Preparation of Neem and Stevia mouthwash and its antimicrobial activity against oral pathogens

Nivedha Valliammai Mahalingam [1], *Vidhya T.K. [2]

1.CRRI,Saveetha Dental College & Hospitalsl,Saveetha Institute of Medical and Technical Sciences (SIMATS),Saveetha University Chennai,Tamilnadu, India
Email Id:151901047.sdc@saveetha.com

2.Senior lecturer,Department of Prosthodontics ,Saveetha Dental College & Hospitals
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Saveetha University Chennai,Tamilnadu, India
Email Id:vidhyatk.sdc@saveetha.com

Corresponding author: Dr.Vidhya T.K.
Senior lecturer ,Department of Prosthodontics
Saveetha Dental College & Hospitals,Saveetha Institute of Medical and Technical Sciences (SIMATS),Saveetha University,Chennai,Tamilnadu, India
Email Id:vidhyatk.sdc@saveetha.com

Abstract:

Introduction: Poor oral hygiene leads to dental caries gum diseases which may lead to systemic illness also. Overall health status of our body depends on maintaining good oral hygiene. Herbal mouthwash which contains plants and their extracts is a therapeutic and traditional method to treat oral diseases. Numerous studies have strongly proved that toothpaste and mouthrinse with herbal formulation are successful in the treatment of various oral diseases like gingivitis, halitosis, ulcers in the oral cavity and tooth decay.

Aim: The main aim of our study is to evaluate the antimicrobial activity of neem and stevia based mouthwash against oral pathogens.

Materials and method: Neem and stevia extract was prepared and condensed to 5ml. Mouthwash was prepared using the extract .

Results: The zone of inhibition caused by the prepared mouthwash in S.aureus, E.faecalis and C.albicans at concentrations 25µL, 50 µL ,100 µL and commercial mouthwash .

Conclusion: The prepared neem and stevia mouthwash shows convincing antimicrobial activity. Even though the Synthetic MouthWash has its gold standard, the herbal mouth wash with natural antimicrobial agents can be easily substituted for long term use, avoiding the side effects of Synthetic Mouthwash.

INTRODUCTION:

Aza- dirachta indica A. Juss commonly known as neem is one of the most commonly available medicinal plants in India. This neem tree holds various medicinal properties which make it a versatile source. All parts of the plant are used in nanoscience to produce natural and traditional products which are commercially available. (1). Neem is now acknowledged as a natural substance with a lot to offer in addressing major issues with public health, the environment, and agriculture around the world.(2) The significance of neem in the agricultural sector is currently being studied by researchers all around the world (3). In the current research field neem is used in various commercial applications, because of its antimicrobial, anti inflammatory, cytotoxic and antioxidant properties (4). Some Neem based commercially available products are oil, skincare, mouthwash and more which have shown promising biological activities and healing processes. Mouthwashes are antiseptic solutions used to remove bacterial colonisation in teeth and gingiva (5). Over the counter mouthwash is usually known for its antibacterial properties. Chlorhexidine is the most commonly available commercial mouthwash which shows high substantivity and antibacterial properties. Using mouthwash can help reduce plaque and calculus formation.

Maintaining good oral hygiene by brushing and flossing keeps the mouth clean and healthy which prevents tooth decay and gum disease (6). Dental caries is an interaction of many factors like diet and environmental factors like social, behavioral, psychological, and biological factors. Dietary carbohydrates, especially sucrose rich food and dental plaque plays a major role in the formation of dental caries. Hence these are considered to be dietable bacterial diseases. The Predominant role played by bacteria in initiation of dental caries and other oral diseases are well established and most of them are multifactorial. (7).Dental caries are caused by the alteration of the demineralization-remineralization cycle, destroys tooth structure and negativities the chewing and aesthetic appearance (8). Cariogenic microflora, fermentable carbohydrates, plaque are the factors and duration play the most effective roles in the formation of dental caries. The bacterial accumulation on dental surfaces known as dental plaque are the major reason for dental caries. Dental caries don't occur, dental plaque or fermentable carbohydrate products are not present.(9)

In this trendy world, tooth brushing can't stand alone in removing the dental caries. Mouthwashes is an added oral hygiene care that supports the control of bacterial plaque to restrain periodontal disease (10). Natural herbs in combination or utilized as a single

ingredient have been strongly and scientifically proven to be safe without any side effects and shows significant effect against various oral diseases such as gingivitis, halitosis, ulcers in the mouth, and preventing dental caries. The major advantage of these natural herbs are that there are no side effects associated and contains no artificial sugar, color, alcohol etc. (11). Thus, herbal mouthwashes are useful in maintaining good oral hygiene and health (12). Management of dental caries mainly depends on restraining tooth demineralization by changing the dietary habits, controlling the growth of microbes, altering the pH of saliva, and its buffering capacity. A mouth rinse or mouthwash is one of the means to promote healthy oral hygiene and prevent dental caries by targeting the bacteria causing caries. (13). Numerous mouthwashes with antimicrobial activity are commercially available in the market used to prevent dental caries. Mouthwashes are identified to be the safest and effective source as they have the ability to deliver therapeutic ingredients to all surfaces in the oral cavity including interproximal surfaces (10).

Herbal medicines are therapeutic and traditional methods to treat diseases using plants and their extracts. Herbal medicines are both promotive and preventive in its approach and have less side effects. (14). Herbal medication uses various secondary metabolites extracted from plants like *Bibhitaka* which is a key ingredient of the ayurvedic preparation Triphala for treating skin disease and respiratory disease. (15,16)Another herb *Nagavalli* has antioxidant, antiinflammatory, antimicrobial activities, *Peppermint* contains methanol that activates the cold receptor in the skin and the mucosal tissues, to maintain good health (17). Numerous Studies have proven the successful treatment of various oral diseases like gingivitis, halitosis, ulcers of oral cavities and dental caries using herbs like Triphala, tulsi patra, green tea, neem, clove oil, pepper, pudina etc. (18)Punica Granatum or pomegranate belongs to the Punicaceae family and is a shrub native to Asia. (19)The peel and arils are the main source of bioactive compounds like polyphenols, flavonoids, tannins, minerals and vitamins and are used as an astringent, hemostatic agent, and drug to control diabetes. The ethanolic extract of *A.comosus* peel includes phytochemicals like alkaloids, flavonoids, saponins, tannins, triterpenoids and phytosterols (20). The presence of abundant polyphenols in pomegranate juice are hydrolyzable tannins called punicalagins, which are observed to have antioxidant properties. (21)Intake of pomegranate plays a vital role as an anti-bacterial and antioxidant agent in the oral and gingival areas. (15)Mouthwash and toothpaste can be used as a carrier for daily exposure to these active agents (22). The present study deals with stevia This study is based on synthesis of neem and stevia based mouthwash and to evaluate the antimicrobial activity.

MATERIALS AND METHODS:

Extract preparation:

1gm of Neem (shown in figure 1) and 1gm of Stevia was added in 100 ml of distilled water (shown in figure 2) and boiled for 15-20 minutes at 40 degrees celsius (shown in figure 3). After boiling, the plant extract was filtered by Whatman No filter paper. Kept for condensation upto 5 ml and the solution is saved in the refrigerator for further use.

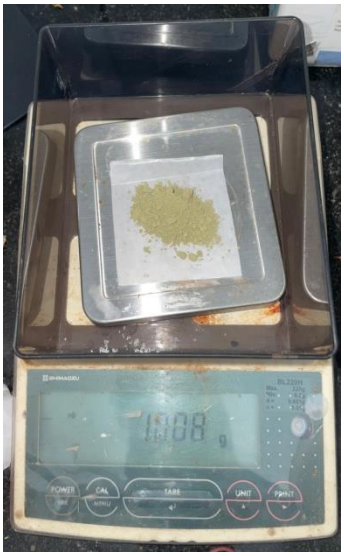


Figure 1:1gm of Neem

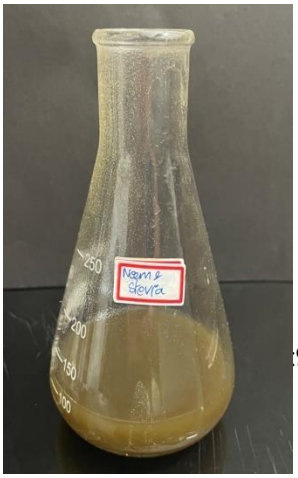


Figure 2:1gm of Stevia was added in 100 ml of distilled water

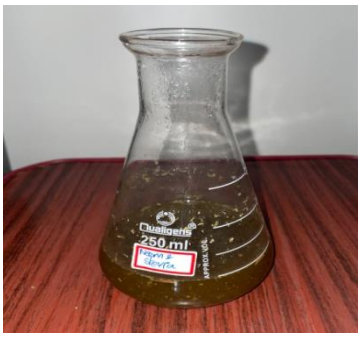


Figure 3: Neem and stevia in ethanol

Mouthwash preparation:
0.3gsucrose, 0.001g sodium benzoate and 0.01g of sodium lauryl sulphate is dissolved in 8ml distilled water. To that extract sample 2ml was added. And flavouring agent peppermint oil was added- 50 microlitres.

Figure 4: Neem and stevia extract

Figure 5: Neem and stevia mouthwash

Antibacterial Activity

Antibacterial activity of respective mouthwash against the strain Staphylococcus aureus, and E.faecalis. Mueller Hinton Agar was utilised for this activity to determine the zone of inhibition. Mueller hinton agar was prepared and sterilised for 15 minutes at 121oC. Media poured into the sterilised plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The mouthwash samples with different concentrations (25µL, 50 µL ,100 µL) were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hours at 37 °C. After the incubation time the zones of inhibition were measured.

Antifungal activity

Candida albicans is used as a test pathogen by agar well diffusion assay. Rose Bengal Agar is used to prepare the fungal medium. The prepared and sterilised medium was swabbed with test organisms and mouthwash samples with different concentrations (25µL, 50 µL ,100 µL) were added to the wells and in the fourth well standard antibiotic fluconazole was loaded. The plates were incubated at 37°C for 48-72hours. After the incubation time the zone of inhibition was measured.

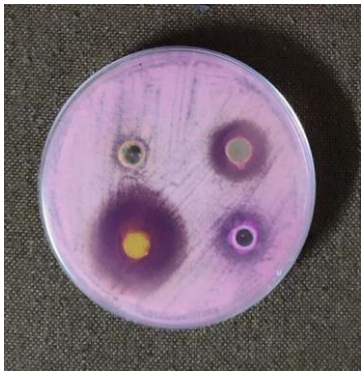
RESULTS AND DISCUSSION:

Organisms	Zone of inhibition

	25 µL	50 µL	100 µL	Commercial mouthwash
S.aureus	9	9	12	28
E.faecalis	9	12	14	13
C.albicans	10	14	16	22

S.aureus

E.faecalis



C.albicans

Figure 6: Antimicrobial activity of neem and stevia based mouthwash on S.aureus, E.faecalis and C.albicans

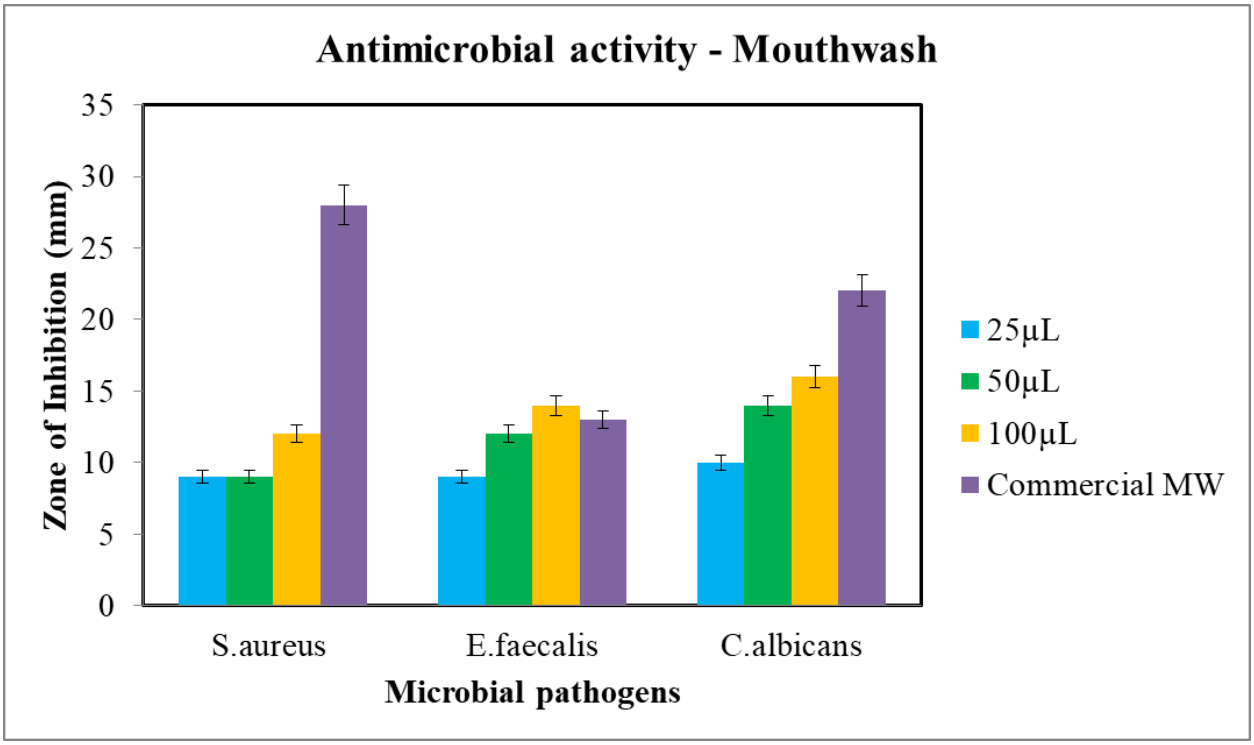


Figure 7: Antimicrobial activity of neem and stevia based mouthwash

The relatively evident fact is that bacterial biofilms play a major role in the etiology of dental caries and periodontal diseases. Since mechanical methods of plaque control have some limitations, chemical methods of removal were projected. Consequently, the use of mouth rinses regularly are found to be beneficial in reducing the biofilm formation in conjunction with mechanical methods. (23).

Streptococcus mutans, a commensal bacteria of the oral cavity, is considered one of the potent initiators of dental caries. Herbal mouthwash like Hiora are made of natural herbs with medical properties like anticariogenic and antiplaque properties The main constituents of this mouth wash are S. persica which contains trimethylamine, salvadorine, chlorides, sulfur, vitamin C, tannins, saponins, flavonoids and sterols, Piper betle and Elettaria cardamomum with antibacterial activity, *Terminalia Billerica with* anti-inflammatory and immunity booster effects and natural flavorings agents Mentha and Trachyspermum ammi. Nagavalli (piper betle) present in Hiora mouthwash assert to inhibit Streptococcus and Actinomyces species and its aqueous extract shows plaque inhibitory action. The current study is in line with the study done by Charuta Sadanand Dabholkar et al (23,24).

The herbal formulations such as triphala, *Gaultheria procumbens*, *Allium sativu*m, *Salvadora persica*, *Zingiber officinale*, *Citrus limon* and *Mentha piperita* are used as

indigenous ingredient in herbal mouthwash, each of which are strongly proven to have antimicrobial activity on the oral pathogens. A study conducted by Vrushali Ramdas Khobragade et al, clearly states the efficacy of the herbal mouthwash against organisms from supra gingival dental plaque in healthy and chronic periodontitis patients (25) which is in a similar line with the present study.

In a study conducted by Mehta S et al, it was observed that *A. actinomycetemcomitans* was most resistant to mouthwash containing pomegranate extract at lower concentration as similar to the result of our present study where it became effective at higher concentrations (25,26). The results of our present study are also similar to the study conducted by Vahabi , et al on Streptococcus .mutans which showed that Punica granatum and S. persica which is one of the components of Hiora mouthwash showed significant antibacterial activity. At 100% w/v, Punica granatum had strong antibacterial activity against S.mutans, and its antimicrobial activities were more significant than Miswak (S.persica) which is in favor of our article (27).

CONCLUSION:

The properties of synthesising mouthwash are further implied in nano science and nano technology which may be useful for future medicinal purposes. Even though the Synthetic MouthWash has its gold standard, the herbal mouthwash containing neem , and stevia with natural antimicrobial agents can be easily substituted for long term use, avoiding the side effects of Synthetic Mouthwash.

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