"**COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF LICORICE AND TRIPHALA LOLLIPOPS ON STREPTOCOCCUS MUTANS COUNT IN CHILDREN AGED 4 TO 8 YEARS: AN EXPERIMENTAL STUDY"**

Dissertation submitted to Maharashtra University

of Health Sciences, Nashik

in the Partial Fulfillment of

Regulations for the award of the Degree of

MDS

IN

PAEDODONTICS AND PREVENTIVE DENTISTRY

BRANCH VIII

2019-2022

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LIST OF ABBREVIATION

S. mutans	Streptococcus mutans
S. sobrinus	Streptococcus sobrinus
S. salivarius	Streptococcus salivarius
S. anginosus	Streptococcus anginosus
S. intermedius	Streptococcus intermedius
S. sanguini	Streptococcus sanguini
L. casei	Lactobacillus casei
FDA	Food and Drug Administration
GRAS	Generally Regarded as Safe
T. bellerica	Terminalia bellerica
T. chebula	Terminalia chebula
E. officinalis	Emblica officinalis
Chlorhexidine	CHX
PCR	Polymerized Chain Reaction
DMFS	Decayed Missing Filled Surfaces
DMFT	Decayed Missing Filled Teeth
defs	Decayed extracted filled surfaces
HPLC	High-Performance Liquid Chromatography
BHI	Brain Heart Infusion
WHO	World Health Organization
DG-LRE	Deglycyrrhizinated Licorice Root Extract
LRE	Licorice Root Extract

CFU	Colony Forming Units
MSBA	Mitis Salivarius Bacitracin Agar
ELISA	Enzyme-Linked Immunoassay
RCT	Randomized Control Trial
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MHPE	Mixed Herbal Powder Extract
SEM	Scanning Electron Microscope
ID	Intellectually Impaired
AAPD	American Academy of Pediatric Dentistry
AAMD	American Association of Mental Deficiency
S	Significant
NS	Not significant

INTRODUCTION

"The great thing about Ayurveda is that its treatments always yield side benefits, not side effects"

-Shubhra Krishan

Dental caries is one of the most prevalent disorders in children.[(1)] Over the past 30 years, it has increased in low socioeconomic populations. It is an irreversible microbial disease of the calcified tissue of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.[(2)] It is a major public health issue around the world due to its multifactorial origin. It is a complicated and chronic illness caused by acidogenic bacteria present in the dental plaque, also called the dental biofilm which causes progressive degradation of the hard tooth components such as enamel, dentin, and cementum.[(3)] Streptococcus mutans (S. mutans) and other Streptococcus species like

Streptococcus sobrinus, S. mutans, and other Streptococcus species such as Streptococcus sobrinus, Streptococcus salivarius, Streptococcus anginosus, Streptococcus intermedius, Streptococcus sanguinis; Veillonella spp., Actinomyces spp. such as Actinomyces gerencseriae, Bifidobacterium spp.[(4)] S. mutans has a significant action in the initiation and progression of dental caries. The quantities of S. mutans in plaque or saliva are strongly linked to existing or future caries experiences. As a consequence, the proportion of S.mutans in plaque or saliva counts might be utilized to predict caries activity and susceptibility.[(1)] In young children risk factors for dental caries include frequent exposure to dietary sugar and refined carbohydrates, low saliva flow rates, high levels of cariogenic bacterial colonization, improper bottle feeding, developmental defects of tooth enamel, previous caries, low socioeconomic status, high maternal levels of cariogenic bacteria, and poor maternal oral hygiene. Further risk factors include low fluoride levels in community water, poor tooth brushing or use of fluoride-containing toothpaste, a lack of dental care, and parental oral health negligence.[(5)]

Dietary counseling, oral hygiene, and fluoride treatment are the most common preventive strategies now available for the prevention of dental caries. New management approaches particularly among the primary dentition, must be developed as a supplement to the current preventive method. When the preventive approaches of dental caries are both antibacterial and child-acceptable, it will be highly valuable for children.[(6)] Dental caries management has evolved over the ages and has followed numerous courses, culminating in a paradigm shift toward a preventive approach. Caries prevention has progressed from a surgical to a medical approach with the

current suggestion for early caries detection and monitoring rather than waiting until a cavity develops.[7]

Increased disease incidence, opportunistic infections in immunocompromised individuals, increased resistance by pathogenic bacteria to presently prescribed antibiotics as well as chemotherapeutics, and financial constraints in developing countries have all contributed to the universal need for alternative prevention options.[8]

There has been a movement in global thinking, with a growing tendency to 'go natural.' Spirituality, yoga, nutritious organic foods, and the use of medicinal herbs are all instances of how western civilization is looking to old eastern culture for ways to revive their whole way of life.[9] Many plants are utilized in Ayurveda to prevent caries. Various traditional plants and plant products have been used to treat oral problems. Several phytochemicals, including antibacterial compounds, were isolated from edible plants and found to have antibacterial activity against S. mutans. Natural phytochemicals obtained from plant extracts have been used in traditional therapy and may be a useful replacement for synthetic chemicals.[10]

Natural goods have various benefits, including ease of availability, minimal toxicity, and a lack of microbial resistance.[11] Licorice (mulethi), also known as Glycyrrhiza glabra, is a medicinal plant that has been utilized for thousands of years by many civilizations. It is 50 times sweeter than sucrose and has several advantages, including the treatment of coughs, sore throats, and gastrointestinal difficulties, as well as the improvement of a child's memory. It may also be used as a sweetening and food flavoring agent. The FDA has designated Licorice as GRAS (generally regarded

as safe). Licorice, often known as the "Grandfather of Herbs" has been utilized for thousands of years by people from all over the world. It is widely available in the market and affordable to people.[12,13] Recent studies disclose that Licorice and its many bioactive components, such as Glycyrrhizin, Licoricidin, Licorisoflavan A., Licochalcone A., and Glabridin, may be helpful in the treatment of oral disorders including dental caries.[14] The safest amount of Licorice is 25 g/ml per day, which is 0.015-0.22 mg glycyrrhizin/kg body weight per day, and should not exceed 2 mg per day.[1,15]

Triphala is another herbal plant to consider. It has been a major element in Ayurveda from the beginning of time. *Terminalia bellerica (T. bellerica)* (Bibhitaki), *Terminalia chebula (T. chebula)* (Haritaki/Harada), and *Emblica Officinalis (E. Officinalis)* (Amalaki) are three herbal fruits that make up this botanical mixture.[16] It has antibacterial, antiviral, and antifungal properties, as well as being antioxidant, anti-inflammatory, antitumor, antihistamine, digestive, cholesterol-lowering, diuretic, and laxative. In children, a safe dose of Triphala is 2-3 grams.[17] Dental caries, gingivitis, spongy bleeding gums, stomatitis, etc., are among the diseases of the oral cavity that Harada may prevent and cure. Triphala extract efficiently reduces the development of plaque on the tooth surface by two mechanisms that promote colonization of organism i.e, by inhibiting sucrose-induced adhesion and glucan-induced aggregation.[18]

A gold standard antimicrobial agent widely used in dentistry is Chlorhexidine. The side effects like staining and altered taste limit its long-term use and acceptability by the patients.[19] Despite several over-the-counter therapeutic agents available for the prevention of caries, natural remedies are warranted. Even if there was no

evidence of tooth structure remineralization, 0.6% Triphala mouth rinse was demonstrated to have anti-caries effect equivalent to chlorhexidine without the disadvantages of tooth discoloration and at a significantly lower cost.[20] Triphala is a one-of-a-kind medicine with several medicinal properties that Ayurveda has bestowed upon the world. It has the property to heal various systemic diseases with little or no side effects. Dentistry is still in a need of a medicine to treat problems with the mouth's hard and soft tissues. When compared to currently available commercial alternatives, Triphala appears to achieve the majority of these goals with little deleterious effects on oral tissues and at a reasonable cost.[21] As a result, more research into Triphala's diverse therapeutic effects in dentistry should be promoted.

Consumption of lollipops is common in children. So, in the present study, it was decided to incorporate the above ayurvedic herbs in lollipops to make them beneficial to children. In addition, lollipops are good stimulators of saliva which has self-cleaning properties.[22] Sugar substitutes such as xylitol, mannitol, sorbitol, lactitol as well as maltitol are often used in place of sugars in lollipops since typical sucrose-sweetened lollipops may add to the caries etiology of the diet. Sugar replacements are beneficial in avoiding dental caries across a variety of sugar-free lollipop tastes. Oral bacteria do not use these sugar substitutes to produce acids that cause demineralization of enamel and dentin.[10] These sugar-free lollipops were now employed as a control group.

Hence, the current study was carried out to assess the antibacterial activity of Licorice and Triphala in vivo in a community of Indian schoolchildren with high caries risk. The study investigated the antimicrobial effects of Licorice and Triphala herbal lollipop in reducing the levels of salivary S. mutans.

AIM AND OBJECTIVES

AIM:

To evaluate and compare anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years.

PRIMARY OBJECTIVE:

1. To evaluate the anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years.

2. To compare the anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years.

SECONDARY OBJECTIVE:

1. To evaluate the palatability of Licorice and Triphala lollipops in children.

2. To compare the palatabilty of Licorice and Triphala lollipops in children.

REVIEW OF LITERATURE

Hwang J, Shim J, Chung J et al (2004)[23] investigated the anticariogenic activity of few tropical medicinal plants against S.mutans and discovered that Methanol extracts of five tropical plants, including Glycyrrhiza glabra Baeckea frutescens, Physalis angulata, Kaempferia pandurata, and Quercus infectoria, demonstrated strong antibacterial activity against cariogenic bacterium. At 50 mg/ml extract concentration, K. pandurata, G. glabra, and P. angulata displayed a rapid deadly bactericidal action against S. mutans in 2 minutes. He found that the test for bactericidal shows that methanol extracts of G. glabra kill S. mutans in 2 minutes at a dosage of 50 mg/ml.

Isbrucker R.A and Burdock G.A. (2006)[24] investigated the risks and benefits of using Licorice root, extract, and powder as a food additive, focusing on pharmacology and toxicity of glycyrrhizin. Glycyrrhizin, the major component of licorice extracts, is often used in foods, cigarettes, and herbal and traditional

medicine. So as a result, licorice and glycyrrhizin are widely utilised in the United States, with an estimated daily glycyrrhizin intake of 0.027–3.6 mg/kg. Both of the products are approved for use in meals by the majority of national and international regulatory bodies. Ingestion of licorice and glycyrrhizin possesses anti-viral, anti-ulcer and hepatoprotective activities, according to several in vivo and clinical studies. Several genotoxic studies have revealed that glycyrrhizin is neither mutagenic nor teratogenic and that under some conditions, it may have anti-genotoxic properties. Based on clinical observations and in vivo they recommended a daily dose of 0.015–0.229 mg glycyrrhizin / kg body weight / day.

Carounanidy U, **Satyanarayanan R, Velmurugan** A **(2007)**[9] carried out a clinical study on the utilization of an aqueous extract of the T. chebula as an anticariogenic drug. For preparing an aqueous extract, dried ripe fruit of T. chebula was crushed into a fine powder and mixed with ten times its volume of sterile distilled water in a flask with round-bottom. Saliva samples were collected from the individuals between the ages of 18 and 25 who were at high risk for caries. Salivary samples were taken from each of the 32 patients twice, once before and once after washing with T. chebula extract.

Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE (2008)[14] carried out a research with the goal of using molecular approaches to discover all of the bacterial species related with caries in both primary and permanent teeth, as well as to evaluate the bacterial profiles associated with different disease stages. For collection of Plaque 39 healthy controls as well as intact enamel and white-spot lesions, dentin lesions, and deep-dentin lesions in each of 51 severe caries patients. To determine species identities, 16S rRNA genes went under PCR amplified, cloned, and

sequenced. A reverse-capture checkerboard test was used to look for 110 common bacterial species in 243 samples. A total of twenty-two new phylotypes were discovered. Additional species, such as those from the genera Propionibacterium, Atopobium, and Lactobacillus, were found in patients with S. mutans at much greater levels than S. mutans. In white-spot lesions, species of Actinomyces and non-S. mutans streptococci predominated, although recognised acid producers were identified at their maximum levels later in illness. And determined that half of the bacteria linked to tooth cavities have yet to be cultured. Bacterial profiles shift as illness progresses and differ from both primary to secondary dentition. The current findings support that the ecological plaque concept in caries disease and induce bacterial composition changes.

Touyz LZG (2009)[(15)] published a case report on Health Check Liquorice Oro-Dental Implications in 2009. This paper assesses liquorice and offers a reality check on its properties such as chemical structure, botanical sources, active liquorice ingredient physiological or pharmacological activity, and some common liquorice-containing consumables, systemic impact on health, a typical case report of liquorice-induced hypertension, and effects of liquorice consumption on oro-dental structures. It is suggested that important clinical therapy principles for reducing licorice consumption be followed. They also discovered that severely discoloured teeth, a stained tongue, or other orodental indications of intraoral chewing tobacco usage, when accompanied with elevated blood pressures, should alert dentists to the risk of licorice toxicity or overuse-related morbidity. In Dental clinics blood pressure should be measured before starting any surgery and regular medical history updates are essential with regular dental maintenance. A few people may also reveal information

about their high blood pressure. All hypertensive patients' diets should be examined by health care practitioners, and in addition to offering advice about avoiding licorice products, affected people should be referred for early hypertension diagnosis and treatment.

Peters MC, Tallman JA, Braun TM, Jacobson JJ (2010)[(1)] conducted a pilot trial on clinical S. mutans reduction in children going to pre-school using a new lollipop using liquorice root extract. To demonstrate the notion, this pilot trial used sugar-free lollipops containing liquorice root extract to provide a therapeutic intervention. Using baseline SM levels as a risk indicator, children aged 2 to 5 years were classified as having a low, medium, or high caries risk. Bacterial counts were compared at baseline, throughout intervention, and 9 weeks after intervention. In addition, he concluded that usage of a herbal Liquorice lollipop twice daily considerably decreased the quantity and relative % of S. mutans in high-risk kids.

Tandon S, Gupta K, Rao S, Malagi KJ (2010)[(20)] investigated the impact of Triphala mouthwash on dental caries. The study included 1501 children between the ages of 8 and 12 who had the same socioeconomic level and dental hygiene practises. Randomization was used to divide these pupils into three groups. 514 students in Group 1 received Triphala mouthwash (0.6 percent), 495 students in Group 2 received chlorhexidine (0.1 percent) mouthwash, and 492 in Group 3 students received distilled water. In hospital's pharmacy production facility, 0.6 percent Triphala mouthwash, 0.1 percent chlorhexidine mouthwash, and distilled water were made. To eliminate prejudice, all solutions were created of the same colors. The DMFS/dmf index was used to assess the caries state. Each group rinsed their mouths with the appropriate mouthwash after lunch on a regular, supervised basis. After lunch,

children were encouraged to rinse their mouths with 10 ml of solution for 1 minute before being asked not to rinse their mouths with water or drink anything for the next half hour. They were told to bring the mouthwash bottles home for the weekend. The well diffusion agar technique was used to test the impact of Triphala extract on Streptococcus mutans and Lactobacillus. It was discovered that 0.6 percent Triphala extract had the greatest antibacterial efficacy against S. mutans and Lactobacillus. At one occasion, 10 millilitres of mouthwash were administered. For two minutes, the mouthwash was swished in all quadrants of the mouth. The defs of incipient caries was performed at 3, 6, and 9 months after baseline, and the caries evaluation was performed at 9 months after baseline. They determined that, while the usage of Triphala resulted in an increase in DMFS scores, it was not statistically significant. The caries status trended in the same direction as chlorhexidine. Because this was a nine-month long-term trial, dmfs scores could not show the efficacy of mouthwash, and a few of the carious teeth reported at baseline were exfoliated at end of the study, dmfs ratings could not indicate the efficacy of mouthwash. No new carious lesions were discovered. Also there was a reduction in incipient caries scores, the effect of mouthwash in the remineralization process could not be determined. No significant rise in the incipient caries score at the end of the research, implying that mouthwash of Triphala can help prevent the development of incipient lesions.

Hu C, He J, Eckert R, Wu X, Li L, Tian Y et al (2011) (25) studied the evaluation and development of a sugar-free herbal lollipop that destroys cavity-causing bacteria. For determining high-performance liquid chromatography was used the quality of large volumes of effective licorice extracts. S. mutans, strain UA159; S. sobrinus, strain ATCC27607; and L. casei, strain ATCC334, were the bacterial

strains used in the study. All bacteria were grown in BHI broth under anaerobic conditions at 37°C. The antibacterial activity of licorice extract was examined using the microdilution broth technique developed by the Clinical and Laboratory Standards Institute. A traditional sugar-free candy mix from Dr. John's Candy was used to make the herbal lollipop. Depending on the amount of Glycyrrhizol A in a batch of licorice extract, 7-15 mg of licorice extracts were added to each lollipop to provide a homogenous dosage of Glycyrrhizol A. Two tests were used to investigate the bioactivities of herbal lollipops against bacteria: overnight growth suppression and time killing. This study found sugar-free lollipops are safe and have consistent antibacterial activity in the formulations recommended for administration. A application of these lollipops twice daily for 10 days resulted in a considerable decrease in cariogenic bacteria, according to two pilot human studies. All of the visits of the children scheduled during morning, before the lunch. Finally, they discovered that while lollipop consumption is beneficial to oral health, it does not replace current dental preventative procedures such as tooth brushing and fluoride treatments provided by dental clinics.

Taheri JB, Azimi S, Rafieian N, Akhavan Zanjani H (2011)[26] published a review study on herbs in dentistry in 2011. Herbs have been used to prevent and control disease for millennia. Herbal extracts are useful because they interact with certain chemical receptors in the body and, in a pharmacodynamic sense, are medications in their own right. Patients have avoided the various adverse effects associated with standard pharmaceuticals by utilising herbal remedies, but side effects do occur with them also. Only competent practitioners may prescribe the appropriate plant and dose. Herbal treatments were formerly considered in every culture, but

pharmaceutical industries changed their minds. Pharmaceuticals are now referred to as "traditional," whereas herbs are referred to as "alternative." The greatest obstacle and concern is a lack of knowledge regarding the effect of herbs on oral tissues, the mechanism of action, and side effects. Herbs are the source of some prominent conventional medications on the market. Aspirin, digitalis, and Sudafed are examples (modelled after a component in the plant ephedra). The potency of herbal products might vary. As a result, caution must be exercised while picking herbs; nonetheless, herbal medicines have far fewer adverse effects and are far safer to use than the conventional pharmaceuticals. And determined that the plants detailed in this article are Peppermint, Bloodroot, Caraway, Chamomile, Myrrh, Rosemary, Sage, Thyme, Echinacea, Aloe Vera, Propolis, and a list of additional herbs effective in dentistry. Herbs may be effective alternatives to conventional therapies for oral health issues, but further study is clearly required.

Jeon JG, Rosalen PL, Falsetta ML, Koo H (2011)(27)[7)] reviewed Natural Products in Caries Research: Current (Limited) Knowledge, Challenges, and Future Prospects. According to this research, while fluoride, administered in multiple modalities, remains the cornerstone for caries prevention, new techniques are needed to improve its effectiveness. Antiplaque techniques now available are based on the use of broad-spectrum microbicidal drugs, such as CHX. Natural products provide a rich supply of structurally varied molecules with a wide variety of biological activities that might be beneficial in the development of alternative or complementary anticaries therapy. To extract active molecules from natural products, however, is a difficult technique due to intricate chemistry and separation procedures. Furthermore, the majority of studies have focused on the general inhibitory effects on glucan synthesis

as well as bacterial metabolism and growth, frequently using methods that do not address the pathophysiological aspects of the disease (e.g. bacteria in biofilms) and the length of exposure/retention in the mouth. As a result, the genuine efficacy of natural compounds in caries prevention, as well as their precise mechanisms of action, are mostly unclear. Nonetheless, natural compounds that are possibly efficacious against the virulence of cariogenic microbes have been found. This paper has focused on information gaps and proposes a paradigm for evaluating the use of natural ingredients in anticaries treatment.

Ramakrishna Y, Goda H, Baliga M, Munshi A. (2011)[10] designed a research to reduce cariogenic bacteria using a natural, alternative preventative strategy based on Phytochemistry (Plant Extracts). They described the antimicrobial efficacy of several plant extracts against cariogenic bacteria in this investigation. The significance of essential oils and essential oil components found in plant extracts is also discussed. Natural goods, particularly plant-derived chemicals, have recently piqued the interest of researchers looking for anti-cariogenic characteristics. Plant extracts' antimicrobial activities against cariogenic bacteria give substantial evidence stating that plant extracts, essential oils, and pure phytochemicals have the potential to be used in preventative or therapeutic therapy for oral illnesses. Furthermore, there are other hurdles to overcome, particularly due to a lack of reliable scientific information about the cost effectiveness of these treatments. They found that while phytosome technology is not now cost effective, with advancements in dentistry research, this natural-based therapy might be both helpful and cost effective.

Nadimi H, Wesamaa H, Janket S-J, Bollu P, Meurman JH (2011)[28] gave a review on Are sugar-free confections really beneficial for dental health? To avoid

dental decay caused by sugar and other fermentable carbohydrates, many sugar alternatives have been produced and are commonly used in confections and drinks. Sugar alcohols or polyols are one type of sugar replacement. Polyols have been utilised specifically in diabetic diets because they are not quickly absorbed in the gut and bloodstream, limiting post-prandial glucose rise. They may also reduce calorie consumption. A search was conducted with the search terms 'sugar alcohol' or 'sugar-free' or 'polyols' and combined with a search with the terms 'dental caries' or 'dental erosion And they found that while polyol-based sugar-free products may reduce the incidence of dental caries, they may introduce another oral health concern, tooth erosion, if they contain acidic flavouring. In this field, there is a need for carefully conducted clinical research.

Messier C, Epifano F, Genovese S, Grenier D (2012)[3] performed a review research on Licorice and its prospective positive advantages in common oro-dental illnesses. Species of Glycyrrhiza has been used as a traditional herbal remedy since ancient times, according to WHO Licorice, which includes a variety of secondary metabolites, has been related to a number of positive health effects in humans. Glycyrrhizin, glabridin, licochalcone A, licoricidin, and licorisoflavan A, among other bioactive components of licorice, may be useful in the treatment of oral problems. This study looked at the effects of licorice and licorice ingredients on oral microbial pathogens and the host immune response in common ora-dental illnessses . It also highlighted the results of clinical studies into the potential advantages of licorice and its constituents for prevention and treatment of oro-dental diseases. Licorice and its bioactive constituents have the potential to be employed in the treatment of oral ailments, particularly periodontal diseases, according to several in vitro studies.

Inconsistent results were regularly discovered in human clinical research. The experimental design of some of the in vivo studies is flawed. As a result, they concluded that licorice extracts and licorice components found in mouthwash, toothpaste, gel, and chewing gum should be explored further in clinical studies to confirm the positive results obtained in in vitro testing. Given the potential side effects of consuming high quantities of licorice for a lengthy period of time, such as hypertension, a localised application of these bioactive compounds may be preferable.

Omar HR, Komarova I, El-Ghonemi M, Fathy A, Rashad R, Abdelmalak HD, et al (2012)[29] provided a comprehensive review on Licorice abuse: time to issue a warning message, emphasising the necessity of researching the dietary habits and herbal medicines that are utilised globally on cultural and habitual basis rather than trustworthy scientific data. Licorice is a dietary supplement permitted by the US FDA that is used in a variety of products with no strict controls in place to prevent toxicity. Licorice extracts are frequently employed as flavouring ingredients in pharmaceutical treatments to hide the bitter taste. DGL is available in capsules, lozenges, wafers, and liquid and has been designed to minimise the adverse effects of licorice by eliminating the active ingredient glycyrrhizin. The authors have provided a complete analysis of licorice as well as the documented problems associated with excessive use. Despite its apparent efficacy in a few therapeutic settings, daily licorice administration is never justified since the advantages are minimal in comparison to the negative effects of chronic ingestion. The active metabolites in licorice extract, glycyrrhizic acid and glycyrhetic acid, can cause an apparent mineralocorticoid excess syndrome [Stewart et al. 1987]. These adverse effects are caused by the suppression of the enzyme 11-ß-HSD and the concomitant increase in cortisol activity. As a result,

this study will serve as a warning message to patients to avoid excessive licorice use, as well as a message to the FDA to begin regulating the use of this drug

Srinagesh J, Krishnappa P, Somanna S (2012)[16] carried out an in vivo study to determine antibacterial activity of Triphala (6%) in mouthwash formulation and the on salivary streptococci levels after 48 hours and 7 days of twice-daily use with 0.2 percent chlorhexidine . Sixty undergraduate student volunteers, ranging in age from 18 to 25, were allocated to one of three research groups at random. a) 6 percent Triphala mouthwash, 15 mL twice daily (active control group); b) 0.2 percent chlorhexidine mouthwash, 15 mL twice daily (passive control group); c) passive control group directed to rinse with plain water two times a day. By inoculating blood agar with saliva samples after 48 hours and again after 7 days, CFUs/ml of oral streptococci were measured. Triphala group had a 17% and 44% reduction after 48 hours and 7 days, respectively, whereas the chlorhexidine group had a 16 percent and 45 percent reduction. The CFUs/ml reduction seen in the Triphala group was substantially identical to that seen in the chlorhexidine group. As a result, they discovered that while Triphala is been used in Ayurveda for millennia and numerous potential systemic benefits, promising findings shown by Triphala encourage future investigation of its antibacterial properties against diverse oral bacteria.

Ahn S-J, Song Y-D, Mah S-J, Cho E-J, Kook J-K (2012)[30] examined the lowest inhibitory concentration and minimum bactericidal concentration of deglycyrrhizinated licorice root extracts (DG-LRE) in planktonic and biofilm phases, as well as time-kill kinetics, growth, adhesion, and biofilm tests, against Streptococcus mutans UA159. According to the findings of this study, DG-LRE had significant antibacterial action against S. mutans in the planktonic phase and

significantly inhibited the production of S. mutans biofilms, regardless of carbohydrate availability or saliva-coating. The oral cavity's microbial population, on the other hand, is a diverse microbial community. Since S. mutans does not exist in a monoculture, but rather in diverse microbial consortia containing thousands of microorganisms and bacterial species, the impact of DG-LRE on growth, adhesion, and survival has been investigated. As a result, a biofilm development assessment in vivo is required.

Jain E, Pandey RK, Khanna R (2013)[14] planned an in vitro and in vivo study in children to see how efficient and tolerable ethanolic and aqueous extracts of licorice are at preventing caries. The lowest bactericidal dosages of licorice aqueous and ethanolic extracts against mutans streptococci were calculated, and their toxicity was evaluated using Caenorhabditis elegans as a model organism. A total of 60 children aged 7 to 14 were randomly assigned to one of three groups: Group 1 received 15% aqueous licorice mouthwash, Group 2 3.75 percent ethanolic licorice mouthwash, and Group 3 received 0.156 percent chlorhexidine gluconate as a positive control pH alterations and mutans streptococci colony counts were measured in three pre-rinse saliva samples and one post-rinse saliva sample. A self-designed questionnaire was used to assess the palatability of licorice extracts. The ethanolic licorice group exhibited a significant drop in colony counts as compared to the control group. Licorice extracts similarly generated a quick rise in salivary pH and had a poor retentivity antibacterial activity at first. As a result, they found that licorice extracts, both aqueous and ethanolic, are effective cariostatic medications that are also appealing to pediatric patients.

Anagha K, Manasi D, Priya L, Meera M (2014)[31] conducted a review study on the antibacterial activity of Yashtimadhu (Glycyrrhiza glabra L.) to define the extent of Glycyrrhiza glabra as an antimicrobial agent. More than 200 research articles and a few review papers were searched and categorized according to Glycyrrhiza glabra's varied antibacterial properties and gathered in different parts in this review. Glycyrrhiza glabra antimycobacterial activity was discovered at a concentration of 500 microg/mL. Four studies were discovered that investigated the antibacterial impact of Helicobacter pylori. This study looked at the in vitro and in vivo cariostatic efficiency of aqueous and ethanolic extracts of licorice, as well as its acceptability among pediatric patients. The results of this investigation demonstrated that licorice was safe, well-tolerated by kid patients, and resulted in a decrease in mutans streptococci colony counts and an increase in pH following a rinse with both aqueous and ethanolic extracts. The effectiveness of licorice extracts was also tested in vitro. They concluded, based on the examples in this research, that Glycyrrhiza glabra extracts have the potential to be turned into preventative or curative antibacterial agents.

Bhattacharjee R, Nekkanti S, Kumar NG, Kapuria K, Acharya S, Pentapati KC (2014)[32] conducted a randomized trial on the effectiveness of aqueous extract of Triphala which is 0.6% prepared to 0.12 % chlorhexidine on plaque and gingivitis to assess baseline and follow-up plaque and gingivitis in 60 school children aged 8 to 12 years (n = 30 in each group using plaque and gingival indices; Triphala and chlorhexidine groups who had a plaque index score of at least 0.9 and were ready to participate with parental consent (fair category of plaque index). Antibacterial activities of Triphala mouth rinse are effective against both Gram-positive and Gram-

negative microorganisms. It also has antioxidant properties and a substantial inhibitory impact on the polymorphonuclear leukocyte-type matrix metalloproteinase, which is connected to periodontitis extracellular matrix disintegration. As Triphala has multiple beneficial effects on oral health, Triphala is a cost-effective and excellent treatment for reducing plaque and gingivitis. Without the risk of chlorhexidine side effects, Triphala mouth rinse can be used in short-term treatment regimens.

Elumalai M, Bhuminathan S, Tamizhesai B (2014)[33] carries out a study on herbs used in dentistry. The sole goal of any area of medicine is to use available resources to make human existence happier and healthier. Herbs are making a return, and a herbal renaissance is taking place all over the world. When compared to synthetics, which are harmful to persons and the environment, herbal products represent safety. An herb is a plant portion other than woody tissues that can be used to cure a variety of ailments. Herbal extracts have been utilized as antimicrobials, antiseptics, and antibacterials in dentistry. Herbal extracts have been utilized to reduce plaque in gingivitis and periodontitis cases. Furthermore, plant extracts, essential oils, and purified phytochemicals have the potential to be transformed into compounds that may be employed as preventative or therapeutic agents for dental problems.

Chavan S, kemparaj U, Lakashminarayan N (2015)[34] analyzed and compared the reduction in salivary Mutans Streptococci counts after chewing Xylitol, herbal, and placebo gums in high school students in a randomized control experiment. A contemporaneous parallel design was used to conduct a clinical follow-up study on high school pupils. A preliminary study's data was used to calculate the sample size. A contemporaneous parallel design was used to conduct a clinical follow-up study on high school pupils. A preliminary study's data was used to calculate the sample size.

A frank carious cavity and salivary Mutans Streptococci levels of 10 CFU/ml or above were required for the study. On mitis salivarius bacitracin agar plates the diluted material was streaked and incubated. The saliva of children with a Mutans Streptococci count of 10 was deemed, potential candidates. The chewing gums that were employed in this investigation were: Group A consisted of herbal chewing gum ("Orbit white"), 100 percent Xylitol chewing gum ("Xylitol"), and placebo chewing gum ("gum base"). After breakfast, lunch, evening snacks, and supper, patients were instructed to chew one pellet for 10 minutes four times a day for 21 days. All subjects were encouraged to maintain their normal eating habits, daily oral hygiene routines, and avoid chewing gum other than that provided to them throughout the study. It was also mandated that class teachers urge children to chew their gums every day. On the 22nd day, a non-stimulated salivary sample was collected and analyzed for salivary Mutans Streptococci. All participants were requested to fill out a compliance questionnaire on that day only, which consisted of three questions about their compliance and experience during the trial. According to the findings of this study, eating 100 percent Xylitol sweetened chewing gum for 10 minutes four times a day for 21 days dramatically lowers salivary S. mutans levels, which may be effective in managing dental caries in high-risk individuals.

Shanbhag VKL (2015)[(35)] gave a review on 'Triphala as an antibacterial in the oral cavity for prevention of dental caries and oral infections like periodontal disease, as well as oral candidiasis add considerably to the global economic burden each year. Though there are conventional management approaches for many disorders, they are not cost-effective and have side effects. Ayurveda is a traditional Indian medical system that has been used for centuries on the Indian peninsula.

Triphala has a regal place among the numerous herbal remedies used in Ayurveda due to its extensive positive systemic activities. Triphala is a blend of Terminalia bellirica, Terminalia chebula, and Emblica Officinalis fruits. Triphala's antibacterial properties are widely reported in the literature. However, there is a scarcity of review publications on Triphala as an antibiotic against oral infections. It was considered that this component of Triphala should be revisited. The goal of this article is to explore how Triphala and its components can help prevent and treat tooth caries and other common oral illnesses. A thorough analysis of the literature revealed that Triphala can be used effectively to treat dental caries, gingival disease, and periodontal disease. It can also be used for a root canal irrigant and to combat oral Candida species.

Yamaguchi M, Tezuka Y, Takeda K, Shetty V (2015)[36] studied the assessment of disposable saliva collection kits for speedy, dependable, and reproducible saliva sample collection. from 10 healthy persons to obtain complete saliva samples the saliva collection kit was used who agreed to take part in the study. From them separately each of the five subjects had a passive drool saliva sample (3 ml) obtained. After saliv settles at the bottom of the mouth then expectorating it into a commercially available polypropylene cup in this way each subject delivered the sample. The native saliva collected by micropipetting was processed In the same way as a cortisol ELISA kit the native saliva collected by micropipetting was processed and served as a control to guarantee consistency. Finally, the sCortisol levels from the individual aliquots (both saliva collection kit and micropipetting) were measured by using a cortisol ELISA kit and concluded saying that The disposable saliva collection kit allows accurate and repeatable collection of fixed amounts of the whole saliva and does not interfere with subsequent measurements of salivary cortisol.

Adyanthaya A, Ismail S, Sreelakshmi N(2016)[37] gave a review titled "Indian traditional medicinal plants against dental caries - an unsung past to a bright future." The goal of this study was to provide a few lesser-known traditional Indian medicinal herbs, their bioactive phytochemicals, the section of the herb employed, and the minimum inhibitory concentration (MIC) against Streptococcus mutans in particular. This review of the literature summarises the scientific foundation of a few indigenously used medicinal herbs in India that have demonstrated effectiveness against oral bacteria such as Streptococcus mutans. India has diverse biodiversity and indigenous knowledge, notably in traditional ethnomedical techniques. According to reports, the Indian folk medicine system utilizes over 5000 distinct plant species with approximately 25,000 formulations as treatments for various diseases, whilst tribal healers use approximately 8000 wild plants with almost 1,75,000 concoctions. The ancient indigenous Indian medical system recommends over 10,000 recorded medicines.

Almaz ME, Sönmez IŞ, Ökte Z, Oba AA (2016)[38] carried out a study on The benefits of a herbal lollipop containing licorice root extract on salivary Streptococcus mutans in caries-free and high-risk children aged 5–11. After clinical and radiographic examinations, as well as S. mutans levels, both caries-free and high-caries-risk youngsters were enrolled in the study. Caries were removed from all of the children's teeth. The members of the groups were the children who are caries-free (n=36), and high-risk caries and did not comply with dental treatment(n=36). Herbal (A-1, B-1, C-1) and placebo lollipops (A-2, B-2, C-2) were the two groups of the participants. One of the ingredients in the herbal lollipop was Glycyrrhiza uralensis. All of the kids ate lollipops twice a day for ten days, as instructed by the

manufacturer. One lollipop was consumed at school in the morning under the supervision of the examiner, and the other was consumed at home in the evening under the supervision of the parent/guardian. The amount of S. mutans in the saliva was determined using the Dentocult SM Strip Mutans test, which uses a dip-slide approach. As a result, he concluded that herbal lollipops were effective in lowering salivary S. mutans levels in children with high caries risk (who did not comply with dental care).

Hegde R, Kamath S (2017)[39] carried out a study on a comparison of the changes in Streptococcus mutans and Lactobacillus colony count in saliva after chlorhexidine (0.12 percent) mouth rinse, combination mouth rinse, and green tea extract (0.5 percent) mouth rinse in 75 school children aged 8–12 years with at least dmft 4 and attached to an at least once daily tooth brushing routine and no other professional or home-based oral hygiene measures study employed the following mouth rinses: (1) Chlorhexidine mouth rinse (0.12%) (2) Combination mouth rinse: commercially available Thermokind (3) Green tea extract mouth rinse (0.5%). All of the children were given a demonstration of Tooth brushing and mouth rinsing demonstration at the start of the study was given to all the children, and they were advised to brush their teeth before breakfast in the morning and after meals at night. The media had been prepared. Using a colony counting grid, the bacterial colonies were then counted as CFUs. Finally, the researchers come to the end that for dental caries and periodontal illness green tea mouth rinse could be an excellent alternative to mouth rinse and a promising preventative therapy over the world.

Saxena S (2017)[11] studied Terminalia Chebula, Terminalia bellirica, Emblica Officinalis, and Triphala's antibacterial effectiveness against Salivary Streptococcus

Mutans. Count the number of patients aged 15 to 40 who have active carious lesions that have not yet been treated. The four species were T. chebula, T. bellirica, E. of icinalis, and Triphala, and each patient received 15 mL of the freshly made 10% rinse and all four therapies: T. chebula, T. bellirica, E. of icinalis, and Triphala are the four species. Patients were instructed to eat breakfast at least two hours before visiting the department on the scheduled day. Unstimulated salivary samples were obtained by directing pre-test salivary samples. The patients were told to retain the rinse in their mouth for one minute, then rapidly swish it over their teeth and oral cavity before expectorating it. salivary samples which were not stimulated were obtained at 5 and 60-minute intervals after the rinse. Between the collection of salivary samples, the participants were not allowed to eat or drink anything. Within one hour, all salivary samples were sent in sterile containers to a microbiological laboratory for microbiological investigation. They also discovered that using a 10 percent concentration of Triphala as a mouth rinse for 60 minutes once a day significantly reduced the number of oral streptococci

Aparna M, Hegde V (2018)[(6)] did a study on the efficiency of Licorice and Triphala mouth rinses against streptococcus mutans and to compare the impact of Licorice, Triphala, and Chlorhexidine mouthwash among 75 individuals with DMFT scores more than 5. They were divided into three equal groups: Licorice, Triphala, and Chlorhexidine, which were labeled Licorice, Triphala, and Chlorhexidine, respectively. On Day 1: Unstimulated saliva samples were collected in labeled saliva collection tubes for each subject at least 2 hours after meals. On Day 7, each patient's unstimulated saliva samples were collected in labeled saliva collecting vials. The sample was inoculated onto mitis salivarius agar and incubated at 370 degrees Celsius

for 24 hours. The colony count was then calculated and compared to the prior count. After one week of washing, the Streptococcus mutans colony count was significantly lower in the licorice, Triphala, and chlorhexidine groups. It was also shown that licorice and Triphala mouthwashes had an antimicrobial effect comparable to chlorhexidine.

Krishnakumar G, Gaviappa D, Guruswamy S (2018)[(40)] conducted research to evaluate the anti-bacterial efficiency of licorice lollipop extract and its remineralizing potential. The volunteers were chosen among the institution's undergraduate students between the ages of 18 and 21. The licorice lollipops were obtained from the LOLOZTM Company, which was 7 to 15 mg of the active component, Glycyrrhizol A. 2–2.5 mL unstimulated saliva was collected. The samples were obtained in the morning, at least 1 hour after any meal or drink had been consumed. The obtained samples were delivered to the laboratory within 3 to 4 hours for processing and determination of S. mutans and Lactobacillus bacterial populations. Saliva samples were inoculated into MSB agar and Rogosa agar, and colony counts were performed using a digital colony counter. On, the Remineralizing Potential was evaluated. SEM study of the enamel block embedded on a Hawley's appliance worn by volunteers for 14 days following intake of the licorice lollipop was performed on twenty premolars excised for orthodontic purposes. The drop in S. mutans count was statistically significant, confirming that licorice is effective in lowering salivary S. mutans counts, however, LRE failed remineralizing capacity.

Sankeshwari R, Ankola A, Bhat K, Bolmal U, Rao M (2018)[(12)] conducted a study in which, locally produced Licorice varnish (LV), commercially available Fluoride varnish (FV), and a hybrid of the two varnishes (CV) were examined in

terms of physical attributes. The cold maceration method was used to obtain Licorice extract from certified Licorice roots. CV was created in six different concentrations of both varnishes using commercially available FV (Bifluorid12) as a positive control. Antibacterial activity testing methods such as disc diffusion and broth dilution were found to be ineffective. As a result, a novel evaluation method was developed, in which the varnish was mixed with Brain Heart Infusion broth and Streptococcus mutans and immediately incubated. pH, evaporation rate, viscosity, film-forming ability, and prep cost were all evaluated and compared. The extract was filtered using a muslin cloth and Whatman No.1 filter paper. The extract was evaluated for antibacterial activity against Streptococcus mutans ATCC 25175, using the broth dilution technique. The researchers discovered that LV, FV, and CV exhibit antibacterial properties against the Streptococcus mutans normal strain. All three varnishes had the same viscosity, evaporation rate, pH, and film-forming properties. The most cost-effective of the three was LV, but it had the shortest shelf life. To confirm these findings, more research employing an in vivo study design would be required.

Prakash S, Shelke A (2018)[18] reviewed the role of Triphala in dentistry. This study concentrated on a thorough examination of Triphala and its many uses in dentistry. In the prevention and treatment of a variety of oral illnesses, such as dental cavities, spongy and bleeding gums, gingivitis, and stomatitis Terminalia chebula is useful. T. chebula extract may be a beneficial agent In the treatment of carious teeth T. chebula extract may be a beneficial agent Because of its potential to limit the growth and accumulation of S. mutans on the surface of the tooth. Although there was no indication of re-mineralization of tooth structure, Without the drawbacks of tooth

staining and at a significantly lower cost of 0.6 percent Triphala mouthwash had strong anti-caries action equivalent to that of chlorhexidine. Triphala as a root canal irrigant, Triphala's anti-collagenase activity, Triphala's anti-microbial and anti-oxidant capabilities, and Triphala as a mouth rinse were also discussed in this review. When compared to currently available commercially available alternatives, Triphala appears to cover the majority of needs with no negative effects on oral tissues and at a very low cost. As a result, more studies into Triphala's diverse therapeutic activities in dentistry should be promoted.

Nair SK (2018)[41] discussed "Triphala: a wonder plant in the realm of dentistry: A mini review" briefly. Ayurveda seeks to foster a healthy mind and body by not just treating ailments but also developing strategies for health preservation. Ayurveda attempts to rectify body humor imbalances and derangements, as well as re-establishment. Balance conditions can be attained by utilizing all available spiritual and material resources. to the human species Despite significant advances in contemporary medicine, effective treatment of "Oral diseases" is provided by current drugs and chemicals. Chemicals have shown the issue. Because of the wide range of undesirable consequences, there is a pressing need to develop an effective therapy. Herbal remedies are both safe and efficient in treating any illness.

Megalaa N, Thirumurugan K, Kayalvizhi G, Sajeev R, Kayalvizhi E, Ramesh V et al (2018)[42] carried out an RCT to determine the effectiveness of Tulsi herbal rinses and Black myrobalans on S. mutans count and compared it with sodium fluoride (SF) mouth rinse among school children of 6 to 12 years ago as well as children with high caries risk as per the Caries Risk Assessment Tool for children aged >6 years old. A total of 105 youngsters were screened and their information was

entered on the case sheet. The case sheet included vital information, and oral hygiene index, a plaque index, decayed filled teeth/decayed filled surfaces, and an index of missing, decayed, and filled teeth/missing, decayed, and filled surfaces. Children were randomly assigned to one of three groups: with a control group of sodium fluoride rinse, some children in the experimental group of Tulsi mouth rinse (4%), and Black myrobalans (2.5%). Mouth rinses were distributed to each group. Salivary pH and microbial count data were collected and put into a data-collecting form. The control group (Sodium fluoride) had the highest increase in salivary pH, followed by Tulsi leaf extract, while Black myrobalans mouth rinse had the lowest. However, Black myrobalans mouth rinse exhibited a greater reduction in S. mutans count, indicating that it has a long-lasting impact on S. mutans. Tulsi and Black myrobalans mouth rinses have anticaries effectiveness by raising salivary pH and decreasing S. mutans numbers. All three mouthwashes significantly improved salivary pH while decreasing S. mutans numbers.

Chen Y, Agnello M, Dinis M, Chien KC, Wang J, Hu W (2019)[43] did research on 96 Chinese preschool children aged 3 to 6 years, licorice which had an extract of Glycyrrhiza uralensis which lowers S.mutans colonization as well as preserves oral microbial diversity. S. mutans cells/ml of the high-risk youngsters included in the study were estimated by an antibody-based technique. Children included in the treatment group got lollipops and oral health care counseling. They were encouraged to ingest 2 lollipops each day for 3 weeks, whereas the control group received only oral health care counseling and no placebo. As a result, visits of the control group youngsters were the same as that of the treatment group but without lollipops. Unstimulated saliva was collected from each participant. Salivary S. mutans

levels were quantified using 1ml. Samples were collected from the treatment group at various intervals i.e baseline visit (before lollipop uptake), 1 week, 2 weeks, and 3 weeks after initiating lollipop therapy, as well as at a follow-up visit of 1 week after discontinuation of use of lollipop. Samples of all participants were collected at the same time and concluded For three weeks, Chinese preschool children were given herbal lollipops twice a day, which drastically lowered S. mutans levels in their saliva.

Anushya P, Priya AJ, Arivarasu L et al. (2020)[44] did a review of herbal extracts and their impact on oral health. Pubmed, Scopus, and Google Scholar were used to find the articles. The conclusion is expected based on prior research that discusses the function of herbal medicines in dentistry, and the data obtained is examined using proper statistical procedures. Herbal medication is useful in dentistry for lowering inflammation and minimizing plaque buildup. According to this review study, Ginger, Garlic, Aloe Vera, and Miswak outperformed standard dentifrices in terms of reducing dental plaque and gingival irritation. The drawbacks of utilizing herbal medication such as clove oil include major difficulties such as pharyngitis, vomiting, cytotoxicity, convulsions, and trouble breathing. And they concluded that herbal dentifrices have potential advantages in plaque and inflammation reduction as additions to gingivitis patients' daily oral care. Before herbal medication may be prescribed definitely for oral care, preclinical and clinical investigations are required to assess biocompatibility and safety.

Ramalingam K, Amaechi BT (2020)[21] researched the antimicrobial efficacy of an Acacia arabica herbal extract combined with Triphala on cariogenic bacteria that generate biofilms. The MBC, kinetics of killing, MIC, biofilm disruption, along with the anticaries impact of MHPE against biofilm-producing cariogenic bacteria such as

L. casei S. mutans, C. albicans, and A. viscosus were determined. The structural quality of the efficacy was tested using a Scanning Electron Microscope (SEM). SEM pictures were taken of biofilm that had been produced in 6-well plates that had glass slides at the bottom of each well. Before SEM photography, MBC concentration of MHPE was used to treat the biofilm at room temperature for 30 minutes. The establishment of a caries-like lesion in a continuous flow biofilm model indicated the anticaries effect which lead to the conclusion that the MHPE formula has effective antibacterial activity and thus, it could be a useful source of anti-cariogenic agents in the coming years, as well as the faster killing activity suggests that MHPE formula has effective antibacterial activity and highlights that this material could be a useful source of anti-cariogenic agents.

Nuvvula S, Nunna M, Almaz ME, Mallineni SK (2020)[45] did a Systematic Review to investigate the efficacy of Licorice lollipops in reducing caries in children. A thorough database search was carried out. Randomized controlled trials, prospective clinical trials with healthy children in which licorice lollipops were used to decrease caries, and research published in English were all considered. Children with systemic illnesses and elderly people were not included in the studies. Before final eligibility was decided, narrative reviews, systematic reviews, conference abstracts, and letters to editors were also removed. The Cochrane Collaboration's risk of bias tool was used to assess the quality and risk of bias of the chosen studies. And found that licorice candies/ lollipops exhibited a potential benefit in preventing caries by lowering the CFUs of S. mutans in saliva with very little evidence obtained. Licorice lollipops cannot replace preventive measures such as healthy eating habits, adequate mouth hygiene, and fluoride administration. Further study using RCT

designs with high sample numbers is needed to determine the efficacy of licorice candies/lollipops in the prevention of dental caries.

Tharakan AP, Pawar M, Kale S (2020)[46] carried out a comprehensive study of the efficacy of licorice in reducing dental caries in children. All in vivo research, randomized controlled trials, and clinical studies involving licorice as a cariostatic drug in children, all types of licorice such as dentifrice, chewing gum, gels, restorations utilized as an intervention, and studies done among children aged 3–15 years were included. Licorice extracts are beneficial as an antibacterial agent by lowering the number of S. mutans in youngsters. Its impact on biofilm prevents an acidic environment that raises the risk of dental cavities by limiting the pH drop there. Furthermore, youngsters enjoy licorice in the shape of la ollipops.

Chen X, Daliri EB-M, Kim N, Kim J-R, Yoo D, Oh D-H (2020)[7] conducted a study on Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. One of the most frequent microbe-mediated oral illnesses in humans is dental caries. Caries etiology is now based on a paradigm that has four factors that involve oral bacteria, oral environment, host along time. Excessive consumption of dietary carbohydrates promotes the growth of acid-producing and acid-resistant bacteria in the oral cavity. Dysbiosis of the dental biofilm adhering to the enamel surface causes dental caries. Inhibiting cariogenic bacteria, using an anti-biofilm agent, as well as limiting the intake of sugar are all effective prophylactic measures. The objective was to lower the overall quantity of biofilm as well as the levels of individual infections. Naturally occurring products may be suggested for reducing dental caries since they may have fewer negative

effects than synthetic antimicrobials. Furthermore, natural antibacterial agents such as herbs probiotics, and spices appear to be useful in decreasing tooth caries.

Deshpande M, Baliga S, Thosar N, Rathi N, Jyothishi S, Deulkar P et al. (2021)[47] conducted a randomized controlled experiment to assess the antibacterial activity of Triphala tooth wipes on oral S.mutans counts in intellectually impaired (ID) children of 6–13 years of age with a modest degree of ID AAMD and placed in a moderate- and high-risk category for dental caries according to AAPD standards. A Triphala decoction was made. The single sachet wet wipes machine was used to manufacture the wet wipes. Strawberry flavoring ingredient was added to the tooth wipes. Within the limits of the study, the author found that Triphala tooth wipes are more effective than placebo tooth wipes against oral S. mutans, although both tooth wipes are similarly effective in decreasing dental plaque.

MATERIAL AND METHODS

The current experimental study was conducted in rural schools in Nagpur, Maharashtra, after ethical committee permission from the Institutional Ethics Committee (IEC) before the beginning of the study. Children aged 4 to 8 years old were assessed and chosen for the research based on inclusion and exclusion criteria. The goal and procedures of the study were communicated to the parents of the chosen child in the local vernacular language, and written informed consent was acquired.

SAMPLE SIZE:

The sample size was determined considering pre-test & post-test mean difference as the main outcome measure and the following assumptions were made by the study done by Aparna et al (2018).[6]

$$n = \frac{(Z\alpha + Z\beta)^2 Sd^2}{d^2}$$

where;

Zα =Standard normal value corresponding to a specified level of α

Zβ =Standard normal value corresponding to a specified level of β

Sd=Standard Deviation of the [pre-post] differences

d=Effective size or mean difference

ASSUMPTIONS:

1. Pre test mean CFU/ml =6.4
2. Post test mean CFU/ml =3.8
3. SD in pre test =4.5
4. SD in post test =4.34
5. Effect size (mean difference) =2.6
6. α error = 5%
7. Power (1- β) =80%

Required sample size(n) = 25(per group)

Therefore, a total of n=75 subjects were included in this study.

SAMPLING:

A random sampling method was used for sampling. Randomization was done by the lottery method. This is a single-blind study where patients were blinded.

INCLUSION CRITERIA:

- Healthy children of 4-8 years of age.
- Children with parental consent.
- defs index ranging from 0-5

EXCLUSION CRITERIA:

- Uncooperative children.
- Children with any systemic illness.
- Children on antimicrobial therapy in last one month.

WITHDRAWAL CRITERIA:

The calculated sample size was n=75, and the final sample size including a 10% drop out was n=83. This was a school-based study where subjects were selected by a random sampling method.

DATA COLLECTION TOOLS:

1) Sterile disposable gloves, mouth mask, and head cap **(Fig.1)**

2) Sterile swab

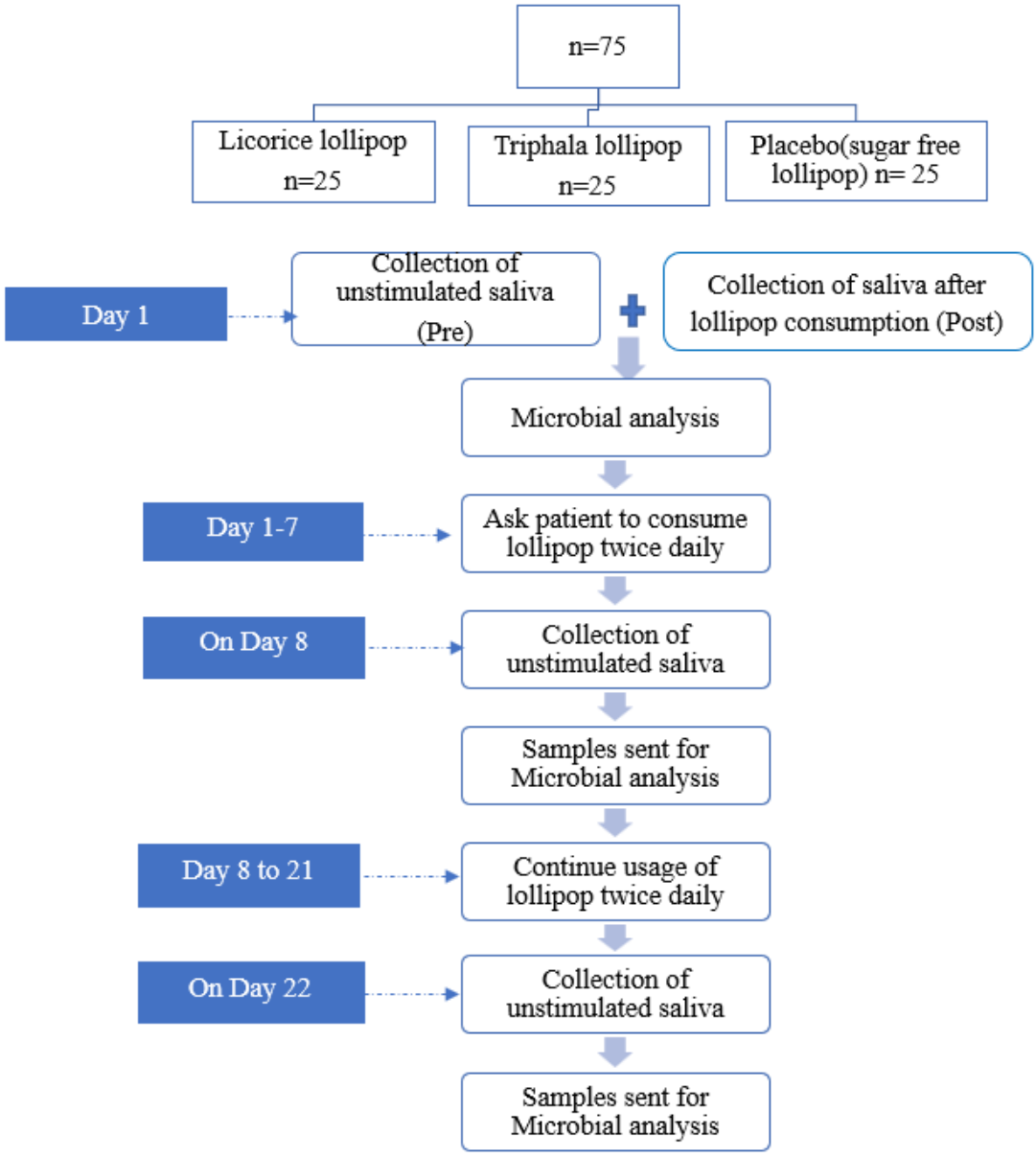
3) 0.5 ml micropipette

4) Disposable cup/Vial **(Fig.2)**

5) Test tube

6) Diagnostic Instruments: Mouth mirror, probe, and tweezers, Cotton rolls **(Fig.1)**

PROCEDURE:



To measure the defs index in children, an oral examination was done by using a mouth mirror and probe **(Fig.1).** Children with def scores ranging from 0 to 5 were included in the research as per the inclusion criteria.

BASELINE EXAMINATION:

Children were screened at the baseline, and those who met the inclusion criteria were enrolled in the research after their parents submitted informed consent. Children were told to arrive on an empty stomach the day before. Saliva samples were obtained in the early morning, just after the children arrived at school and before they ate breakfast. The kids were asked to spit normal saliva into a disposable cup **(Fig. 3),** then transfer it with a 0.5 ml pipette into a test tube containing a fixing solution and shake it for 5 seconds. The first unstimulated baseline measurement was taken the day before the lollipop regimen began. Each kid was then offered a lollipop.

	Group 1	Group 2	Group 3
Interventions	Triphala lollipop	Licorice lollipop	Placebo (Sugar-free lollipop)

After eating lollipops, the children were requested to spit saliva into a disposable cup, and 0.5 ml of saliva was pipetted into a test tube with a fixing solution and shaken for 5 seconds. Both the unstimulated saliva and the saliva following lollipop intake were kept in the fridge until they were transported to the lab. Then S. mutans count was calculated on (CFU) plate. Until the saliva sample collection on days 7,14 and 21; children were asked to eat lollipops twice a day. And the same procedure was carried out for saliva sample collection in all the follow-up days i.e.,7 14, and 21.

METHOD OF DATA MEASUREMENT:

Selective Culture Assay was used for sample culturing.

Preparation of the extract (Fig 4):

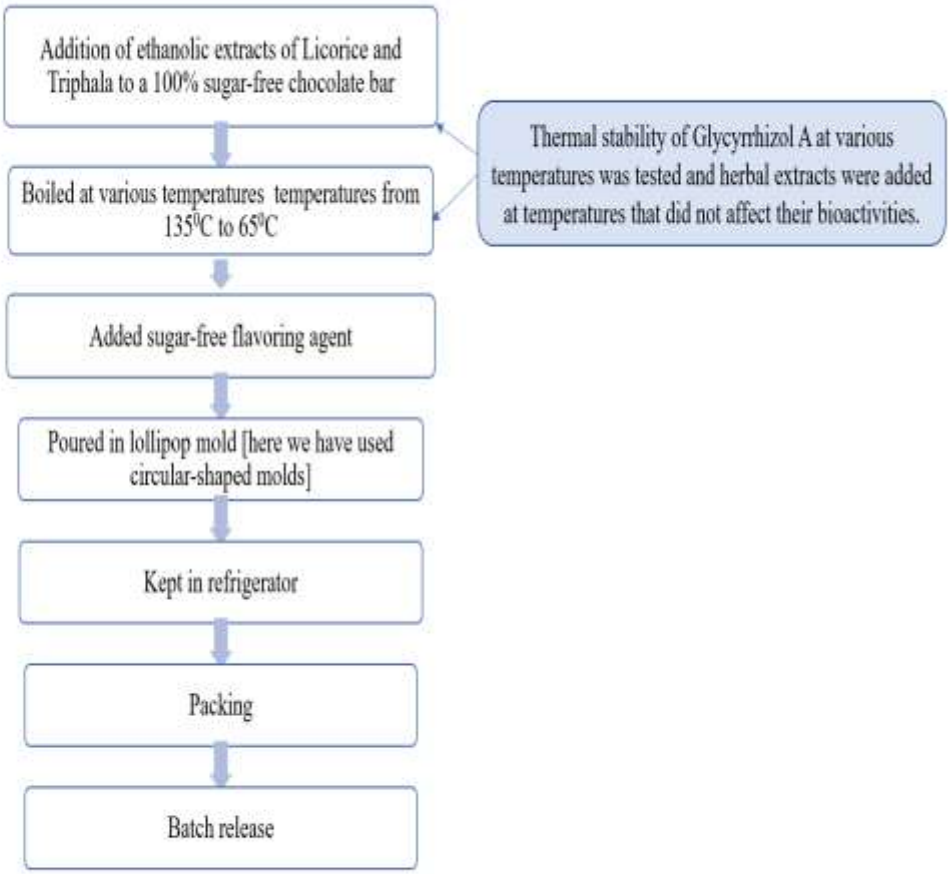
Triphala extract (Fig 5):

Triphala dried ripe fruit was gathered and processed into a fine powder. It was combined in a round-bottomed flask with 10 times the volume of sterile distilled water, and the suspension was maintained at 4°C for 72 hours. Decantation of the aqueous extract was done followed by its purification by muslin cloth filtration and evaporated at 40°C in a flat-bottomed porcelain dish.[9] The dried extract was suspended in polyethylene glycol (20% v/v) and evaporated with distilled water to get the final concentration.

Licorice Root Extract (Fig 6):

Licorice root was sliced into thin slices and steeped in extraction containers with 95% ethanol in a 1:8 ratio for 72 hours at 37^0 C. The resultant ethanol solutions (containing extracts) were filtered three times. The extract was then concentrated by evaporating ethanol followed by drying using a microwave dry distillation process, which combines microwave heating and dry distillation at air pressure.

Preparation of lollipop (Fig. 8):



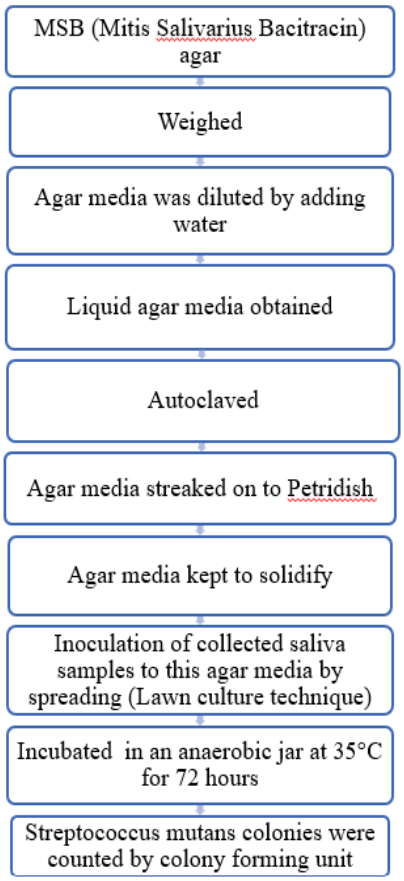
Lollipops were wrapped in three different colorful wrapping papers to distinguish them from one another **(Fig.9).**

Lollipop use (Fig 10, and Fig. 11):

Children were asked to consume the lollipop for 10 minutes. Then saliva sample was taken after 10 minutes and 90 minutes of lollipop consumption in the afternoon.[9] To ensure that the lollipops were enjoyed securely, the children were placed in a circle. To assist the youngsters, comprehend that these lollipops are not the same as others and are not delicious candy, education materials and recommended activities were offered. Puzzles, storybooks, films, and games kept the kids interested in the research and on track with the procedure.[1]

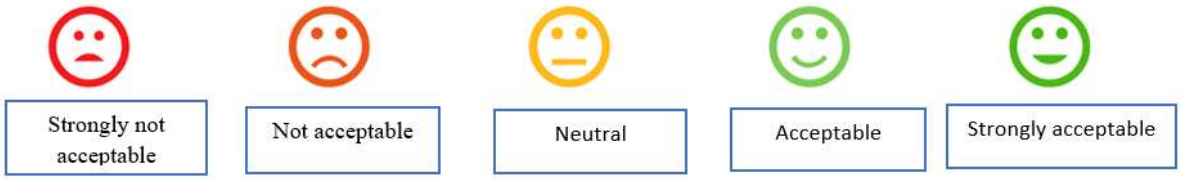
Salivary microbial analysis (Fig.12, to Fig 23)[9]

In the present study, MSB agar was used for culturing of S. mutans as a special media.



Likert scale:

The palatability of lollipops among children was assessed by using 5 points Likert scale.



COLOR PLATE I

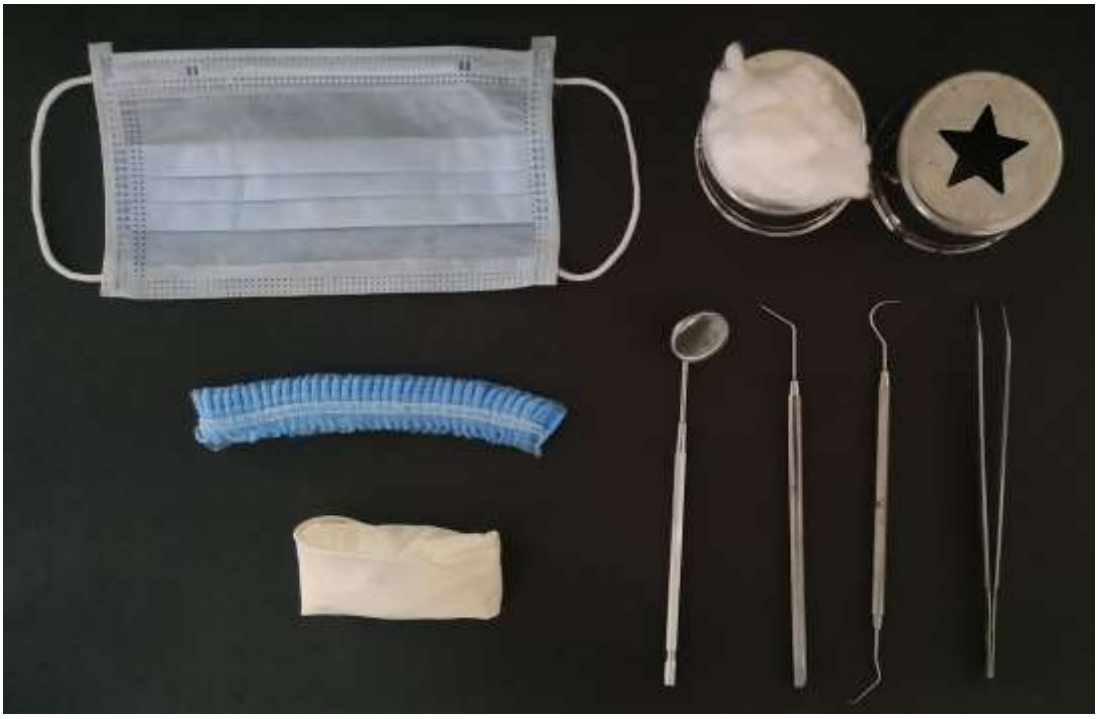


Fig 1. The diagnostic instrument, gloves, face mask, headcap

COLOR PLATE II

Fig. 2 Vials for collection of saliva samples

Fig. 3 Collection of saliva samples

COLOR PLATE III

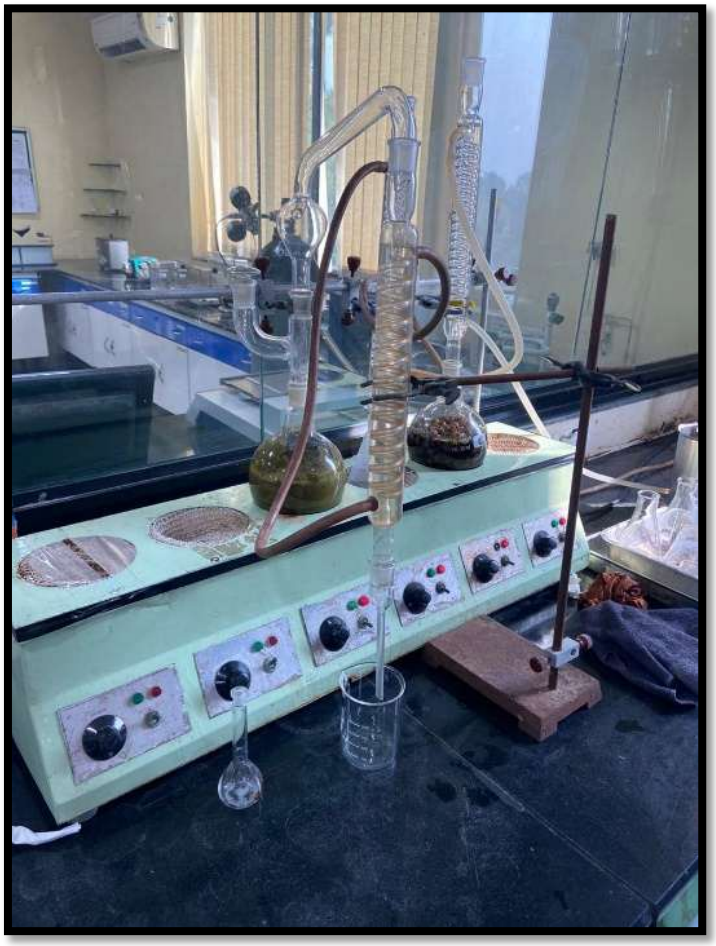


Fig. 4 Extraction

(Round bottom flask, Y junction, condensator connector, spiral condenser beaker)

COLOR PLATE IV

Fig.5 Triphala extract

Fig.6 Licorice extract

Fig. 7 Sugar free sugar

COLOR PLATE V

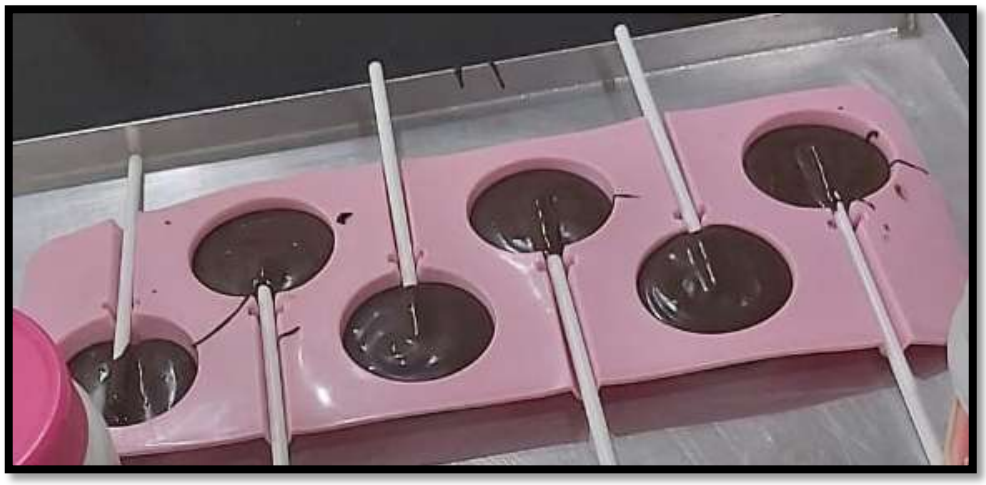


Fig. 8 Preparation of lollipops

Fig. 9 Triphala Lollipop, Licorice Lollipop, Placebo Lollipop

COLOR PLATE VI

Fig.10 Distribution of Lollipops

Fig.11. Children enjoying a lollipop

COLOR PLATE VII

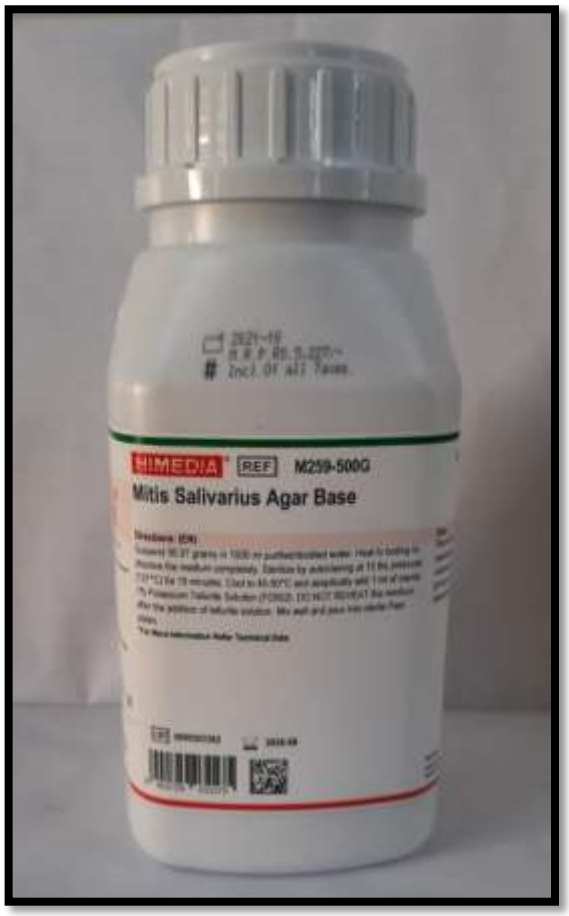


Fig.12 Mitis Salivarius Agar Base (selective media for S. mutans)

COLOR PLATE VIII

Fig.13 Anaero Gas Pack pouch for incubation of anaerobic organisms

COLOR PLATE IX

Fig. 14 Media before autoclaving

Fig.15 Media after autoclaving

COLOR PLATE X

Fig.16 Preoperative saliva samples

Fig.17 Postoperative saliva samples

COLOR PLATE XI

Fig. 18 Pouring Media

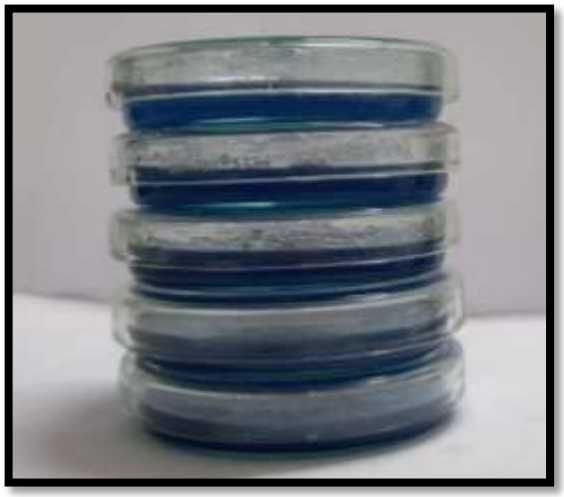


Fig. 19 Media plates after solidification

COLOR PLATE XII

Fig. 20 Addition of Sample- 100 µl

Fig. 21 Spreading (Lawn culture technique)

COLOR PLATE XIII

Fig. 22 Anaerobic jar with Gas packs

Fig. 23 Incubated for 24-48 hours

COLOR PLATE XIV

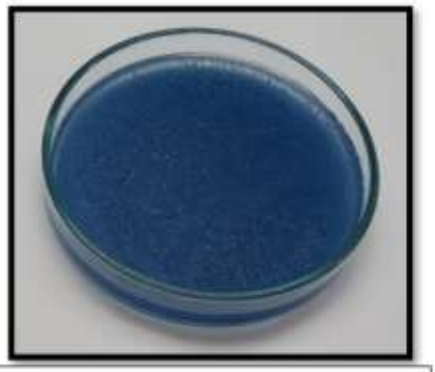


Fig.24 (a) S. mutans CFU before consumption of Triphala Lollipop

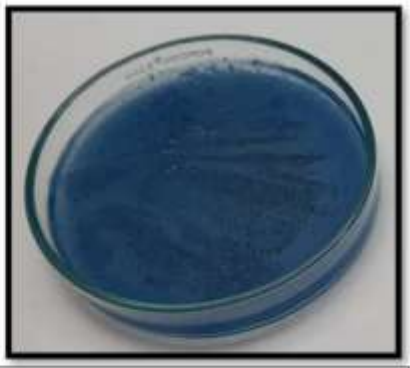


Fig.24 (b) S. mutans CFU After consumption of Triphala Lollipop

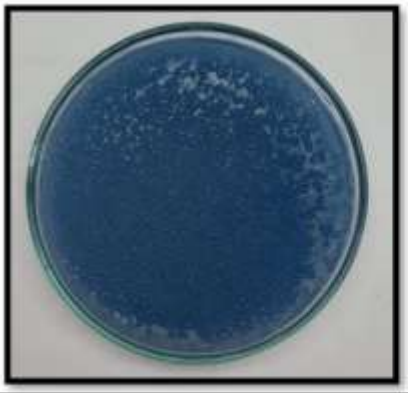


Fig.25 (a) S. mutans CFU before consumption of Licorice Lollipop

Fig.25 (b) S. mutans CFU after consumption of Licorice Lollipop

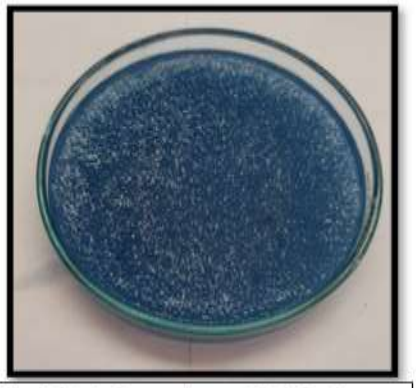


Fig.26 (a) S. mutans CFU before consumption of sugar free lollipop (Placebo)

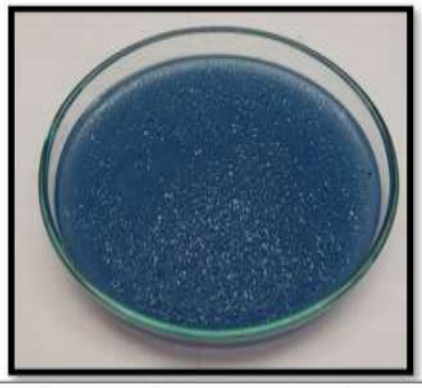


Fig.26 (b) S. mutans CFU after consumption of sugar-free lollipop (Placebo)

RESULTS

The present study was single blind, randomized controlled clinical study. 75 children aged 4-8 years, were enrolled based on the inclusion criteria and were randomly divided into 3 groups of 25 each. The antimicrobial efficacy of Triphala, Licorice and Placebo was compared.

Group A: Triphala

Group B: Licorice

Group C: Placebo

Children were screened at the baseline, and samples were obtained in the early morning just after the children arrived at school and before they ate breakfast. The first unstimulated baseline measurement was taken the day before the lollipop regimen began. Each child was then offered a lollipop. After consumption of lollipops, saliva samples were collected. Both the unstimulated saliva and the saliva following lollipop intake were kept in the fridge until they

were transported to the lab. Then S. mutans count was calculated on (CFU) plate. Till the saliva sample collection on days 7,14 and 21; children were asked to eat lollipops twice a day. And the same procedure was carried out for saliva sample collection in all the follow-up days i.e.,7 14, and 21.

Statistical methods:

Statistical analysis of **comparative evaluation of anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years: An Experimental Study** was carried out to find the significant difference between those values. Analysis of the data was done by using descriptive and inferential statistics both.

The software used in the analysis was SPSS 24.0 and Graph Pad Prism 7.0 version and $p<0.05$ is considered as the level of significance.

The statistical tests used for the analysis of the result were:

1. One way ANOVA
2. Tukey Multiple Comparison Test
3. Chi-square Test

Descriptive Statistics :

1. **Arithmetic Mean**: The arithmetic mean, or average, is the sum of the values divided by the number of values.

Formula:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where:

$\overline{X}$ = Sample arithmetic mean

n = Sample size

$X_i = i^{th}$ Observation of the random variable X

$\sum_{i=1}^{n} X_i$ = Summation of all the X_i values in the sample

2. Standard Deviation (SD) =

$$\sqrt{\frac{\Sigma(X - \overline{X})^2}{(n - 1)}}$$

where:

X = each score
$\overline{X}$ = the mean or average
n = the number of values
Σ means we sum across the values

3. Mean percentage=Total Score/no of questions

4. Max/Min = Maximum/Minimum value of score

Inferential Statistics :

1. One way ANOVA

A One-Way Analysis of Variance is a way to test the equality of three or more

$$\bar{X}_{GM} = \frac{\sum n\bar{x}}{\sum n} \qquad \bar{X}_{GM} = \frac{\sum x}{N}$$

Total Variation

The total variation (not variance) is comprised of the sum of the squares of the differences of each mean with the grand mean.

$$SS(T) = \sum (x - \bar{X}_{GM})^2$$

Between Group variation

The variance due to the interaction between the samples is denoted MS(B) for Mean Square Between groups. This is the between-group variation divided by its degrees of freedom. It is also denoted by.

$$SS(B) = \sum n(\bar{x} - \bar{X}_{GM})^2$$

Within Group Variation

The variance due to the differences within individual samples is denoted MS(W) for Mean Square Within groups. This is the within-group variation divided by its degrees of freedom. It is $SS(W) = \sum df \cdot s^2$ also denoted by. It is the weighted average of the variances (weighted with the degrees of freedom).

2. Tukey Multiple Comparison Test

$$HSD = \frac{M_1 - M_2}{\sqrt{MS_w \left[\frac{1}{n}\right]}}$$

Where

HSD - Honestly Significant Difference

M1 ,M2 are mean values

MSw - Mean Square Width

n - Number per mean

3. Chisquare Test

Chi Square Test

- Formula
  - o = observed data in each category
  - e = observed data in each category based on the experimenter's hypothesis
  - ∑ = Sum of the calculations for each category

$$X^2 = \sum \frac{(o - e)^2}{e}$$

Results

Distribution of children according to their age

4% of patients in the Triphala Lollipop group and 12% in the Licorice Lollipop group were 4 years of age, each 20% of the patients in the Triphala Lollipop and Licorice Lollipop group and 16% in the placebo group were 5 years of age, each 16% of the patients in Triphala group and placebo group and 20% in Licorice

Lollipop group were 6 years of age, each 36% of the patients in Triphala Lollipop and placebo group and 20% in Licorice Lollipop group were 7 years of age and 24% of the patients in Triphala Lollipop group, 28% in Licorice lollipop group and 32% in the placebo group were 8 years of age. By using the **Chi-square test statistically, no significant difference was found in the ages** of the patients of three groups(χ2-value=5.47,p=0.70) **[Table 1,Graph 1]**

Distribution of children according to their gender

56% of patients in the Triphala Lollipop group, 48% in the Licorice Lollipop group, and 52% in the placebo group were males and 44% of patients in the Triphala Lollipop group, 52% in Licorice group, and 48% in the placebo group were females. By using the **Chi-square test statistically, no significant difference was found in the gender of the patients of three groups**(χ2-value=0.32,p=0.85) **[Table 2,Graph 2]**

Comparison of defs index in patients of three groups

The mean defs index in patients of the Triphala Lollipop group was 2.76±1.64, in the Licorice group it was 2.84±1.57, and in patients of the placebo group, it was 2.64±1.65. By using one-way **ANOVA statistically no significant variation was found in the defs index score of patients of three groups** (F=0.096, p=0.908). By using multiple comparison **Tukey Test statistically no significant difference was found in defs score among** patients of Triphala Lollipop and Licorice Lollipop group(p=0.983), Triphala Lollipop and placebo group(p=0.963) and between Licorice Lollipop and placebo group(p=0.901) **(Table 3, Graph 3).**

Comparison of Streptococcus mutans count in Triphala Lollipop group with baseline

Mean Streptococcus mutans count at baseline was 122.32±15.56, within 24 hours it was 80.72±19.34, at 7 days it was 31.24±7.68 and at 21 days it was 10.96±4.48. By using **Student's paired t-test statistically significant difference was found in Streptococcus mutans counts at 24** days(t=10.45,p=0.0001), at 7 days(t=26.59,p=0.0001) and at 21 days(t=35.08,p=0.0001) when compared score at baseline **(Table 4,Graph 4).**

Comparison of Streptococcus mutans count in Licorice Lollipop group with baseline

Mean Streptococcus mutans count at baseline was 129.84±33.45, within 24 hours it was 53.48±13.79, at 7 days it was 9.12±3.50 and at 21 days it was 3.52±2.56. By using **Student's paired t-test statistically significant difference was found in Streptococcus mutans counts at 24** days(t=11.53,p=0.0001), at 7 days(t=17.99,p=0.0001) and at 21 days (t=18.97,p=0.0001) when compared score at baseline **(Table 5,Graph 5).**

Comparison of Streptococcus mutans count in Placebo group with baseline

Mean Streptococcus mutans count at baseline was 122.40±33.36, within 24 hours it was 129.36±34.62, at 7 days it was 131.76±32.71 and at 21 days it was 147.24±26.98. By using **Student's paired t-test statistically significant difference was found in Streptococcus mutans counts at 24** days(t=6.63,p=0.0001), at 7

days(t=2.47,p=0.021) and at 21 days (t=3.67,p=0.001) when compared score at baseline **(Table 6,Graph 6).**

Comparison of Streptococcus mutans count in patients of three groups

Mean streptococcus mutans count in patients of the Triphala Lollipop group was 122.32±15.56, in the Licorice group it was 129.84±33.45, and in the placebo group, it was 122.40±33.56. **By using one-way ANOVA statistically no significant variation was found in Streptococcus mutans count among patients of three groups(F=0.62). By using multiple comparisons: Tukey test statistically no significant difference was found** between Triphala Lollipop and Licorice group(p=0.62), Triphala Lollipop and placebo group(p=1.0), and between Licorice Lollipop and Placebo group(p=0.63) **(Table 7,Graph 7).**

Within 24 hrs mean streptococcus mutans count in patients of the Triphala Lollipop group was 80.72±19.34, in the Licorice group it was 53.48±13.79, and in the placebo group it was 129.36±35.62. **By using one-way ANOVA statistically, significant variation was found in Streptococcus mutans count among patients** of three groups(F=62.83,p=0.0001)). By using multiple comparisons: the Tukey test statistically significant difference was found between Triphala Lollipop and Licorice group(p=0.0001), Triphala Lollipop and placebo group(p=0.0001), and between Licorice Lollipop and Placebo group(p=0.0001) **(Table 7,Graph 7).**

Within 7 days mean streptococcus mutans count in patients of the Triphala Lollipop group was 32.24±7.68, in the Licorice group it was 9.12±3.50, and in the placebo group it was 131.76±32.71. By using **one-way ANOVA statistically, significant variation was found in Streptococcus mutans count among patients of**

three groups(F=280.73,p=0.0001)). By using multiple comparisons: the Tukey test statistically significant difference was found between Triphala Lollipop and Licorice group(p=0.0001), Triphala Lollipop and placebo group(p=0.0001), and between Licorice Lollipop and Placebo group(p=0.0001). **(Table 7,Graph 7).**

Within 21 days mean streptococcus mutans count in patients of the Triphala Lollipop group was 10.96±4.48, in the Licorice group it was 3.52±2.56, and in the placebo group it was 147.24±26.98. **By using one-way ANOVA statistically, significant variation was found in Streptococcus mutans count among patients of three** groups(F=650.46,p=0.0001)). **By using multiple comparisons: Tukey test statistically no significant difference was found between Triphala Lollipop and Licorice group(p=0.228) and a significant difference was found between Triphala Lollipop and placebo group(p=0.0001) and between Licorice Lollipop and Placebo group(p=0.0001). (Table 7,Graph 7).**

Comparison of Palatability among patients of three groups

Palatability score was acceptable in each 88% of the patients of Triphala Lollipop and and 44% in the placebo group, palatability was strongly acceptable in all(100%) patients of Licorice Lollipop group and 12% patients of the placebo group and was neutral in 12% patients of Triphala Lollipop group and 28% of the placebo group. By **using the Chisquare test statistically significant difference was found in the Palatability score among patients of three** groups(χ2-value=77.33,p=0.0001). **(Table 8 ,Graph 8).**

DISCUSSION

Dental caries is an infectious condition that occurs due to some cariogenic bacteria and certain dietary habits.[48–50] Some well-known cavity-causing bacteria are S. mutans, S. sobrinus, and L. casei with S. mutans being the most important contributor to acid production. The majority of bacteria that cause cavities inhabit the surfaces of teeth and produce acids that destroy tooth structures. Repeated episodes of disintegration cause cavities and necessitate restoration of the affected sections.[25] Salivary S. mutans levels have previously been utilized as a potential risk factor for dental caries, with individuals showing higher risk of developing caries with higher levels than those with lower levels.[51] In spite of strategies that prevents caries; such as pit and fissure sealants, fluoride, antibacterial agents, as well as probiotics have considerably contributed to reducing the prevalence of caries, which are simpler preventive approaches and would encourage more general adoption and improve accessibility.[52,53] A high sugar diet increases the risk of caries, an ideal therapy

would be to replace sugar consumption with nutritive sources while also restricting bacterial development.[43]

Long-term use of chemicals in various forms would adversely affect oral health such as alteration in taste, staining, increase in tartar formation, dry mouth, mouth irritation, etc., Conventional antibiotics are often effective for treating bacterial infections, but because of increased incidences of antibiotic resistance, there is a continuing need for alternative medicines. As a result, natural medicines are now chosen over synthetic antibiotics.[18] There are various chemicals used to prevent caries. Ayurvedic herbs have minimalistic adverse effects henceforth, we could use these ayurvedic herbs possessing anti-cariogenic properties for prevention of dental caries. Ayurvedic herbs have been employed as traditional treatment methods for several human illnesses in many regions of the world since ancient times.[54] These natural products and plant extracts contain high concentration of physiologically active chemicals, making them viable alternatives to commonly used medicines.[55] According to WHO, four billion individuals in the developing world rely on ayurvedic herbal goods as their major source of healthcare and traditional medical practice.[56] There is a movement toward ayurvedic herbs because they operate by boosting the organisms' innate physiological activities and defensive healing responses. Many contemporary medications, on the other hand, block vital biological activities and obstruct natural healing processes.

The primary advantages of employing ayurvedic herbs are no side effects, easy availability, low cost and toxicity, lack of microbial resistance along improved shelf life.[11,57] Several phytochemicals, including antibacterial substances, isolated

from edible plants have been proven to exhibit antibacterial action against S.mutans by reducing their numbers, including Licorice and Triphala.[58]

Licorice has anti-cariogenic properties with no adverse effects when used within a safe/recommended dose. In several countries, it is frequently used as a sweetening agent.[43] The presence of active principles in the phytochemical analysis also validates the clinical findings. The antimicrobial activity may be mostly due to tannins, triterpenoid saponins, and flavonoids. The presence of saponins also confirmed the extracts to be positive for glycyrrhizin, the active principle known to reduce bacterial growth and acid production.[14]

Triphala is a pronounced polyherbal formulation in Ayurveda.[6] Triphala has a noble place among the numerous herbal remedies used in Ayurveda due to its broad favorable systemic activities. Triphala is a blend of T. bellirica, T. chebula, and E. officinalis fruits.[35] Triphala's antibacterial properties are widely described in the literature. This effect of Triphala is because of the tannic acid, which gets adsorbed well to the hydroxyapatite of the tooth or the salivary mucins, alternately it binds to anionic groups on the surface of the bacterial cells.[59,60] Gupta et al. reported that all the concentrations of ethanolic and aqueous extracts of Triphala inhibited the growth of S. mutans, S. sanguis, and S. salivarius.[61]

Licorice when delivered as a mouth rinse, the duration was found to be too short to provide optimum contact of the herbal extracts with the oral cavity. Moreover, the sweet taste promoted early swallowing of saliva and consequent washout of the residual drug from the oral cavity. Therefore, the property of substantivity was not evident in Licorice extracts. The consumption of lollipops is

common in children. In addition, lollipops are good stimulators of saliva which has self-cleaning properties. As a result, sugar-free candies were developed, with the herbal extract that served as a carrier to deliver the naturally occurring antibacterial component. The objective was to supply antimicrobial components through a similar mechanism that causes dental caries, i.e., to utilize a behavior that creates the issue (sugar intake) to solve the problem (dental caries). This strategy allows access to the at-risk population while requiring no behavioral changes. Among the several forms of sweets, lollipops are acceptable and can be safely delivered to children they were selected.

The mode in which Licorice is delivered to the subjects is a major criterion to effectuate prolonged duration of action. Licorice, delivered as a hard candy or chewing gum, would have an extended release in the mouth, which would significantly enhance its sustained action.[14,25]

As there are various studies in which Triphala has been used as the main ingredient in preparing mouth rinse because of its antimicrobial properties.[6,9,11,16,20,32] But there is not a single study on Triphala in the form of lollipop. Hence the present study was aimed to evaluate antimicrobial efficacy of Triphala in the form of lollipop.

Furthermore, sucking on lollipops stimulates salivary secretion, which benefits remineralization, and because of the dissolving rate and volume; teeth receive the most exposure time to the herbal extracts. Because a lollipop takes longer to disintegrate (10–15 minutes) than other candy shapes, this adds substance to the distribution mechanism.[62]

Hence, these two ayurvedic herbs were incorporated in lollipops to make them beneficial to children.

In our study, each child was given lollipop for 21 days. This was in similar with the pilot study done by Janet et al, in which decrease in S. mutans with increased lollipops ingestion was seen in observe period for 21 days. Furthermore, in our study, administration of herbal Licorice lollipop twice a day lead to a significant reduction in the levels of S. mutans. This was in accordance with the findings of the pilot study in which they carried out 3 weeks follow up study in which children were asked to consume lollipop twice daily.[(63)] No side effects were found in this study. Hence, we intended to deliver the antimicrobial effects of Licorice in the form of lollipops.

Results of the current study showed a difference that was statistically significant in the decrease of S. mutans counts within 24 hours when compared to the baseline score. Furthermore, in the reduction of S. mutans count, a statistically significant difference was seen after 7 and 21 days of follow-up, respectively. This was in accordance with the study done Chen et al. in 2019,who found a significant reduction in salivary S. mutans levels in Chinese preschool children using Licorice lollipop twice daily for 3 weeks which lead to a conclusion that household use of Licorice lollipop should be considered as a simple and effective way to reduce dental caries risk in children.[(43)] Mentes et al in the year 2012, carried out a study where no increase in the S. mutans was observed after 21 days i.e after withdrawal of lollipop. The reason for this may be the substantivity of the Licorice. Therefore, in the present study, it was planned a 7 and 21 days follow up study.[(63)]

Results of the randomized control trial by Almaz et al 2016 showed that herbal lollipops containing licorice root extract were efficient in reducing salivary S. mutans levels in high-risk children when asked to consume it twice a day for 10 days.[38] Furthermore, Sug-Joon Ahn (2012) investigated the antimicrobial effects of DG-LRE on S. mutans in both the planktonic as well as biofilm phases, leading to the conclusion that LRE can be used in the development of oral hygiene products to help prevent dental caries as DG-LRE showed significant antimicrobial activity against S. mutans in the planktonic phase.[30]

Unlike CHX, antibacterial substantivity of Triphala was not evaluated so far. Naiktari et al 2019 had done a study to Determine the antibacterial substantivity of Triphala mouthwash and comparing it with CHX promoting its frequency of usage for the maximum efficiency. Triphala mouthrinse has an antibacterial effect for 3–4 h after a single rinse. It can be used for at least 3 times daily for its maximum antibacterial effect. CHX remains the gold standard providing maximum antibacterial substantivity of 7–8 h.[59]

Deshpande et al. conducted another study in 2021 to test the antibacterial performance of Triphala tooth wipes on oral S. mutans counts and discovered the effectiveness of Triphala tooth wipes against oral S. mutans in decreasing dental plaque.[47] As a result, this helped our research in documenting Triphala as an anti-cariogenic agent in lollipops. In the present study, a group of children who consumed Triphala lollipop showed a reduction of S. mutans counts.

In our study, a sugar free lollipop without any ayurvedic herbal formula was used as control group (Placebo). As this placebo had the same ingredients as the other

two herbs i.e. Licorice and Triphala but with no active component. A sugar free lollipop was used as a control group to rule out whether reduction in S. mutans count is because of the salivary stimulation only or due to the main antimicrobial ingredient that reduced the S. mutans count.

Results of the present study showed the reduction of S. mutans count with the Triphala lollipop group was statistically significant when compared to the Licorice lollipop group and placebo. However, Licorice lollipop showed comparatively maximum reduction in S. mutans count at baseline, within 24 hours, at 7 and 21 days.

A five-point Likert scale was chosen to assess the palatability of these sugar-free herbal lollipops. While in a study by Jain E et al a self-designed questionnaire was taken to assess the palatability of licorice extracts among children.[(14)]

Despite of the boon of anticariogenicity of the Licorice and Triphala, taste may hamper the use of them. Being an ayurvedic natural herb both Licorice and Triphala has an ayurvedic tint. Although, Licorice is sweet in taste but Triphala tastes a bit bitter. To alter the bitterness of the herb, a flavoring agent can be added. Also, papermint can be a good choice as a flavoring agent. But papermint being a natural oil, it has to undergo testing to see whether it can be use it for taste alternation.

Limitations

1. Sample size per group should have been large to provide stronger and more accurate results. Study could have been done in other geographic areas too.

2. As ayurvedic herbs possesses a bit pungent taste and not very palatable, so taste enhancers like sugar free sweetening agents, flavoring agents can be added to increase the acceptance among children.

3. Long-term follow up study could be done for more accurate results.

Future scope:

Future is in the phase of increasing demand and fast-growing market of herbal medicines and other herbal healthcare products, in both developing and developed countries of the world.[64,65]

-Few Ayurvedic herbs has to undergo some procedures before coming to market as an extract which might make ayurvedic product costly.

-Ayurvedic herbs are beneficial hence should be incorporated with regular consumable products in day-to-day life so various other delivery systems need to be explored.

-To get these ayurvedic herbs in regular usage; promotion and awareness should be done .

SUMMARY AND CONCLUSION

The current study was conducted to assess and compare the anti-microbial activity of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years. The children were assigned randomly to one of three groups as per the inclusion and exclusion criteria: Triphala is in Group 1, Licorice is in Group 2, and Placebo is in Group 3. Before distributing lollipops to the various groups, baseline saliva samples were collected (pre saliva samples). Within 24 hours, saliva samples were taken again but after delivering the lollipops (post saliva samples). After that, follow-ups were taken for 7 and 21 days. During follow-up, children were asked to eat lollipops twice a day for 7 and 21 days respectively. Saliva samples were collected again on day 7 and 21 and sent for microbial analysis. After the collection of data, the master chart was prepared and sent for statistical analysis.

The results of a 21-day follow-up investigation revealed a reduction in S. mutans count in all three groups. However, the greatest reduction in S. mutans count

was found in the Licorice lollipop group followed by the Triphala lollipop group and the placebo group. Furthermore, a statistically significant difference was found. In addition to this, it can also be concluded that,

1. Both Licorice and Triphala lollipops had antimicrobial effect on S. mutans count.

2. Licorice lollipop had enhanced antimicrobial effect as compared to Triphala lollipops.

3. Though the acceptance due to taste can be modified in future studies.

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TABLES AND GRAPHS

Table 1: Distribution of children according to their age

Age(yrs)	Triphala Lollipop	Licorice Lollipop	Placebo	χ^2-value
4 yrs	1(4%)	3(12%)	0(0%)	
5 yrs	5(20%)	5(20%)	4(16%)	
6 yrs	4(16%)	5(20%)	4(16%)	
7 yrs	9(36%)	5(20%)	9(36%)	5.47
8 yrs	6(24%)	7(28%)	8(32%)	P=0.70, NS
Total	25(100%)	25(100%)	25(100%)	
Mean±SD	6.56±1.19	6.32±1.40	6.84±1.06	
Range	4-8 yrs	4-8 yrs	5-8 yrs	

Graph 1: Distribution of children according to their age

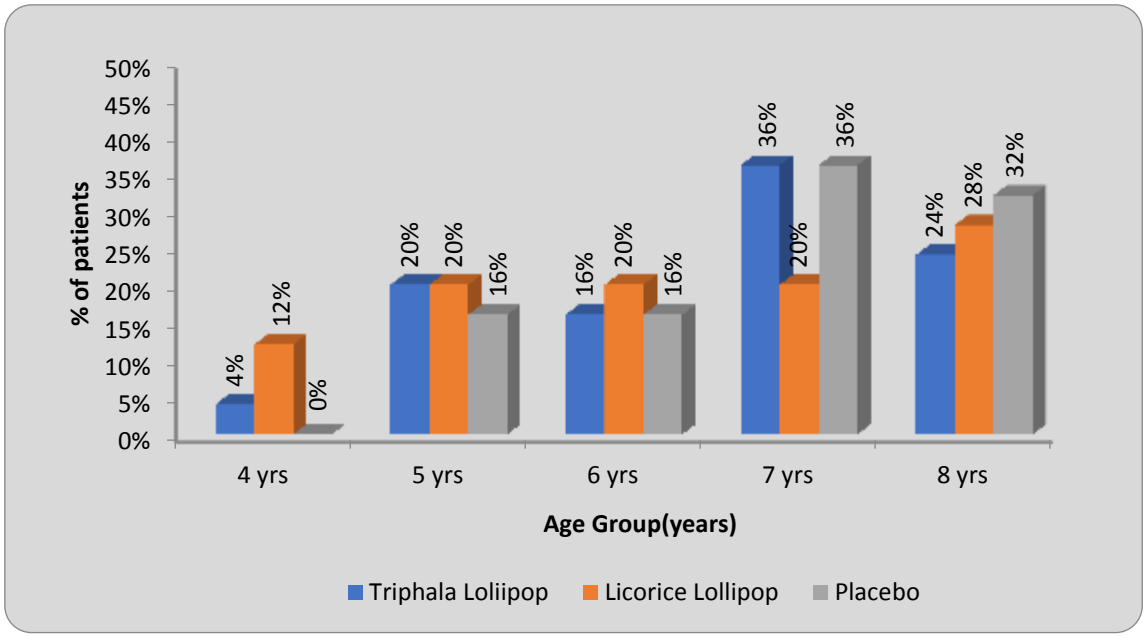


Table 2: Distribution of children according to their gender

Gender	Triphala Lollipop	Licorice Lollipop	Placebo	χ^2-value
Male	14(56%)	12(48%)	13(52%)	0.32
Female	11(44%)	13(52%)	12(48%)	P=0.85, NS
Total	25(100%)	25(100%)	25(100%)	

Graph 2: Distribution of children according to their gender

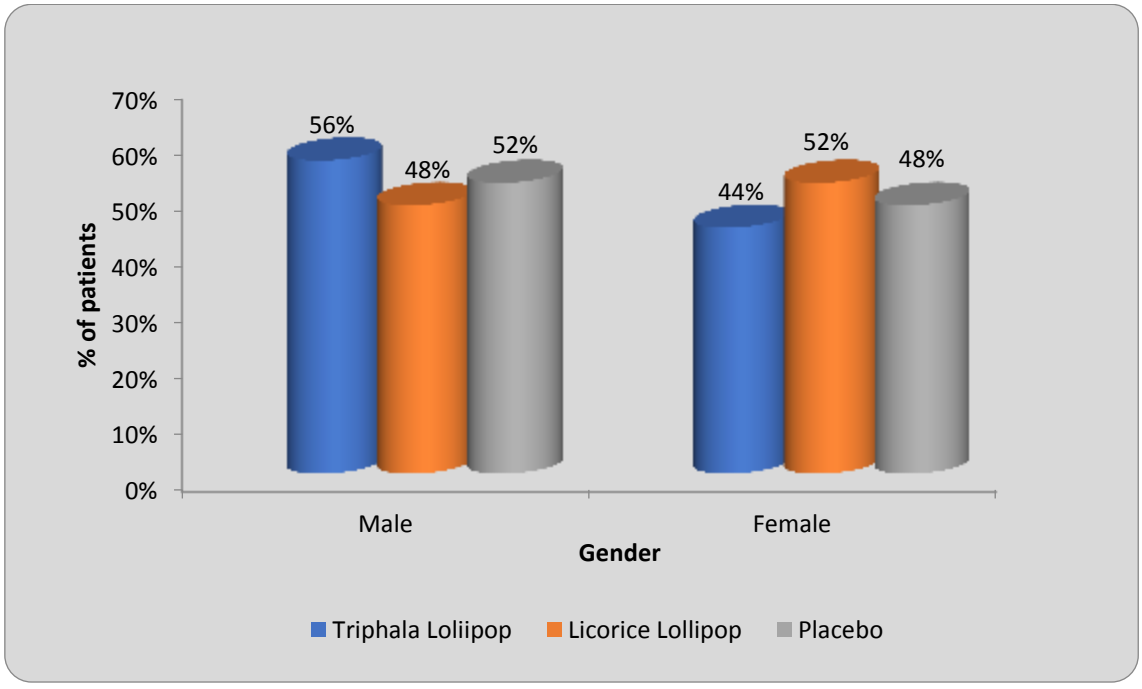


Table 3: Comparison of defs index in patients of three groups

Descriptive Statistics

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Triphala Lollipop	25	2.76	1.64	0.32	2.08	3.43	0.00	5.00
Licorice Lollipop	25	2.84	1.57	0.31	2.19	3.48	0.00	5.00
Placebo	25	2.64	1.65	0.33	1.95	3.32	0.00	5.00

One Way ANOVA

Source of variation	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.50	2	0.25	0.096	0.908, **NS**
Within Groups	189.68	72	2.63		
Total	190.18	74			

Multiple Comparison: Tukey Test

Group	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound

Graph 3: Comparison of defs index in patients of three groups

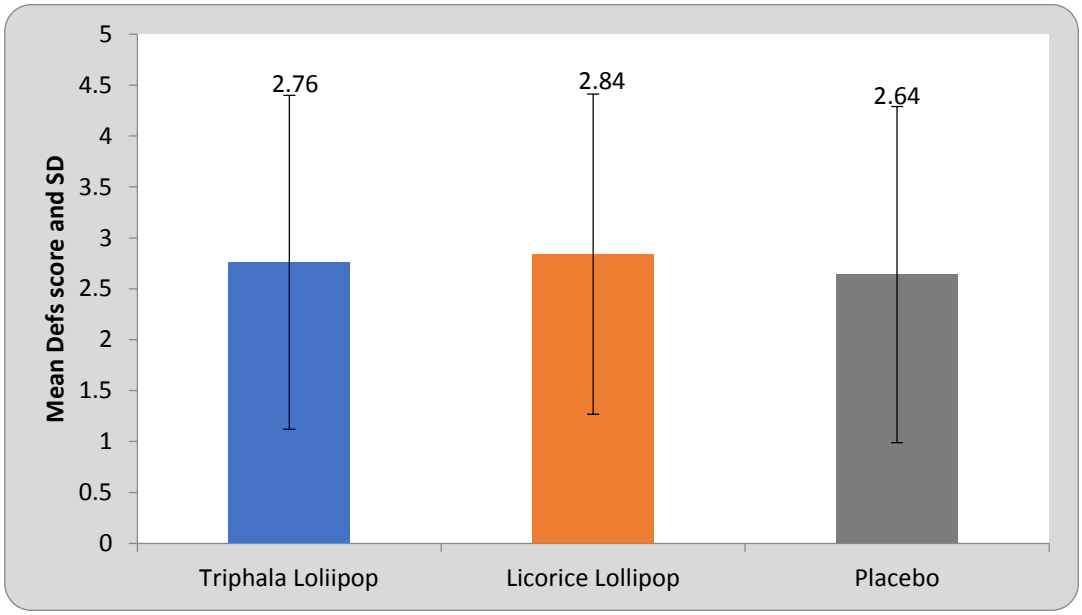


Table 4: Comparison of Streptococcus mutans count in Triphala Lollipop group with baseline

Student's paired t-test

Time Period	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	t-value
Baseline	122.32	25	15.56	3.11	-	-
Within 24 hrs	80.72	25	19.34	3.86	41.60±19.89	10.45 P=0.0001, S
7 days	31.24	25	7.68	1.53	91.08±17.12	26.59 P=0.0001, S
21 days	10.96	25	4.480	0.89	111.36±15.87	35.08 P=0.0001, S

Graph 4: Comparison of Streptococcus mutans count in Triphala Lollipop group with baseline

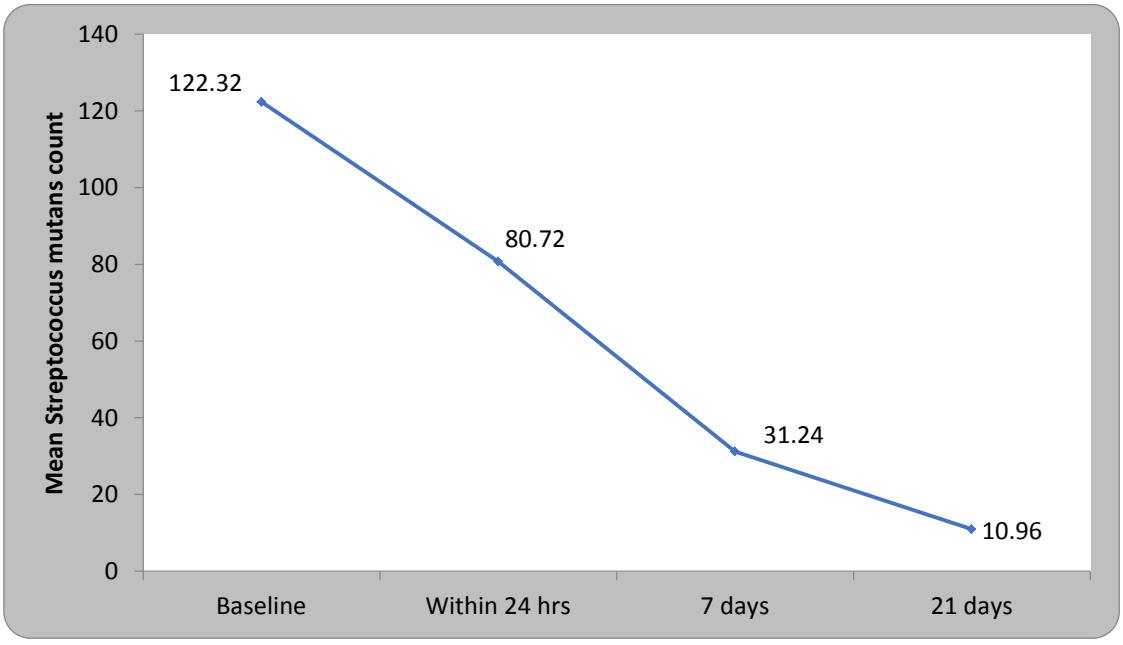


Table 5: Comparison of Streptococcus mutans count in Licorice Lollipop group with baseline

Student's paired t-test

Time Period	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	t-value
Baseline	129.84	25	33.45	6.69	-	-
Within 24 hrs	53.48	25	13.79	2.75	76.36±33.10	11.53 P=0.0001, S
7 days	9.12	25	3.50	0.70	120.72±33.54	17.99 P=0.0001, S
21 days	3.52	25	2.56	0.51	126.32±33.29	18.97 P=0.0001, S

Graph 5: Comparison of Streptococcus mutans count in Licorice Lollipop group with baseline

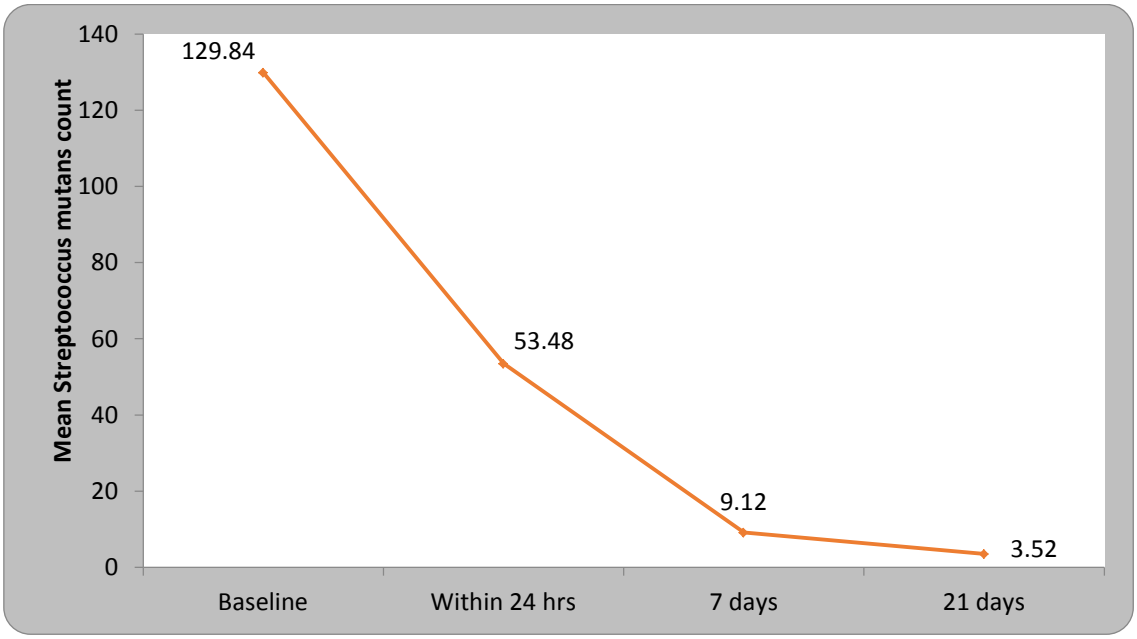


Table 6: Comparison of Streptococcus mutans count in Placebo group with baseline

Student's paired t-test

Time Period	Mean	N	Std. Deviation	Std. Error Mean	Mean Difference	t-value
Baseline	122.40	25	33.36	6.67	-	-
Within 24 hrs	129.36	25	34.62	6.92	6.96±5.24	6.63 P=0.0001, S
7 days	131.76	25	32.71	6.54	9.36±18.94	2.47 P=0.021, S
21 days	147.24	25	26.98	5.39	24.84±33.84	3.67 P=0.001, S

Graph 6: Comparison of Streptococcus mutans count in Placebo group with baseline

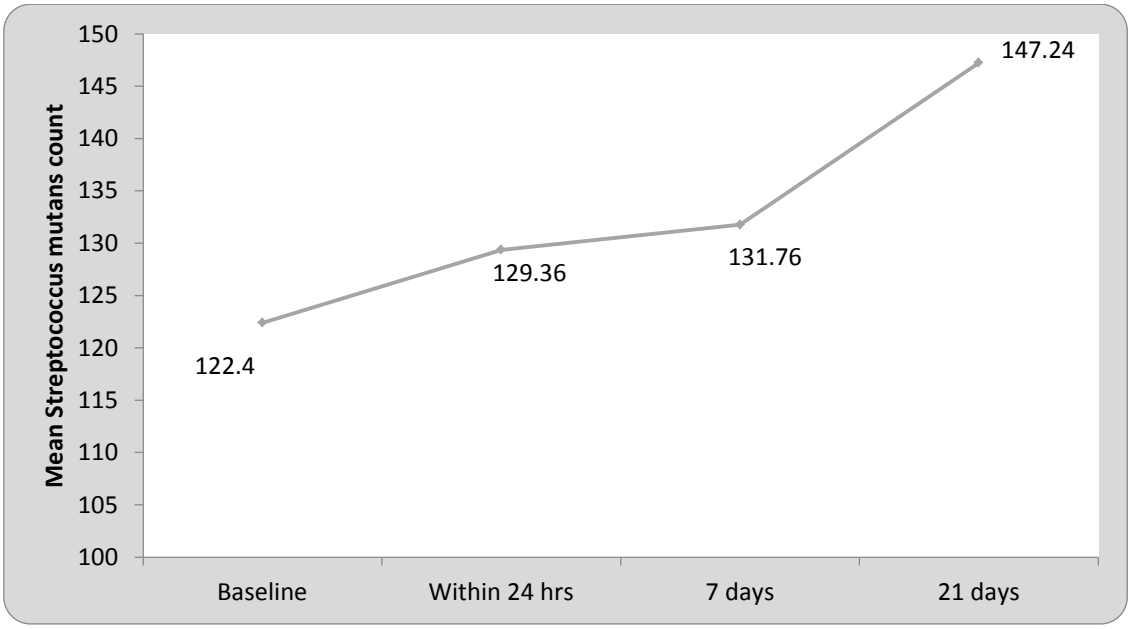


Table 7: Comparison of Streptococcus mutans count in patients of three groups

Student's paired t-test

Time Period	Triphala Lollipop	Licorice Lollipop	Placebo	F-value	Multiple Comparison: Tukey Test		
					Triphala Lollipop Vs Licorice Lollipop	Triphala Lollipop Vs Placebo	Licorice Lollipop Vs Placebo
Baseline	122.32±15.56	129.84±33.45	122.40±33.36	0.56 P=0.57, NS	0.62, NS	1.00, NS	0.63, NS
Within 24 hrs	80.72±19.34	53.48±13.79	129.36±34.62	62.83 P=0.0001, S	0.0001, S	0.0001, S	0.0001, S
7 days	32.24±7.68	9.12±3.50	131.76±32.71	280.73 P=0.0001, S	0.0001, S	0.0001, S	0.0001, S
21 days	10.96±4.48	3.52±2.56	147.24±26.98	650.46 P=0.0001, S	0.228, NS	0.0001, S	0.0001, S

Graph 7: Comparison of Streptococcus mutans count in patients of three groups

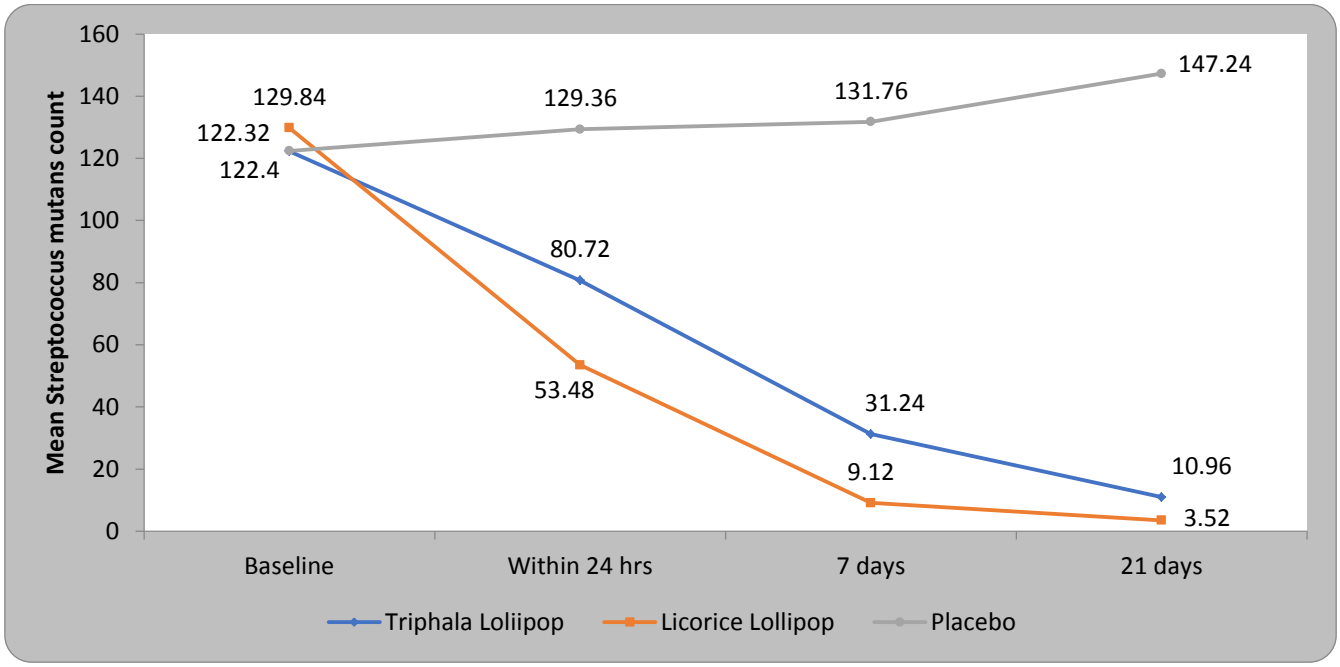
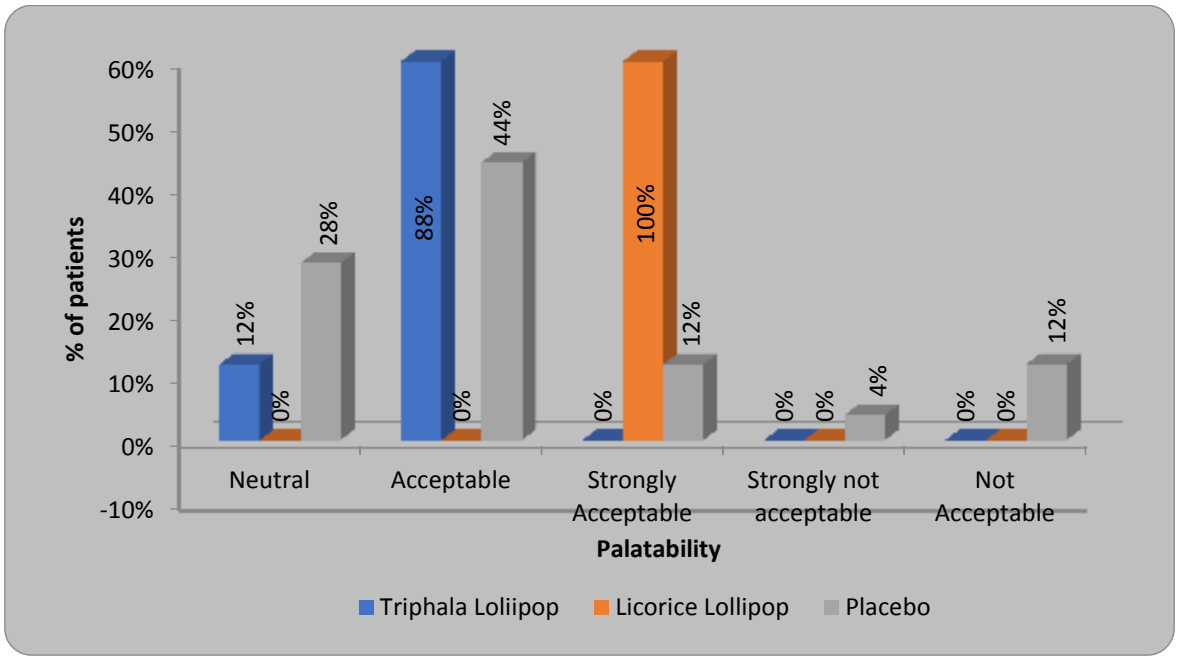


Table 8: Comparison of Palatability among patients of three groups

Palatability Score	Triphala Loliipop	Licorice Lollipop	Placebo	χ^2-value
Neutral	3(12%)	0(0%)	7(28%)	
Acceptable	22(88%)	0(0%)	11(44%)	
Strongly Acceptable	0(0%)	25(100%)	3(12%)	77.33
Strongly not acceptable	0(0%)	0(0%)	1(4%)	P=0.0001, S
Not Acceptable	0(0%)	0(0%)	3(12%)	
Total	25(100%)	25(100%)	25(100%)	

Graph 8: Comparison of Palatability among patients of three groups



ANNEXURES

DEPARTMENT OF PEDIATRIC AND PREVENTIVE DENTISTRY

"Comparative evaluation of anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years: An Experimental study"

EXAMINATION PROFORMA/CASE RECORD FORM

Date of examination

Identification No-

Day -

Date -

Group –

General information:

Name of child_____ Gender-☐

1=M, 2=F

Day Month Year

Date of Birth- ☐☐☐☐☐☐☐☐

Age in years-☐☐

Name and Address of parent:

Contact No. of Parent: …….

Chief Complaint:

H/O Present illness:

Past Medical History:

Past Dental History:

Frequency of tooth brushing per day:

1) Once

2) Twice

3) After every meal

Extra- Oral examination:

1. **Swelling on face:**

2. **Lymph nodes**:

Intra- Oral examination:

A. **Examination of soft tissues:**

1.Gingiva:

2.Floor of mouth

3. Tongue:

B. Examination of hard tissues:

1.Teeth present(FDI) –

2.Dental caries status –

- **defs index –**

16	**55**	**54**	**53**	**52**	**51**	**61**	**62**	**63**	**64**	**65**	**26**

46	**85**	**84**	**83**	**82**	**81**	**71**	**72**	**73**	**74**	**75**	**36**

Total score =

D	**E**	**f**	**s**	**Defs score**

Radiographic interpretation:

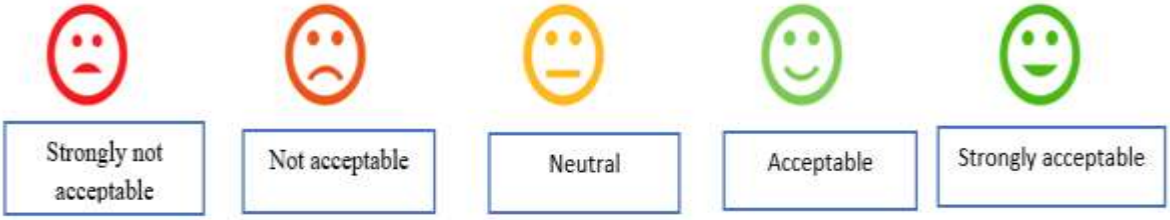
Diagnosis:

Treatment plan:

tability of lollipops among children was done by using 5 points Likert scale.

tability of lollipops among children was done by using 5 points Likert scale.

The palatability of lollipops among children was assesed by using 5 points Likert scale.



DEPARTMENT OF PEDIATRIC & PREVENTIVE DENTISTRY

Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

I acknowledge the "Specially designed proforma", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my child's oral examination to be taken as required and also agree for the required treatment. I agree to take part in this project. I shall co-operate with the doctors, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so. I hereby record my consent for participation in the said project.

.....	.....	.....	.....
Parent's/guardian's name	Signature/thumbprint	Date	Time

.....	.....	.....	.....
Investigator's name	Signature	Date	Time

If illiterate a literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____

Signature of witness _____

Date _____

Day/month/year

Thumb print of participant

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

"Comparative evaluation of anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years: An Experimental study".

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Informed Consent Form

(Confidential)

"Comparative evaluation of anti-microbial efficacy of Licorice and Triphala lollipops on Streptococcus mutans count in children aged 4 to 8 years: An Experimental study"

Mr./Master/Mrs./Miss._____

Resident of: _____

_____ _____aged _____ years,

Exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted by

I acknowledge the receipt of "patient's information sheet", and also that the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I allow to perform the examination of my ward's oral cavity and thereby agree for the treatment. I agree to take part in this project and will not mix any other projects during the period of this trial. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I hereby record my consent for participation in the said questionnaire.

_____	_____	_____	_____
Patient's name	Signature/thumbprint	Date	Time

_____	_____	_____	
Principal Investigator	Signature	Date	Time

DEPARTMENT OF PEDIATRIC & PREVENTIVE DENTISTRY

संमती प्रमाणपत्र

मी खाली दिलेली माहिती वाचली आहे, किंवा मला वाचन दिले गेले आहे. मला प्रश्न विचारण्याची संधी दिली गेली आणि मी जे विचारले होते ते समाधान माझ्या समाधानानुसार देण्यात आले. मी या संशोधनात सहभागी म्हणून स्वेच्छेने सहभाग घेण्यास सहमती देतो.

मी "विशेष रूपाने डिझाइन केलेले प्रोफार्मा" मान्य करतो आणि डॉक्टरांनी मला या संशोधन प्रकल्पाबद्दल योग्य आणि पुरेशी माझी समाधानकारक माहिती दिली आहे. माझ्या मुलाच्या तोंडी परीक्षा आवश्यकतेनुसार घेण्यास मी सहमत आहे. मी या प्रकल्पात भाग घेण्यास सहमती देतो. मी सर्व बाबतीत, डॉक्टरांशी सहकार्य करू. या अभ्यासात मी माझ्या सहभागाचे निकाल प्रकाशित करण्यास परवानगी देतो. मी कोणत्याही परतावा किंवा नुकसान भरपाई दिली जाणार नाही. असे करण्यासाठी कोणत्याही कारण न देता मला कोणत्याही वेळी या संशोधन प्रकल्पातून बाहेर पडण्याचा अधिकार मिळालेला आहे. मी याद्वारे प्रोजेक्टमध्ये सहभागाबद्दल माझी संमती नोंदवित आहे.

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पालकांचे / संरक्षक नाव	सही / अंगठा मुद्रण	तारीख	वेळ
.....	.....	.....	.....
अन्वेषणकर्त्याचे नाव	स्वाक्षरी	तारीख	वेळ

निरक्षर असल्यास साक्षर साक्षीदारांनी सही करणे आवश्यक आहे (जर शक्य असेल तर, या व्यक्तीला निवडकाने निवडले पाहिजे आणि त्यास शोध पथकाशी कोणतेही कनेक्शन नसावे). निरक्षर असणार्या सहभागीांमध्ये त्यांचे थंब मुद्रण तसेच त्यांचा समावेश असावा.

मी संभाव्य सहभागास संमती फॉर्मचे अचूक वाचन केले आहे, आणि व्यक्तीस त्याला प्रश्न विचारण्याची संधी मिळाली आहे. मी पुष्टी करतो की व्यक्ती स्वतंत्रपणे संमती दिली आहे.

सहभागी व्यक्तीचे अंगठा

साक्षीदारांचे नाव _____

साक्षीची स्वाक्षरी _____

तारीख _____

दिवस / महिना / वर्ष

MASTER CHART

Demographic Details					Assesment					Demographic Details					Assesment					Demographic Details					Assesment				
					Triphala Loliipop										Licorice Lollipop										Placebo				
					Streptococcus mutans count										Streptococcus mutans count										Streptococcus mutans count				
Sr No.	ID No.	Gender	Age	defs Index	Baseline	Within 24 hrs	168 hrs(7 days)	504 hrs(21 days)	Likert scale (Palatability)	Sr No.	ID No.	Gender	Age	defs Index	Baseline	Within 24 hrs	168 hrs(7 days)	504 hrs(21 days)	Likert scale (Palatability)	Sr No.	ID No.	Gender	Age	defs Index	Baseline	Within 24 hrs	168 hrs(7 days)	504 hrs(21 days)	Likert scale (Palatability)
1	5	F	5 yrs	2	150	95	30	9	Strongly acceptable	1	30	F	6 yrs	4	180	50	5	5	Strongly acceptable	1	51	M	8 yrs	2	182	190	150	160	Neutral
2	7	M	7 yrs	1	102	62	29	11	Strongly acceptable	2	45	M	8 yrs	2	160	40	4	0	Strongly acceptable	2	72	F	7 yrs	4	160	165	160	172	Acceptable
3	3		6 yrs	4	109	80	35	12	Strongly acceptable	3	35	F	5 yrs	5	120	45	10	2	Strongly acceptable	3	64	M	5 yrs	3	79	80	90	180	Strongly acceptable
4	11	M	4 yrs	0	140	54	20	6	Accepatable	4	42	M	8 yrs	3	110	65	11	1	Strongly acceptable	4	65	F	6 yrs	5	86	86	80	105	Acceptable
5	2	F	8 yrs	5	130	92	25	5	Strongly acceptable	5	38	F	8 yrs	0	80	65	12	9	Strongly acceptable	5	57	F	7 yrs	2	105	120	182	109	Acceptable
6	14	M	7 yrs	4	120	75	25	15	Strongly acceptable	6	49	F	6 yrs	4	170	60	10	2	Strongly acceptable	6	73	M	8 yrs	1	75	80	90	100	Not acceptable
7	1	F	8 yrs	3	110	89	32	13	Strongly acceptable	7	27	M	5 yrs	3	150	45	6	4	Strongly acceptable	7	61	F	8 yrs	0	150	158	150	160	Neutral
8	17	F	6 yrs	1	136	109	40	19	Strongly acceptable	8	34	M	4 yrs	5	110	65	11	8	Strongly acceptable	8	52	M	7 yrs	3	142	150	120	135	Strongly acceptable
9	22	M	7 yrs	0	142	111	35	12	Strongly acceptable	9	40	F	7 yrs	2	90	40	4	0	Strongly acceptable	9	59	M	5 yrs	5	175	185	170	182	Acceptable
10	15	F	5 yrs	5	118	89	42	17	Strongly acceptable	10	50	M	7 yrs	3	100	52	7	6	Strongly acceptable	10	60	F	7 yrs	4	119	138	130	145	Acceptable
11	13	M	7 yrs	4	104	57	29	5	Neutral	11	28	M	8 yrs	1	165	65	9	3	Strongly acceptable	11	70	M	8 yrs	4	89	89	95	120	Neutral
12	4	M	7 yrs	2	147	95	31	8	Strongly acceptable	12	47	F	6 yrs	0	118	38	5	0	Strongly acceptable	12	74	F	5 yrs	3	109	112	120	140	Not acceptable
13	24	F	6 yrs	0	133	49	25	11	Strongly acceptable	13	44	M	5 yrs	4	97	55	10	3	Strongly acceptable	13	63	M	6 yrs	2	125	130	125	155	Acceptable
14	3	M	5 yrs	3	129	111	35	20	Strongly acceptable	14	36	F	7 yrs	3	153	40	6	4	Strongly acceptable	14	69	M	7 yrs	0	80	84	90	180	Acceptable
15	19	F	7 yrs	4	104	60	42	6	Strongly acceptable	15	46	F	8 yrs	2	172	55	11	3	Strongly acceptable	15	53	M	7 yrs	2	138	140	160	172	Acceptable
16	23	M	8 yrs	5	113	93	33	13	Accepatable	16	31	M	5 yrs	3	140	99	8	5	Strongly acceptable	16	62	F	8 yrs	1	165	170	181	109	Neutral
17	20	M	8 yrs	2	126	80	35	7	Strongly acceptable	17	41	F	4 yrs	4	123	45	11	3	Strongly acceptable	17	66	M	6 yrs	5	95	101	110	138	Strongly acceptable
18	25	M	6 yrs	1	109	87	29	10	Strongly acceptable	18	32	M	7 yrs	1	138	59	15	4	Strongly acceptable	18	75	F	7 yrs	4	84	92	97	169	Neutral
19	6	F	7 yrs	3	145	100	45	14	Strongly acceptable	19	26	F	6 yrs	0	182	70	15	8	Strongly acceptable	19	67	M	8 yrs	3	136	144	150	170	Strongly not acceptable
20	21	M	8 yrs	3	106	59	29	4	Strongly acceptable	20	29	F	8 yrs	4	175	57	10	3	Strongly acceptable	20	56	F	7 yrs	1	95	100	99	110	Acceptable
21	9	F	7 yrs	2	139	55	45	7	Accepatable	21	33	M	6 yrs	3	99	35	3	0	Strongly acceptable	21	54	M	5 yrs	0	149	150	160	150	Not acceptable
22	12	M	5 yrs	4	105	79	19	12	Strongly acceptable	22	43	F	7 yrs	5	90	50	11	2	Strongly acceptable	22	71	F	8 yrs	1	100	110	115	128	Acceptable
23	10	F	5 yrs	5	110	55	20	8	Strongly acceptable	23	37	M	4 yrs	5	93	40	15	3	Strongly acceptable	23	68	F	7 yrs	5	170	175	180	182	Neutral
24	8	M	8 yrs	4	113	93	33	13	Strongly acceptable	24	48	M	5 yrs	2	88	45	7	3	Strongly acceptable	24	75	M	6 yrs	4	137	155	162	170	Acceptable
25	2	M	7 yrs	2	118	89	42	17	Strongly acceptable	25	39	F	8 yrs	3	143	60	12	7	Strongly acceptable	25	55	F	8 yrs	2	115	130	128	140	Neutral