ORIGINAL ARTICLE

COMPARISON OF THE ANTIBACTERIAL EFFICIENCY OF HERBAL EXTRACTS OF TULSI LEAVES, CINNAMON, PEPPER SEEDS AND GUAVA LEAVES AGAINST STREPTOCOCCUS MUTANS- AN IN VITRO STUDY

[1]Smita Agrawal, [2]Swapna DV, [3]Roopa R Nadig

[1] PG Student, Department of Conservative Dentistry and Endodontics, Dayananda Sagar College of Dental sciences, Bangalore, Karnataka
[2,3] Reader, Department of Conservative Dentistry and Endodontics, Dayananda Sagar College of Dental sciences, Bangalore, Karnataka

ABSTRACT

Aim:This study evaluated and compared the antimicrobial efficacy and Minimal Inhibitory Concentration (MIC) of chlorhexidine 0.12% and extracts of - Tulsi leaves, Cinnamon, Guava leaves and Pepper seeds against Streptococcus mutans.Materials & Methodology:The agar disc diffusion method and broth micro-dilution method was used to check the antimicrobial activity of the aqueous and methanolic extracts of various medicinal plants. The test samples were divided as: Group I: Negative control – methanol, Group II: Positive control - 0.12% Chlorhexidine solution, Group III: Aqueous extract of tulsi leaves, Group IV: Alcoholic extract of Tulsi leaves, Group V: Aqueous extract of Guava leaves, Group VI: Alcoholic extract of guava leaves, Group VII: Aqueous extract of cinnamon, Group VIII: Alcoholic extract of cinnamon, Group IX: Aqueous extract of Pepper seeds and Group X: Alcoholic extract of pepper seeds .The Zone of Inhibition and MIC values were tabulated and thedata was statistically analyzed using ANOVA and Bonferroni post-hoc tests. Results:Methanolic extracts of guava leaves and cinnamon were found to be most effective against S. mutans with the zone of inhibition of 22.45±0.82 mm&20.51 ± 1.05 mm respectively and MIC value of 62.5µg/ml for both the extracts.Conclusion:Herbal products have potent antimicrobial activity that can be an alternative to commercially available antimicrobial agents thus overcoming their drawbacks. Further in vitro and long-term in vivo studies are recommended studies need to be conducted before it can be recommended for routine clinical usage.

INTRODUCTION

Among the common oral diseases, dental caries is one of the pathology that remains widely prevalent and affect all populations throughout the lifespan [1]. It is steadily increasing in the underdeveloped and developing countries due to low socio- economic level, lack of basic sanitation, access to medication and malnutrition. The increase in caries index is

Access this article online

Quick Response Code:

Website: www.jiadsr.org

Address for correspondence:
Dr.Swapna D.V,
Reader, Department of Conservative Dentistry and Endodontics,
Dayananda Sagar College of Dental sciences,
Bangalore, Karnataka, India
Email id- drsapnadv@gmail.com

linked to changing diet, particularly to an increase in sugar and refined food consumption.

Dental caries is an infectious microbial disease that results in localized demineralization of tooth structure caused by acids, which is produced by plaque bacteria through the carbohydrate metabolism.[2] Dental caries is a multifactorial disease and requires the presence of sugars, bacterial biofilm and the quantity and quality of the saliva.[3]

Dental biofilm is the main etiological factor of dental caries and it is characterized as a community of microorganisms like Streptococcus mutans, Lactobacilli, Porphyromonasgingivalis, Enterococcus feacalis, Actinomycetes and Candida albicans that adheres to the tooth surface. There is substantial evidence that suggests Streptococcus mutans, a gram-positive facultative anaerobe[4,5] as the major pathogen and the initiator of dental caries.[6] This is because, MS are frequently isolated from cavitated caries lesions, induce caries formation in animals which are fed with a sucrose-rich diet, highly acidogenic and aciduric and able to produce surface antigens I/II and water-insoluble glycan, which promote bacterial adhesion to the tooth surface and to other bacteria.[7] Bacterial fermentation of dietary sugars produces acids in the oral cavity. Sugar is also used by S. mutans to produce a sticky, extracellular, dextran-based poly saccharide that allows them to cohere, thus forming dental plaque. One of the most important contributing factors for the progression of these oral diseases is the biodiversity and complex nature of the dental plaque; it acts as a biological film where various bacterial species interact and may form a protective barrier against anti-microbial agents, resulting in resistance to common antibacterial agents used in clinical dentistry.[8,9] Controlling the levels of these microorganisms in the oral plaque will aid in the prevention of these diseases. Hence, a caries prevention program primarily should be aimed at reducing the cariogenic bacterial plaque.

Many attempts have been made to eliminate Streptococcus mutans from the oral cavity. Preventive treatments such as topical Fluoride and fissure sealants have given good results but these procedures require the help of dentist and are expensive. On the other hand, curative treatments by restoring the tooth are an expensive and invasive procedure and still have the risk of secondary caries occurrence.[10] Antimicrobial mouthwashes are used alternative as a preventive treatment against caries. It inhibits plaque formation, reduce gingival inflammation and prevent dental caries. Chlorhexidine, the most common mouth rinse and is used as a gold standard against which other

antimicrobial agents are compared. It has been studied extensively and is currently the most potent chemotherapeutic agent against Streptococcus mutans and dental caries.[11,12]

However, their excessive use can result in alterations of the oral and intestinal flora and cause undesirable side effects such as the development of bacterial tolerance, vomiting, unpleasant taste, staining of teeth and restorations, discoloration of tongue, desquamation and soreness of the oral mucosa.[13,14]

The advantage of traditional medicine is that it is less likely to form allergies and side effects as compared to pharmaceutical drugs. Recently because of their high antimicrobial, anti- inflammatory, anti-oxidant and biocompatible properties, their use in dentistry is becoming more popular.[15] Judicious use of formulations mitigates the S.Mutans count which would in turn reduce its cariogenic potential in the oral cavity and can be considered as one of the possible alternatives or rather a replacement for the synthetic chemical formulations and also prove to be cost effective in developing economies

Tulsi, scientifically known as Oscimum sanctum, belongs to the family Labiateae, which comprises a large proportion of medicinal plant species. Different parts of the plant have shown antimicrobial, anti-inflammatory, analgesic, antipyretic, antiulcer, antidiabetic, antioxidant and anticancer activity due to the presence of active constituents like eugenol, ursolic acid, carvacrol etc.[16]

Guava (Psidiumguajava) belongs to the family Myrtaceae. The antimicrobial activity presented by the plants in the family Myrtaceae is related to its high content of essential oils and phenolic compounds, such as tannins.[17] Their aqueous extracts have in vitro antibacterial effect on the growth of plaque bacteria.[18]

Black pepper (Piper nigrum L.) is a flowering vine of the Piperaceae family. Pepper is used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic, diuretic and also for the treatment of cough, rheumatoid arthritis, peripheral neuropathy, melanoderma and leprosy due to the presence of volatile compounds, tannins, phenols, alkaloids, flavonoids etc. Piperine, a pungent alkaloid present in black pepper, enhances the bioavailability of various structurally and therapeutically diverse drugs.[19]

Cinnamon oil is essential oils with antibacterial activity, due to presence of active compound cinnamaldehyde. Cinnamon oil is shown to be effective against biofilm cultures of Streptococcus mutans and Lactobacillus plantarum.[20]

Hence in search of novel antimicrobial agents, a study was done to evaluate and compare antimicrobial efficacy and minimal inhibitory concentration (MIC)of chlorhexidine 0.12% and extracts of - Tulsi leaves, Cinnamon, Guava leaves and Pepper seeds against Streptococcus mutans.

MATERIALS AND METHODOLOGY

The present study was conducted in the Microbiology department of Dayananda Sagar Institution of Dental Sciences, Bengaluru. The herbs viz. tulsi leaves, guava leaves, pepper seeds, cinnamon were collected from the Ayurvedic Garden, Bengaluru, and the herbal extracts were authenticated by the Department of Botany, Bengaluru University, Bengaluru.Pure strains of Streptococcus mutans (MTCC 890) were obtained from microbiology lab.

PREPARATION OF INOCULUM:

Stock cultures of S mutans were maintained at 4°C on slopes of nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter distilled water). Before every antibacterial assay, active cultures for experiments were freshly prepared by transferring them from agar slant with the help of a sterile disposable inoculating loop into a separate sterile test tube containing nutrient broth and incubated with agitation for 24 hrs at 37°C. A 0.5McFarland standard was used for visual comparison to adjust the bacterial suspension to a density equivalent to approximately 1.5×10^8 CFU/mL.

PREPARATION OF THE EXTRACTS:

Healthy, matured leaves/ bark/ fruits were collected and were used for the preparation of Aqueous and solvent (Methanol) extract. Aqueous extracts Thoroughly washed herbs were shade dried and then powdered with the help of blender. 50 gms of powder was boiled in 500ml of deionised distilled water and allowed to boil to a final volume of 10-20 ml. The concentrated mixture was filtered and the clear extract was stored in airtight bottle in refrigerator for antimicrobial studies.

SOLVENT (METHANOL) EXTRACTS:

Thoroughly washed herbs were shade dried and then powdered in the blender. 20 gm of powder was filled in the thimble and extracted with 100 ml of 100 % methanol using a Soxhlet extractor for 8-10 hrs. All the extracts were concentrated using rotary evaporator. 1 grams of residue (extract) was obtained, so the yield was 5% w/w. One gram of extract was dissolved in 10 ml of dimethyl formamide to obtain 10% concentration of extract and preserved in refrigerator in airtight bottle for antimicrobial studies.

Groups:

The groups were divided as follows:Group I: Negative control - methanolGroup II: Positive control - 0.12% Chlorhexidine solution Group III: Aqueous extract of tulsileavesGroup IV: Alcoholic extract of TulsileavesGroup V: Aqueous extract of Guava leavesGroup VI: Alcoholic extract of guava leavesGroup VII: Aqueous extract of cinnamonGroup VIII: Alcoholic extract of cinnamonGroup IX: Aqueous extract of Pepper seedsGroup X: Alcoholic extract of pepper seeds

IN VITRO ANTIMICROBIAL ACTIVITY

1) Disc diffusion method

Antimicrobial assay was performed against pathogenic strain by agar disc diffusion method. All the Mueller Hinton Agar (MHA) plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solutions. 0.1 ml of microbial suspension was spread evenly onto the surface of the sterilized MHA agar plates using sterile glass spreader. The plates were allowed to dry the excess moisture for 3 to 5 min. Sterile Whatmann No. 1 filter paper was used to prepare disks of diameter 6 mm. The disks were saturated with 50 µl of each extract. These were air dried at room temperature to remove any residual solvent that might interfere with the determination of antibacterial activity. The disks were then placed on the surface of MHA plates that had been inoculated with test bacteria using a sterile pair of forceps. 4 disks per plates were placed. The experiment was repeated 3 times per group. So total of 12 disks per group were used (n=12) the plates were sealed with parafilm, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A Vernier caliper was used to measure the inhibition zones in millimeters. Based on the diameter of the inhibition zone, the results were then assigned. The bigger the diameter of inhibition zone, the more susceptible is the microorganism to antimicrobial agent.

Standard Drug Used (positive control)

Chlorhexidine was used as standard antibiotic against Streptococcus mutans.

2) Determination of Minimal Inhibitory Concentration(MIC)

MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of a micro-organism after overnight incubation.MIC was determined for only those groups that were susceptible to S mutans i.e. (Groups II, IV, V, VI, VIII, X). We followed the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) to determine the MIC using serial dilution method in a 96 well sterile microtitre tray. Nutrient broth and

100 µL of Bacterial suspensions (1.5×10^6 CFU/mL) were added to all wells. Serial dilution was performed by adding different concentrations to the wells (500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 µg/ml) of 10% of the selected groups. The plate was then covered with a sterile plate sealer, agitated and incubated at 37 °C for 24 h. The wells were then visually inspected for bacterial growth. The last well that was clear (no haziness) was designated as the MIC.

RESULTS:

Broth microdilution is a quantitative method used for determining the MICs, of antimicrobial agents and is considered to be 'gold standard' reference testing methodology against which other methods, such as disc diffusion, are calibrated.Our study has shown that the least values of MIC were obtained for methanolic extract of guava leaves (62.5 µg/ml) and methanolic extract of cinnamon (62.5 µg/ml) proving their maxixmum antimicrobial action followed by methanolic extract of tulsi and aqueous extract of guava. Methaolic extract of black pepper showed the highest MIC(500 µg/ml) indicating its least effect against s mutans. Whereas aqueous extracts of tulsi, cinnamon, pepper did not show any antimicrobial activity against MS.

Methanolic and aqueous extract of guava leaves demonstrated (22.45±0.82 and12.37±0.67 mm) zone of inhibition respectively. This improved performance could be due to the fact that guava leaves contain essential oil rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts. Psidiumguajava extracts have the competence to modify the surface characteristics of the bacteria by altering the complementary binding sites in

the dental pellicle. Tannins can precipitate proteins due to their ability to act on the cell surface and across the cell membranes. Polyphenols interact with proteins and disturb microbial co- aggregation thereby disrupting the bonding and adhesion of early settlers in dental plaque. Murray et al have demonstrated the antimicrobial potential of aqueous and Methanolic extracts of Psidium guava leaves and its capacity to act against the chief cariogenic pathogen S.mutansi.[28]

We found an MIC of 62.5 µg/ml and (20.51 ± 1.05) zone of inhibition for Methanolic extract of cinnamon. The possible reason could be the presence of cinnamaldehyde which acts on the plasma membrane, similarly to chlorhexidine (inhibition of the same target). Cinnamon oil has a dual mode of action against S. mutans biofilms; it is able to detach adhering bacteria from a substratum surface and it can kill bacteria. The study authenticated antibacterial activity of

cinnamon oil and cinnamaldehyde (MIC 0.21 - 0.63 mg/mL and 0.8 – 0.15 mg/mL respectively) against the tested bacteria viz. Streptococcus mutans, S. mitis, S. salivarius, Aggregatibacter actinomycetemcomitans, Porphyromonasgingivalis and Fusobacteriumnucleatum.[30]

Methanolic extract of tulsi demonstrated MIC of 125µg/ml. The biological properties of the plant has been attributed to the presence of active compounds like Ursolic acid, flavonoids (epigenin, orientin and vicenin), and phenolic compounds (cirsilineol, circimaritin, isothymusin, eugenol). The leaves of Tulsi contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. Eugenol is the most prominent phytoconstituents present in this plant which may be responsible for antimicrobial activity.[59] Results of our study are in agreement with previous studies where different concentrations of Tulsi have been used against S mutans ATCC 890 and Pooja et al concluded that 4% ethanolic extract of Tulsi was most antimicrobial.[16] Bhatt MK et al investigated the anti-microbial activity of Methanolic extract of Ocimum Sanctum against gram positive bacteria like Bacillus subtilis and gram negative bacteria like Escherichia coli and evaluated that zone of inhibition was 2.5 cm and 2.3 cm respectively and MIC was 65 & 64 mg respectively against Bacillus subtilis and Escherichia coli.[20]

Black pepper extract's potential to inhibit the growth of Streptococcus mutans is probably due to its active antimicrobial contents, such as alkaloids (piperine, piperitin, piperidine and chavicine), tannin, essential oil, coumarine and phenol. They obliterate DNA and cell wall synthesis of Streptococcus mutans. Phenol group such as tannin and coumarine work destructively to the bacterial cell wall and interact with DNA. Methanolic extract works by damaging bacteria cell wall. The resultant of work of three substances will be able to destroy cell wall and DNA and lead to bacterial lysis. In a similar study, Rani S K et al evaluated piperine, a pungent bioactive alkaloid present in Black pepper (Piper nigrum L.) for its antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Alternariaalternata, Aspergillusniger, Aspergillusflavus and Fusariumoxysporum using agar well diffusion method. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm.[31] Sidarta Y O et al showed the MIC of 10% of the white pepper extract on growth activity of Streptococcus mutans in vitro with tube dilution method and streaking on the Brain Heart agar plate.[34]

In the present study, Streptococcus mutans was resistant to methanol. This fact attributed that the antibacterial action of different extracts was solely due to the chemicals present in the herbs and not due to the action of alcohol.In the current in – vitro study, the aqueous extracts of tulsi, cinnamon, pepper did not show any antimicrobial activity against MS. Primarily water was used as a solvent but alcoholic extracts of these plants were certainly much better and powerful.

Gp1 methanol	Gp2 Chlorhe- -xidine	Gp3 Aqut ulsi	Gp4 Alcohtulsi	Gp5 Aqu Guava	Gp6 Alcoh guava	Gp7 Aqueous cinnamon	Gp8 alcoh cinnamon	Gp9 Aqu pepper	Gp10 Alcoh pepper
-	12	-	16.8	12	22	-	20	-	8
-	13	-	17.5	11.8	22.5	-	19.5	-	7.8
-	12.4	-	17	11.5	21	-	22	-	9
-	12	-	16	12.8	22.8	-	21.5	-	8.5
-	13.8	-	16.5	11.5	21.6	-	20	-	9.8
-	12.5	-	17	12	22	-	21.6	-	8.5
-	12.4	-	16.8	12.6	22.5	-	19	-	9
-	12	-	16	13.5	24	-	19	-	9.2
-	12.5	-	17	12.5	22.5	-	20	-	8
-	13	-	16.5	12	23.5	-	21	-	10
-	12.8	-	15.8	13.2	22	-	21.5	-	8.5
-	12.5	-	16	13	23	-	21	-	8

TABLE 1 - MINIMUM INHIBITORY CONCENTRATION (MIC) (µG/ML)

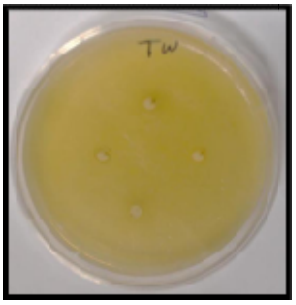
Gp2 Chlorhexidine	Gp4 Alcoholic tulsi	Gp5 Aqueous Guava	Gp6 Alcoholic Guava	Gp8 alcoholic cinnamon	Gp10 Alcoholic pepper
250	125	250	62.5	62.5	500

TABLE 2 - MEAN ZONE OF INHIBITION (MM) RECORDED IN THE GROUPS

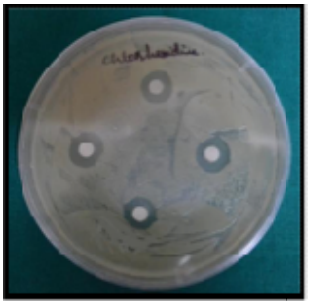
Group	Mean	Std Dev	SE of Mean	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Group 2	12.58	0.52	0.15	12.24	12.91	12.00	13.80
Group 4	16.58	0.53	0.15	16.24	16.91	15.80	17.50
Group 5	12.37	0.67	0.19	11.94	12.79	11.50	13.50
Group 6	22.45	0.82	0.24	21.93	22.97	21.00	24.00
Group 8	20.51	1.05	0.30	19.84	21.18	19.00	22.00
Group 10	8.69	0.72	0.21	8.23	9.15	7.80	10.00

TABLE 3- COMPARISON BETWEEN ZONE OF INHIBITION IN THE GROUPS

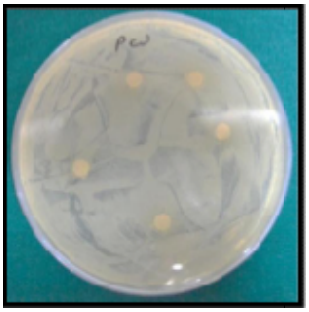
Fig 12- Zone Of Inhibition



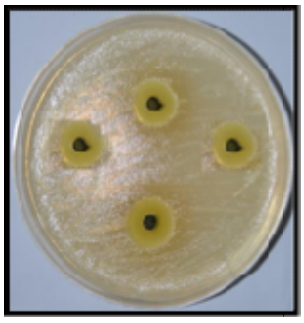
GROUP I



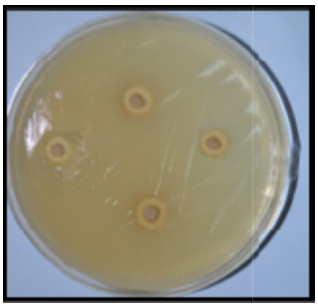
GROUP II



GROUP III

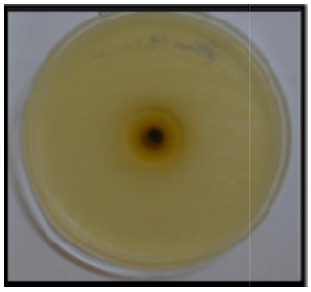


GROUP IV

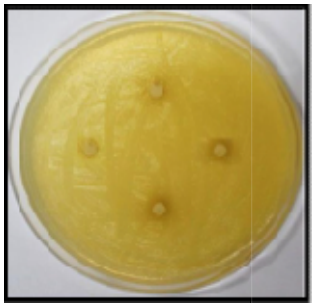


GROUP V

Fig 13- Zone Of Inhibition

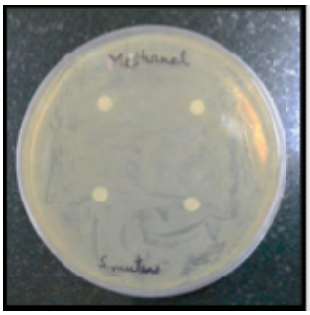


GROUP VI



GROUP VII

GROUP VIII



GROUP IX

BOX-PLOT:

GRAPH 2

We observed that the difference in mean zone of inhibition was found to be statistically significant between

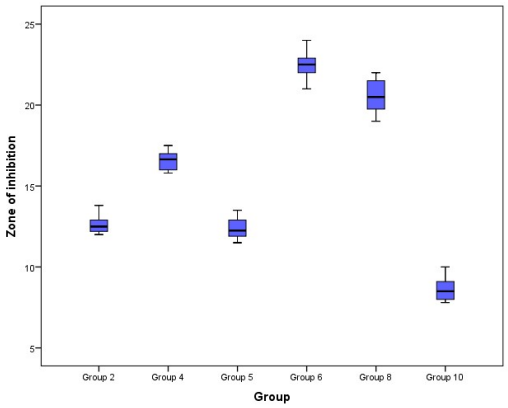
. 1) group 2 & group 4 (P<0.001), group 6 (P<0.001), group 8 (P<0.001) and group 10 (P<0.001)

. 2) group 4 & group 5 (P<0.001), group 6 (P<0.001), group 8 (P<0.001) and group 10 (P<0.001)

. 3) group 5 & group 6 (P<0.001), group 8 (P<0.001) and group 10 (P<0.001)

. 4) group 6 & group 8 (P<0.001), group 10 (P<0.001)

. 5) group8&group10(P<0.001)



DISCUSSION

Dental caries is recognized as major oral health problem throughout the world. Numerous epidemiological studies have shown that tooth decay is the most common sequelae of Dental plaque formation.[41] Dental plaque is a biofilm, i.e. a group of microorganisms embedded in a matrix attached to the tooth surface.[2]

All biofilms have at least one property in common, i.e. the presence of a bacterially derived matrix.[43] Electron histochemistry and transmission electron microscopy of dental plaque reveals microorganisms embedded in a matrix of polysaccharide which appears to increase following exposure to sucrose.[44] The structure of extracellular polysaccharide (EPS) matrix can play a critical role affecting the virulence of plaque bacteria by influencing the physical and biochemical properties of biofilm. Extracellular polysaccharide (EPS) in dental plaque is produced from the interaction of glucosyltransferases (Gtfs) and fructosyltransferases produced by S mutans with sucrose and starch hydrolysates. The presence of highly adherent and insoluble glucans in situ increases mechanical stability by binding the bacterial cells together and to the apatite surface allowing them to persist on tooth enamel in high levels for a prolonged period thereby modulating the development of cariogenic biofilms.[45]

A) Representative confocal images of bacterial cells (in green) and glucans (in red) within biofilms formed by S. mutans.

B) Close-up view of 3-dimensional structural relationship between glucans and S. mutans cells

Streptococcus mutans was chosen as the test organism because it is one of the predominant inhabitants of dental plaque and has long been implicated in the formation of dental caries. Two virulence factors of S. mutans linked to its cariogenicity are its acidogenicity and aciduricity (the ability to produce acid and the ability to survive and grow at low pH, respectively).[46] S. mutans adhere by hydrophobic bonds to the enamel surface and ferment dietary carbohydrates, notably sucrose.[47] This sucrose metabolism promotes the firm adherence and cellular aggregation of bacteria to the tooth surface.[48] Acid production resulting from carbohydrate metabolism by these bacteria and the subsequent decrease in environmental pH are responsible for the demineralization of tooth surfaces.[49] They play a critical role in destabilizing the homeostasis of the plaque by facilitating a shift of the demineralization/ remineralization balance from 'net mineral gain' to 'net mineral loss' (acidogenic stage). Once the acidic environment has been established, MS and other aciduric bacteria may increase and initiate dissolution of the tooth enamel and accumulation of acids in the dental plaque, subsequently leading to localized decalcification, cavitation and breakdown of calcified dental by sustaining an environment characterized by 'net mineral loss' (aciduric stage).[50]

The removal of bacterial biofilms is a decisive component in the prevention and treatment of dental caries. The levels of microorganisms in the biofilm should be controlled for controlling plaque and maintaining gingival health. Thus several methods to reduce the amount of dental plaque have been reported including mechanical (toothbrush, toothpaste, chewing sticks) and chemical methods. The use of mechanical agents is a simple cost effective method. The effectiveness of this method however is influenced by the individual's manual dexterity and motivation.[51] Chemical methods of plaque elimination involve the use of mouthwashes. Mouth rinsing is favored by the public because of its ease of use and breath freshening effect.[52] Among all available mouthwashes, chlorhexidine has good plaque control and an antimicrobial effect.[53,54]

In current era, quick results are expected, hence haphazard use of systematic antimicrobial drugs is now resulting in multiple drug resistances and evidences of serious adverse effects are noted such as alterations of normal flora, vomiting, unpleasant taste, staining of teeth and restorations, desquamation and soreness in the oral mucosa etc.[55] So researches are

trying to pay more attention to herbal drugs. Natural products have been used in folk medicine and shown to be a good alternative to synthetic chemical substances for caries prevention.[35]

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. Various parts of the plants like root, bark, seed and leaves have been an important source of medicine since thousands of years. Herbal substances have become alternative treatment preference regarding its ease to obtain, less side effects and relatively low cost of money.[14] Natural herbs and their varied extracts has been used globally in therapeutic since the distant past. Herbs have shown to possess antibacterial, antiviral, insecticidal and antioxidant properties due to the presence of wide variety of active phytochemicals, including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides.[45]

Herbal extracts have been tested for in vivo and in vitro antimicrobial activity. Their mechanism of action is predominantly on cell membrane by disrupting its structure, blocking membrane synthesis, inhibition of cellular respiration thereby causing cell leakage and cell death.[40] n the current study, we explored four medicinal plants such as O. sanctum (Tulsi), Psidiumguajava(guava leaves), Piper nigrum L (Black pepper) and cinnamon for their antimicrobial effectiveness against S. mutans. The selected plant extracts have shown significant antimicrobial effect against the dental pathogen thus confirming the traditional claim.

For antibacterial susceptibility testing Disc diffusion and broth microdilution methods were followed as per the NCCLS guidelines.

Disk diffusion is a qualitative test. Diameter of the zone of growth inhibition around an antibiotic disk predicts the effectiveness of the antimicrobial agent.

CONCLUSION

An in vitro study was conducted on "Comparison of the antibacterial efficiency of herbal extracts of tulsi leaves, cinnamon, pepper seeds and guava leaves against streptococcus mutans." The results of present study support the traditional usage of plant and plant extracts which posses compounds with antibacterial properties. Within the limitations of this in- vitro study, it can be concluded that

1. All the herbal extracts tested in this study demonstrated antibacterial activity against MS.

2. Except for guava, rest of the experimental agents when used as aqueous extracts did not show any antimicrobial activity against MS

3. On comparing the experimental groups that demonstrated antimicrobial activity, Methanolic extract of guava, Methanolic extract of cinnamon and Methanolic extract of tulsi showed higher antimicrobial activity than chlorhexidine whereas Methanolic extract of pepper showed significantly lesser antimicrobial activity against MS.

4. Herbal products have potent antimicrobial activity that can be looked at as an alternative to chlorhexidine. However, further in vitro and long term in vivo studies are recommended to confirm and correlate the findings of this study to a clinical situation.

REFERENCE:

1.Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. Community Dentistry and oral epidemiology. 2003 Dec; 31:3-24.

2.Samaranayake L. Essential Microbiology for Dentistry-E-Book. Elsevier; 2018 Mar 28.

3.Baum L, Phillips RW, Lund MR. Buku ajar ilmukonservasigigi. Edisi ke-3. Tarigan R, editor. Jakarta: EGC. 1997.

4.Salam MA, Matsumoto N, Matin K, Tsuha Y, Nakao R, Hanada N, Senpuku H. Establishment of an animal model using recombinant NOD. B10. D2 mice to study initial adhesion of oral streptococci. Clinical and Diagnostic Laboratory Immunology. 2004 Mar 1;11(2):379-86.

5.Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. Journal of dental education. 2001 Oct;65(10):1028-37.

6.Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. Australian dental journal. 2000 Dec;45(4):235-45..

7.Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. Journal of dental research. 2011 Mar;90(3):294-303.

8.Wilson M. Susceptibility of oral bacterial biofilms to antimicrobial agents. Journal of Medical Microbiology. 1996 Feb 1;44(2):79-87.

9.Smalley JW. Pathogenic mechanisms in periodontal disease. Advances in dental research. 1994 Jul;8(2):320-8.

10.Meurmann JH. Chemotherapy of Streptococcus mutans. Proc Finn Dent Soc 1986; 82:305-11.

11.Kidd EA. Role of chlorhexidine in the management of dental caries. Int Dent J 1991; 41:279-86.

12.Gold JA. The role of chlorhexidine in caries prevention. Oper Dent 2008 Nov;33(6) :710-716

13.Craig WJ. Health-promoting properties of common herbs. Am J ClinNutr 1999; 70(suppl):491S-9S

14.Goyal P, Kaushik P. In vitro evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, Ocimum sanctum L. British Microbiology Research Journal 2011;1(3):70-78.

15.Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutans: An in- vitro study. Indian J Dent Res 2010; 21:357-359.

16.Saraya S, Kanta J, Sarisuta N, Temsiririrkkul R. Development of Guava Extract Chewable Tablets for Anticariogenic Activity against Streptococcus mutans. Mahidol university journal of pharmaceutical sciences 2008;35(1-4):18-23.

17.Chaudhry NMA, Tariq P. Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. Pak J Pharm Sci 2006;19(3):214-218.

18.Nuryastuti T, Henny C, Busscher HJ, Iravati S, Aman AT, Krom BP. Effect of Cinnamon Oil on icaA Expression and Biofilm Formation by Staphylococcus epidermidis. Applied and environmental microbiology 2009 Nov;75(21):6850-6855.

19.Bhatt MK, Shankar MB, Saluja AK, Dholwani KK. Evaluation of the antimicrobial activity of Ocimum sanctum methanolic extract. Journal of Pharmaceuticals and science 2012;1(4):39-41

20.Gajendrasinh PR, Bhavika MK, Rohit S, Hetal A, Prajapati PK. In vitro Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (Azadirachtaindica L.) and Tulsi (Ocimum sanctum L.).IJPBA 2012; 3(3): 582-586

21.Gupta B, Kumar VN, Mallaiah S. Assessment of Antimicrobial Activity of Various Concentrations of Commercially Available Tulsi (Ocimum Sanctum) Powder against Streptococcus Mutans. Open Journal of Dentistry and Oral Medicine 2013; 1(2): 19-24

22.Sharma A, Meena A and Meena A. Antimicrobial activity of plant extracts ofOcimumtenuiflorum. IntJourn of PharmTech Research 2012 Jan-Mar;4(1):176-180

23.Bhateja S, Arora G. Therapeutic Benefits Of Holy Basil (Tulsi) In General And Oral Medicine: A Review. International Journal of Research in Ayurveda & Pharmacy. 2012 Nov 1;3(5).

24.Biswas b, Rogers K, Fredrick M, Daniels D, Yadav A. Antimicrobial Activities of Leaf Extracts of Guava (Psidiumguajava L.) on Two Gram-Negative and Gram-Positive Bacteria. IntJournOfMicrob 2013; 10(3)

25.Mehta VV, Rajesh G, Rao A,

Shenoy R, BH MP. Antimicrobial efficacy of Punicagranatum mesocarp, Nelumbonucifera leaf, Psidiumguajava leaf and CoffeaCanephora extract on common oral pathogens: an in-vitro study. Journal of clinical and diagnostic research: JCDR. 2014 Jul;8(7):ZC65.

26. Canephora Extract on Common Oral Pathogens: An In-vitro Study. Journal of Clinical and Diagnostic Research. 2014 Jul, 8(7): 65-68

27.Phytochemical and biopharmaceutical aspects of Psidiumguajava(L). essential oil : A review.ResearchJourn of Med Plants 2011;5(4):432-442

28.Prabu G R, Gnanamani A, Sadulla S. Guaijaverin – a plant flavonoid as potential antiplaque agent againstStreptococcusmutans.Journal of Applied Microbiology August 2006;101(2):487-495

29.Lufei, Cheng D, Qian Y. Antibacterial Effect of Cinnamon Oil Combined with Thyme or Clove Oil. Journal of Integrative Agriculture 2011;10(9):1482-1487

30. Zainal-Abidin Z, Mohd-Said S, Adibah F, Majid A, Mustapha WA, Jantan I. Anti-bacterial activity of cinnamon oil on oral pathogens. InThe Open Conference Proceedings Journal 2013 Dec 27 (Vol. 4, No. 1).

31.Oil on Oral Pathogens. The Open Conference Proceedings Journal, 2013;4:12-16 31)Rani S K, Saxena N and Udaysree. Antimicrobial Activity of Black Pepper (Piper nigrum L.)Global Journal of Pharmacology 2013;7(1): 87-90

32.Dorman H and Deans S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 2000, 88, 308–316

33.Fitriani D, Prawiro R Comparative studies on the antimicrobial activity of black Pepper (piper nigrum) and turmeric (curcuma longa) extracts. Aug-Oct -2010; 1(2)

34.Siarta Y O, Prasetyaningrum N. White Pepper Extract (Piper nigrum L.) as Antibacterial Agent for Streptococcus mutans In Vitro. IOSR-JDMS Jan.- Feb. 2013 ;4(6):25-29

35.Bajaj N and Tandon S. The effect of Triphala and Chlorhexidine mouthwash on dental plaque, gingival inflammation, and microbial growth. Int J Ayurveda Res. 2011 Jan- Mar; 2(1): 29–36.

36.Sheen S, Addy M. An in vitro evaluation of the availability of cetylpyridinium chloride and chlorhexidine in some commercially available mouthrinse products. British Dental Journal 2003 ; 194:207-210

37.Strydonck,V, Timmerman M, Velden U et al. Plaque inhibition of two commercially available chlorhexidinemouthrinsesJClinPeriodontol 2005; 32: 305–309

38.Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent. 2008 Nov- Dec;33(6):710-6

39.Prabhakar AR, Ahuja V, Basappa N. Effect of Curry Leaves, Garlic and Tea Tree Oil on Streptococcus Mutans and Lactobacilli in Children: A Clinical and Microbiological Study. Pesq Bras OdontopedClinInteg 2009;9(3):259-63

40.Black GV: Gelatine forming micro-organisms. Indep Practitioner 1886; 7: 546–548.

41.Flemming H-C, Wingender: The biofilm matrix. Nat Rev Microbiol 2010; 8: 623–633

42.Saxton CA. Scanning electron-microscope study of bacterial colonization of the tooth surface. Tooth Enamel (Edited by Stack, MV and Fearnhead, PW) pp. 1971;218.

43.Vacca-Smith AM, Venkitaraman AR, Schilling KM, Bowen WH: Characterization of glucosyltransferase of human saliva adsorbed onto hydroxyapatite surfaces. Caries Res 1996b;30: 354–360

44.Weiss, E., Rosenberg, M., Judes, H. and Rosenberg, E. (1982) Cell-surface hydrophobicity of adherent oral bacteria. CurrMicrobiol 7,125–128.

45.Whittaker, C.J., Klier, C.M. and Kolenbrander, P.E. (1996) Mechanisms of adhesion by oral bacteria. Annu Rev Microbiol 50, 513–552.

46.Hamada, S., Koga, T. and Ooshima, T. (1984) Virulence factors of Streptococcus mutans and dental caries prevention. J Dent Res 63,407–411

47.Beltrami M, Bickel M, Baehni P C. the effect of supragingival plaque control on the composition of the subgingival microflora in human periodontitis. J ClinPeriodontol 1987;14:161-4

48.Simard F, Landry RG. Mouthrinses as an antibacterial adjunct in periodontal treatment. J Can Dent Assoc 1994;60(10):906-11

49.C.A. Gurgan, E. Zaim, I. Bakirsoy, E. Soykan, J. Periodontal 2006, 77, 370-384.

50.N. Jenabian, M. Abedi, P. Tayebi, A.A. Moghdamnia, Int . J. Pharmacol, 2008 , 4, 361- 368. Kubo, I.Muroi, H. and Himejima, M. 1992

51.Bandow J.E., Brotz H.,Leichert L.I.O.,Labishinski H. and HEcker, M.proteomic approach to understanding antibiotic action. Amicro Agents Chemother 2003 47:948- 955. Antimicrobial activity of green tea flavor

52.Leven, M.D., Vanden, B.D.A., Marten,T., Vilientmick , A. and Lomweas,E.C. Screening of higher plants for biological activity.Planta Med. 1979.36:311-312

53.Mimica-Dukic N., Kujundzic S.,Sokovic M, and CouladisM. Essential oil composition and antifungal activity of Foeniculumvulgare Mill obtained by different distillation conditions. Phytother Res.2003.17 (4):368-71.

54.Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian J PhysiolPharmacol 2005;49:125-31.

55.Kumar, B.S.A., Lakshman, K., Tirupathi,M.S. Jayaveera, K.N. et al.Free radical scavenging and antibacterial activities of Armycard power (An Ayurvedic formulation).European Bulltien of Drug Research., 2009. 17:5-9.