zingiber officinale* is also known to have strong anti-adherence activity by inhibiting the sucrose dependent as well as sucrose independent adherence and as adherence is one of the most vital steps in progression of caries, its inhibition impairs the virulence of the bacterial cells. The sucrose-independent adherence is inhibited due to reduction in hydrophobic interactions between the cells and the surface. Sucrose dependent adherence is affected because of reduction in glucan production as flavonoids present

in the extract possess anti- GTFase activity. The reduction in glucan synthesis also results in interference in biofilm formation, thereby disrupting its physical integrity and stability.[30]

Zingiber officinale also has an inhibitory effect on glucosyltransferases activity. It reduces the production of glycolytic acid by the bacteria and inhibits the F-ATPase activity. The suppression of F-ATPase activity gives rise to cytoplasmic acidity resulting in disruption of aciduracity potential leading to decreased acid adaptation of bacteria. Cytoplasmic acidity also leads to the impairment of functioning of enzymes involved in vital physiological processes like glycolysis, production of intracellular and extracellular polysaccharides and cell persistence.[30]

Additionally, it downregulates the functioning of certain virulent genes. It suppresses Gene relA which is involved in the oxidative stress and acid tolerance mechanisms of bacteria, Gene brpA which plays a critical role in maintenance of structural integrity of biofilm. In also suppresses Gene gtfC, affecting the cascades involved in biofilm formation, cell wall integrity, adhesion promotion and surface biogenesis. It also downregulates Gene comDE, which attenuates the internal communication system of the bacteria leading to cell death.[30] These properties give rise to the anticariogenic potential of *Zingiber officinale.*

Salvadora persica also possesses various mechanisms by which it shows antimicrobial activity. It inhibits the cell wall synthesis of the bacteria and has damaging effects on cell membrane resulting in alteration of permeability of the cell membrane. This is mainly due to the presence of benzylisothiocynate which penetrates through the membrane and interferes with the redox systems hampering the ability of the bacteria to maintain its membrane potential. It also hampers the attachment of the

bacteria to the surface.[41] Presence of thiocyanate also acts as a substrate for lactoperoxidase which generates hypothiocyanite in the presence of hydrogen peroxide which further reacts with sulfhydryl groups present in bacterial enzymes leading to cell death.[64] Additionally, it inhibits nucleic acid synthesis and protein synthesis which causes interference in vital cellular functions such as electron transportation, uptake of nutrients and altered enzymatic activity.[38] It can also result in inhibition of oxygen uptake, leading to oxidative stress in bacteria causing immediate toxicity and death. Presence of high mineral content, dissolved inorganic compounds, chlorides, fluorides, sulphur and heavy metals also contributes to its antibacterial effect.[39]

The antibacterial activity of *Cinnamomum zeylanicum* can be attributed to the presence of cinnamaldehyde which is highly electronegative and interferes in biological processes involving electron transfer. It also reacts with nitrogen containing components such as proteins and nucleic acids which results in inhibition of growth of the microorganisms.[45] It also inhibits amino acid decarboxylase activity and acid production ability of the bacteria. It also acts by disrupting the structure of cell membrane causing cell leakage and cell death.[65]

Cinnamomum zeylanicum also possesses antibiofilm activity. This is due to the presence of glucosyltransferase adhesion-inhibition effect. Additionally, it has the ability to interfere with bacterial quorum sensing which leads to inactivation of bacterial cells inducing biofilm dispersion.[66]

A combined effect of these targeted mechanisms against the microorganisms has resulted in the superior antibacterial activity of Herbal Dental gel as compared to Acidulated Phosphate Fluoride gel.

Acidulate Phosphate Fluoride gel also depicted an antibacterial effect against the test organisms. This can be attributed to its tendency to diffuse into the bacterial cell wall as a neutral hydrofluoric acid molecule where it dissociates in the cytoplasm releasing fluoride ions. These ions inhibit key enzymes, ceasing the glycolysis causing acidification of cytoplasm by not allowing the removal of H^+ ions. Overall, it creates a less acidic environment that reduces the driving force for dissolution of mineral content. Additionally, it reacts with the enzyme enolase which directly reduces the production of bacterial acids. It also possesses an indirect effect on the phosphotransferase system pathway that causes reduction in the amount of sugar entering the cell by limiting phosphoenolpyruvate. This leads to less acidogenic conditions altering the long term ecology of the plaque.[67] However, its antibacterial efficacy was found to be less as compared to Herbal Dental gel.

With the clear understanding regarding the demineralization–remineralization theory of caries initiation, it is also vital to assess the remineralization potential of the study groups. The assessment of remineralization was carried out using stereomicroscope wherein the values were tabulated at an interval of 24, 48 and 72 hours.

After an interval of 24 hours, mean remineralization was 4.57 units in Acidulated Phosphate Fluoride gel and 6.07 in Herbal Dental gel group. At an interval of 48 hours, mean remineralization in Acidulated Phosphate Fluoride gel group was 6.83 units as compared to 8.23 units in Herbal Dental gel group. Mean remineralization after 48 hours was 9.63 units in Acidulated Phosphate Fluoride gel group and 11.00 units in Herbal Dental gel group. These results indicate the superior remineralization of Herbal Dental gel over Acidulated Phosphate Fluoride gel at all time intervals.

The reason that can be cited for the same is presence of tannins in *Zingiber officinale* which forms a layer over enamel due to their astringent effect, thus providing protection against demineralization. It also has high fluoride content which can be attributed for the remineralization effect.[68]

Salvadora persica is abundant in polysaccharides, which absorbs directly onto the enamel and form protective layers over the surface. *Salvadora persica* is also abundant in chlorides and fluorides which are important elements to remineralize the enamel structure.[42] It also produces increase in calcium concentration in saliva which induces remineralization.[69]

Remineralization capability of *Cinnamomum zeylanicum* is due to its rich calcium and phosphorus content, both of which are key components of the hydroxyapatite crystals in enamel. These ions get incorporated on the surface and decrease its porosity, which eventually results in an increase in enamel microhardness against demineralizing action of the acids produced by bacteria.[49]

Acidulated Phosphate Fluoride gel also showed comparable remineralization potential. It is known to halt the demineralization process by adsorbing negative fluoride ions on tooth surface and thereby acting as a physical barrier against acids. It also acts as a catalyst for the diffusion of calcium and phosphate into the tooth, which remineralises the crystalline structures in the lesion. The newly formed crystals, called fluorapatite incorporate the more electro-negative fluoride ion at the hydroxyl position resulting in increased hydrogen bonding, a more dense crystal lattice, and an overall decrease in solubility.[7,8]

However, with decades of extensive research and gathered information, the available evidence suggests that fluoride actually possesses unacceptable health risk with virtually no proven benefits. Fluoride has been extensively linked with dental and skeletal fluorosis. Excessive intake of fluoride during the child's early developmental years can also hamper their neurological development as various studies have demonstrated the neurotoxic effect of fluoride as it can generate free radicals creating alterations in the level of neuro-transmitters in the brain.[9] Additionally, studies have reported its association with impaired cognitive development, hypothyroidism, neurological disorders, hormonal derangements, oxidative stress, premature aging and even cancer.[21-24] Because of its detrimental effects, some authors have even cited it as a protoplasmic poison.[25] This calls for the use of safer alternatives with good anticariogenic effect.

As per the results obtained in our study, it can be implied that the Herbal Dental gel prepared by combination of *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* has shown good synergistic action. The formulation showed good antibacterial efficacy against caries causing microorganisms like *Streptococcus mutans* and *Lactobacillus acidophilus* and also depicted good remineralization potential of the enamel surface as compared to Acidulated Phosphate Fluoride gel. Therefore, the alternative hypothesis formulated at the beginning of the study holds true, highlighting the efficacy of this formulation as a potent anticariogenic agent.

Because of the promising results achieved in this study, we advocate the use of Herbal Dental gel containing *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* for prevention of caries in children. In addition to its efficacy as an anticariogenic agent, the Herbal Dental gel has also proven to be a safe formulation.

Hence, it is an effective and safe alternative to commercially available fluoride preparations.

However, as the results obtained in our study were in-vitro analysis, we look forward to use this formulation in clinical scenarios by conducting clinical trials with substantial sample size. We would also like to extend the use of this formulation in the form of other delivery systems such as mouth rinses, dentifrices or lozenges. We look forward to patent this formulation with an aim of making it commercially available so that it can be a boon for prevention of caries which is the most common disease worldwide, and its benefits can be reaped by majority of the population.[2]

Owing to the adverse effects related to synthetic formulations, it is a pressing priority to come up with safer alternatives with promising results, so that this global burden of dental caries can be lessened. There is a need to come up with more alternatives in the form of these herbal preparations to combat this disease. Thus, more meticulous research is needed to be carried out in this direction as there are still abundance of benefits of these herbs which are yet to be explored. This study is an effort to open the gateway to the mother nature and reap its benefits to the fullest, as mother Earths medicine chest is full of healing herbs of incomparable worth and we believe in the fact stated by Hippocrates that-

"*Nature itself is the best physician.*"

CONCLUSION

Despite of the advancements in the field of pediatric dentistry, dental caries still dominates the spectrum of oral diseases. With the recent paradigm shift towards the safer and efficient alternatives in the form of herbal medicine, the present study opens the door for further exploration of these alternatives.

The following conclusions are drawn from the present study:

1. The herbal extracts namely *Zingiber officinale, Salvadora persica* and *Cinnamomum zeylanicum* have shown Minimum Inhibitory Concentration and Minimum Bactericidal Concentration results against *Streptococcus mutans* and *Lactobacillus acidophilus. Zingiber officinale* showed MIC of 11.25 + 2.80 µl/ml and MBC of 27.50 ± 13.69 µl/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus,* its MIC was 10.94 + 9.38 µl/ml and MBC was 22.50 ± 5.59 µl/ml. *Salvadora persica* had a MIC of 1.56 + 0.96 µl/ml and MBC of 13.75 ± 6.85 µl/ml against *Streptococcus mutans*. It showed MIC of 2.50 + 0.86 µl/ml and MBC of 20.00 ± 6.85 µl/ml against *Lactobacillus acidophilus. Cinnamomum zeylanicum* had MIC of 11.25 + 2.80 µg/ml and MBC of 27.50 ± 13.69 µg/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus*, its MIC was 20.00 + 6.85 µg/ml and MBC was 35.00 ± 13.69 µg/ml.

2. During cytotoxicity evaluation, fibroblasts showed 100% viability in presence of Acidulated Phosphate Fluoride gel and Herbal Dental Gel at 100 percent concentration. This signifies the non-toxic nature of the study groups.

3. Acidulated Phosphate Fluoride gel showed a zone of inhibition of 11.50 mm and 16.50 mm against *Streptococcus mutans* and *Lactobacillus acidophilus* respectively. Herbal Dental gel showed a zone of inhibition of 20 mm and 21

mm against *Streptococcus mutans* and *Lactobacillus acidophilus* respectively. On evaluation using Time Kill Assay, Acidulated Phosphate Fluoride gel and Herbal Dental gel and showed comparable antibacterial efficacy at various time intervals.

4. Herbal Dental gel showed better antibacterial efficacy as compared to Acidulate Phosphate Fluoride gel against both organisms i.e. *Streptococcus mutans* and *Lactobacillus acidophilus* by exhibiting the greater zone of inhibition in Agar Well Diffusion test.
5. Acidulated Phosphate Fluoride gel showed 4.57±1.59, 6.83±2.17 and 9.63±3.17 units of remineralization at the end of 24, 48 and 72 hours respectively. Herbal Dental gel showed 6.07±2.41, 8.23±2.43 and 11.00±2.64 units of remineralization at the end of 24, 48 and 72 hours respectively.
6. Herbal Dental gel showed better remineralization potential than Acidulated Phosphate Fluoride gel at 24 hours and 48 hours. However, there was no statistically significant difference in the remineralization potential between both the study groups after 72 hours.

However, we advocate that in future an attempt should be made to corelate the results drawn from the present in vitro study in clinical scenarios with sizeable sample size.

SUMMARY

The present study was conducted with the aim to evaluate and compare the antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel Containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum*.

After the collection of raw herbs, they were powdered and ethanolic extract was prepared by maceration. The MIC and MBC of ethanolic extracts of *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* was determined against *Streptococcus mutans* and *Lactobacillus acidophilus* using Resazurin method and Agar Plate Streaking method respectively. These values were used for the preparation of Herbal Dental gel. The cytotoxicity of both the study groups i.e. Acidulated Phosphate Fluoride gel and Herbal Dental gel 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. This was followed by determination of the remineralization potential of both the study groups.

The sensitivity of all the extracts were found to be similar against both the test organisms, i.e., *Streptococcus mutans* and *Lactobacillus acidophilus*. *Zingiber officinale* showed MIC of 11.25 + 2.80 µl/ml and MBC of 27.50 $\pm$ 13.69 µl/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus,* its MIC was 10.94 + 9.38 µl/ml and MBC was 22.50 $\pm$ 5.59 µl/ml. Salvadora persica had a MIC of 1.56 + 0.96 µl/ml and MBC of 13.75 $\pm$ 6.85 µl/ml against *Streptococcus mutans*. It showed MIC of 2.50 + 0.86 µl/ml and MBC of 20.00 $\pm$ 6.85 µl/ml against *Lactobacillus acidophilus. Cinnamomum zeylanicum* had MIC of 11.25 + 2.80 µg/ml and MBC of 27.50 $\pm$ 13.69 µg/ml against *Streptococcus mutans.* Against *Lactobacillus acidophilus*, its MIC was 20.00 + 6.85 µg/ml and MBC was 35.00 $\pm$ 13.69 µg/ml.

The cytotoxicity evaluation using MTT assay showed that fibroblasts depicted 100% viability in presence of Acidulated Phosphate Fluoride gel and Herbal Dental Gel at 100 percent concentration signifying the non-toxic nature of the study groups.

On comparison of antibacterial effectiveness using Agar Well Diffusion test, Herbal Dental gel showed greater zone of inhibition against both test organisms, i.e., *Streptococcus mutans* and *Lactobacillus acidophilus*. Both the study groups i.e. Acidulated Phosphate Fluoride gel and Herbal Dental Gel gave comparable results during Time Kill Assay by showing similar time dependent bactericidal effect.

On comparison of remineralization potential, Herbal Dental gel showed better remineralization potential than Acidulated Phosphate Fluoride gel at all time intervals, but the difference was statistically non-significant.

These results were obtained due to the synergistic effect of the phytoconstituents present in *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum.* Herbal Dental gel depicted good anticariogenic potential by showing good antibacterial efficacy and remineralization potential which 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
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1429

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of antibacterial efficacy and Remineralization potential of acidulated phosphate fluoride gel with Herbal Dental gel containing Zingiber officinale, Salvadora Persica and Cinnamon – An in vitro study

Submitted by

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

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

Date : 23/3/24

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

Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belgaum

ANNEXURE II a

AUTHENTICATION CERTIFICATE OF *ZINGIBER OFFICINALE* FROM KAHER'S SHRI. BMK AYURVEDA MAHAVIDYALAYA, BELAGAVI

SINCE 1938

K.L.E. SOCIETY'S
K.L.E AYURVED PHARMACY
(GMP CERTIFIED UNIT)
UPPAR GALLI KHASABAG BELGAVI 590004 KARNATAKA
Teaching pharmacy of KAHER'S
Shri B.M.Kankanwadi Ayurveda mahavidyalaya, Belgavi
PHONE NO. 0831-2488865 EMAIL: klepharma@yahoo.com FAX NO. 0831-2424157

KLE AYURVEDA

Ref No : KLE/AP/DR-35/2021-22 **Date :** 27/08/2021

Certificate

Reference No.

Guide:

Name of the PG Scholar: **Mobile No.**

Department: Department of Pediatric & Preventive dentistry, KLE VK Institute of Dental Sciences.

Name of procured Drug: Ginger powder (*Zingiber officinale* Roxb.).

Total quantity of Prepared drug with details of package:

S.No	Name of Product	Qty
1.	Ginger powder	100gms

Method of Storage: Normal Room temperature.
Store in a cool and dry place.
Avoid direct sunlight.

Batch No : I

Time and Date: 27/08/2021 at 5:00 PM

Note: After receiving of Research drug, Pharmacy will not be responsible for any spoilage and remained drugs.

Ayurved Pharmacy * K.L.E Society's * Khasbag Belagavi

Production Supervisor

ANNEXURE II b

AUTHENTICATION CERTIFICATE OF *SALVADORA PERSICA* FROM KAHER'S SHRI. BMK AYURVEDA MAHAVIDYALAYA, BELAGAVI

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit KLE Academy of Higher Education & Research
Deemed–to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By:

Submitted Date: 27/08/2021

Date of Issue: 09/09/2021

S N	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Name	Scientific Name	Family	Part Authenticated
1	Miswak (Pilu)	*Salvadora persica* L.	Asteraceae	Root	CRF/Auth/ 61/2021	Miswak (Pilu)	*Salvadora persica* L.	Asteraceae	Root

Signature:
Authentication Expert Name:
Date: 09/09/2021

CRF SHAHAPUR, BELGAUM

Signature of Coordinator
ASU Drug Testing Laboratory

ANNEXURE II c

AUTHENTICATION CERTIFICATE OF *CINNAMOMUM ZEYLANICUM* FROM KAHER'S SHRI. BMK AYURVEDA MAHAVIDYALAYA, BELAGAVI

SINCE 1938

K.L.E. SOCIETY'S
K.L.E AYURVED PHARMACY
(GMP CERTIFIED UNIT)
UPPAR GALLI KHASABAG BELGAVI 590004 KARNATAKA
Teaching pharmacy of KAHER'S
Shri B.M.Kankanwadi Ayurveda mahavidyalaya, Belgavi
PHONE NO. 0831-2488865 EMAIL: klepharma@yahoo.com FAX NO. 0831-2424157

KLE AYURVEDA

Ref No : KLE/AP/DR-36/2021-22 **Date :** 27/08/2021

Certificate

Reference No.

Guide:

Name of the PG Scholar: **Mobile No.**

Department: Department of Pediatric & Preventive dentistry, KLE VK Institute of Dental Sciences.

Name of procured Drug: Cinnamon powder (*Cinnamomum zeylanicum* Blume.).

Total quantity of Prepared drug with details of package:

S.No	Name of Product	Qty
1.	Cinnamon powder	100gms

Method of Storage: Normal room temperature.
Store in a cool and dry place.
Avoid direct sunlight.

Batch No : I

Time and Date: 27/08/2021 at 5:00 PM

Note: After receiving of Research drug, Pharmacy will not be responsible for any spoilage and remained drugs.

K.L.E. Society's Ayurved Pharmacy Khasbag Belagavi

Production Supervisor

ANNEXURE III:

BIOSTATISTICS CLEARANCE 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

Professor and Head, Department of Pediatric and Preventive Dentistry entitled "**Comparative evaluation of antibacterial efficacy and remineralization potential of Acidulated Phosphate Fluoride gel with Herbal Dental gel containing *Zingiber officinale*, *Salvadora persica* and *Cinnamomum zeylanicum* – 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)**
☎: 0831-2470362 **Web: http://www.kledental-bgm.edu.in**
FAX: 0831-2470640 **E-mail:principal@kledental-bgm.edu.in**

Date : 19.9.2022 **Serial No. :** 107

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 ANTIBACTERIAL EFFICACY AND REMINERALISATION POTENTIAL OF ACIDULATED PHOSPHATE FLOURIDE GEL WITH HERBAL DENTAL GEL CONTAINING ZINGIBER OFFICINALE, SALVADORA PERSICA AND CINNAMOMUM ZEYLANICUM - AN INVITRO 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 .....3.....%, 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