

TO EVALUATE AND COMPARE THE ANTIBACTERIAL ACTIVITY OF FRESHLY PREPARED PUNICAGRANATUM, SYZYGIUMCUMINI EXTRACT MOUTHWASH & A COMBINATION OF CARICA PAPAYA WITH ANANASCOMOSUS IN CONTRAST WITH CHLORHEXIDENE AGAINST STREPTOCOCCUS MUTANS - AN IN-VITRO STUDY.

Research Paper

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ABSTRACT: Mouth wash is usually prescribed in dentistry for prevention and treatment of various oral diseases which are caused by cariogenic and aciduric bacteria. It is widely used as adjuncts to oral hygiene and in the delivery of active agents to the teeth and gums. These mouthwashes have the ability to reduce plaque formation and to alter the course of gingival formation due to their antibacterial, anti-inflammatory and cytostatic effects. Herbs are being widely explored to discover alternatives to synthetic antibacterial agents as they don't have any side effects. In the recent times the use of naturally occurring products are used on a large scale.

Aim: This study aims to evaluate and compare the antibacterial activity of freshly prepared Punicagranatum, Syzygiumcumini extract mouthwash & a combination of Carica papaya with Ananas Comosus in contrast with chlorhexidene against streptococcus mutans.

Material and Methods: Microbiological assay (well diffusion method) to determine zone of inhibition against pure forms of streptococcus was performed. The antibacterial effects of different extracts of Punicagranatum, Syzygiumcumini & a combination of Carica papaya with Ananas Comosus in contrast with chlorhexidene mouthwash were studied.

Results: The results of the present study are in following order Group 1> Group 4> Group 3> Group 2.

Conclusion: The impetus of research now-a-days should shift to herbal components which can be viable & harmless option to oral health.

Keywords:

Mouthwashes,
Chlorhexidine,
Punicagranatum,
Syzygiumcumini,
streptococcus mutans.

Conflict of interest: Nil

No conflicts of interest : Nil

INTRODUCTION: Dental caries is the most prevalent infectious disease which is caused by the bacterial breakdown of the hard tissues of the teeth (enamel, dentin and cementum). The caries process is initiated by activity of bacteria within the biofilm and dental plaque.[1] The mouth contains a wide variety of both commensals and pathogenic organisms, but only a few species of microorganism are believed to play an important role in causing dental caries; Mutans streptococcus and Lactobacilli. The eradication of cariogenic bacteria from the oral cavity using antibacterial agents is one of main strategies for prevention of dental caries.[2] Mouthwashes is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens. It is widely used as adjuncts

to oral hygiene and also in the delivery of active agents to the teeth and gums. These mouthwashes have the ability to influence plaque formation and to alter the course of gingival formation due to their antibacterial, anti-inflammatory and bactericidal effects.[3]

Nowadays herbs are widely used as an alternative to synthetic antibacterial agents.[4] Several antimicrobial chemicals incorporated into these herbal extracts which will help to improve oral health and oral hygiene without any side effects. Thus the purpose of this study was to evaluate and compare the antibacterial activity of freshly prepared Punicagranatum, Syzygiumcumini extract mouthwash & a combination of Carica papaya with Ananas Comosus in contrast with chlorhexidene against streptococcus mutans.

MATERIAL AND METHODS: The present in-vitro study was done in the Department of Paediatric and Preventive Dentistry, Hitkarini Dental College and Hospital, Jabalpur, Madhya Pradesh, in collaboration with Daksh Laboratories and Research Center, Jabalpur. Ethical clearance for the study design was approved by the Ethical committee of Hitkarini Dental College and Hospital, Jabalpur Madhya Pradesh.

Media Preparation: The microorganisms, *Streptococcus mutans* (ATCC 2517), were obtained from American-type culture collection. The bacterial strains from the stock were revived by plating on blood agar medium. After overnight incubation at 37°C, isolated colonies were selected with identification. Isolated colonies were transferred to sterile Brain Heart Infusion (BHI) broth and once again incubated overnight. The growth concentration was adjusted to 10⁵ organisms/ml by using 0.5 McFarland's turbidity standard.

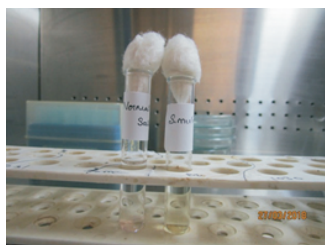


Fig.1 Brain Heart Infusion Broth

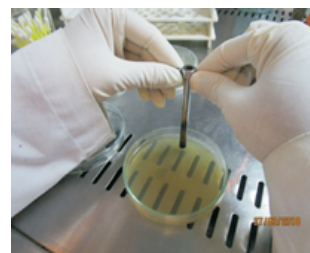
Four groups of mouth washes were used against the bacterial strains after taking minimum inhibitory concentration which are as follows:-

- A. Chlorhexidene Positive Control (Group I)
- B. Syzygiumcuminiextract extract mouthwash (Group II)
- C. Punicagranatum extract mouthwash (Group III)
- D. Carica papaya with Ananascomosus (Group IV)

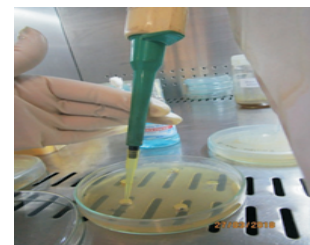
Screening of antibacterial activity [Agar well diffusion technique]: Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes. Well measuring 4mm depth was made on the agar with sterile cork borer. The samples of the mouthwashes (50 µl) was poured in wells by the help of micropipette & then the plates was incubated at 37°C for 24-48hrs and then zone of inhibition of growth was measured in millimetres.



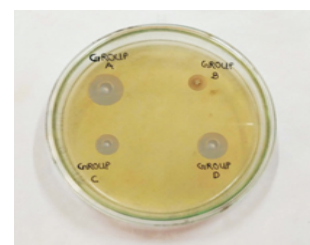
(a)



(b)



(c)



(d)

Fig. 2 (a) Swabbing of test microorganism by sterile cotton bud (b) Well of 4mm depth was made on plate using sterile cork borer(c) 50µl samples are poured in wells with the help of micropipette(d) Plates were incubated at 37°C and zone of inhibition of growth was measured in mm.

RESULTS: For statistical analysis data was entered in Microsoft excel 2016 for Windows. Mean, standard deviation (SD), minimum and maximum values of zone of inhibitions of different mouthwashes against *Streptococcus mutans* were calculated. Shapiro-Wilk test showed that zone of inhibitions followed normal distribution. Hence parametric test one-way ANOVA was applied for comparison. When one-way ANOVA showed significant results, LSD post hoc test was applied for pairwise comparison. P value <0.05 was considered statistically significant. Data analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA).One-way ANOVA showed significant difference between different mouthwashes for antimicrobial activity against *Streptococcus mutans*. After this LSD post hoc test was applied for pairwise comparison, which showed following observations:

1. Zone of inhibition with Chlorhexidine (Gr 1) was significantly higher than Jamun (Gr 2), Pomegranate (Gr 3) and Papaya and Pineapple (Gr 4).
2. Zone of inhibition with Papaya and Pineapple (Gr 4) was significantly higher than Jamun (Gr 2) and Pomegranate (Gr 3).
3. Zone of inhibition with Pomegranate (Gr 3) was significantly higher than Jamun (Gr 2).

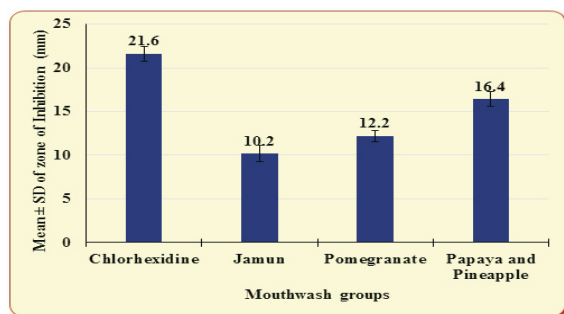
Largest zone of inhibition was observed with chlorhexidine and smallest with Jamun

Table 1: Antimicrobial activity of different mouthwashes against Streptococcus mutans.

Mouthwash groups	Zone of Inhibition (mm)	
	Mean ± SD	Min-Max
Chlorhexidine (Gr 1)	21.60 ± 0.84	20.00-23.00
Jamun (Gr 2)	10.20 ± 0.92	9.00-12.00
Pomegranate (Gr 3)	12.20 ± 0.63	11.00-13.00
Papaya and Pineapple (Gr 4)	16.40 ± 0.84	15.00-18.00
One-way ANOVA	F = 381.800, P = 0.000 (<0.001), Very high significant	
LSD post hoc test	Gr 1 > Gr 4 > Gr 3 > Gr 2	

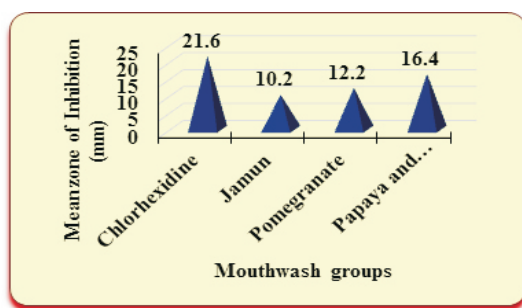
Format 1: Mean and SD value graph

Graph 1: Antimicrobial activity of different mouthwashes against Streptococcus mutans.



Format 2: Only mean value graph

Graph 1: Antimicrobial activity of different mouthwashes against Streptococcus mutans.



DISCUSSION: Bacterial plaques have been proven to have a role in the etiology of dental caries and periodontal diseases. The use of mouthwashes as disinfectants can help mechanical

methods to reduce plaques. In our study the maximum zone of inhibition was seen with chlorhexidine and also it has been proven its antibacterial property by various studies Phatan, et al (2017) showed that CHX showed higher levels of antimicrobial action than the herbal mouthwash against bacterial species similarly. Jayaprakash, et al. (2010) in their study showed that it has strong antibacterial action against streptococcus mutans the reason being is that it is a potent antimicrobial agent, particularly against MS. Chlorhexidine affects the metabolic activity of bacteria because, in low concentrations, it is bacteriostatic and prompts both changes to the functioning of the cellular membrane as well as leakage of the intracellular constituents, while in high concentrations, it acts as a bactericide, prompting irreversible precipitation of the cellular contents. It, likewise, inhibits the action of the glycosyltransferase enzyme responsible for the accumulation of bacteria on the dental surface and has effects on sugar transport and acid production in oral bacteria.[7]

Traditional Indian medicines like Ayurveda and Unani prescribe jamun for different health problems ilike diabetes, dental issues, digestive disorders, liver trouble and skin ailments. The leaves have antibacterial properties and used for strengthening teeth and gums. Essential oils extracted from the Jamun leaves have been reported to exert antibacterial properties against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Samonellatypimum. In this study we observed similar activity of jamun tree bark extract against streptococcus mutans Vasudevan Patturajan et al. (2018) showed that a, Jamun tree bark extract effectively inhibited the growth of all dental caries.[8]

Pomegranate showed maximum zone of inhibition after chlorhexidine and scygiumcuminextract mouthwash which is in accordance with the study conducted by Umar D et al. (2016) which shows that pomegranate has a potential anticariogenic mouthrinse. The anticariogenic property is because of its strong antioxidant activities, including radical scavenging ability, ferrous ion chelating, and ferric.[9]

The next maximum zone of inhibition was seen by the combination of Carica Papaya and Ananascomosus group which is similar to the study conducted by Fareen HF (2017) which shows that it has strong antibacterial property against streptococcus mutans. The reason being is that Ananascomosus contains bromelain enzyme which helps to break up plaque and the sticky film which accumulates on teeth.[10]

CONCLUSION: The efficacy of herbal mouthwash was equally effective in reducing plaque and gingivitis as compared to chlorhexidine mouthwash and may be considered as a good alternative. The chlorhexidine mouthwash was reported with many side effects such as discoloration of teeth, altered taste sensation, mucosal irritation, parotid swelling, and enhanced supra-gingival calculus formation which limits its acceptability and long-term use, whereas the presently tested herbal mouthwash has no side effects.

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