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All Herbal Mouthwash: A Retreat to Nature

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Abstract:

Mouthwashes are an integral part of proper oral hygiene apart from brushing and use of other components. They have the ability to percolate within cracks and small gaps where a brush cannot clean. However, most mouthwashes are laden with chemicals which might induce an allergic reaction and furthermore is not suitable for young kids or patients who have a compromised immune system or are undergoing chemotherapy. In such cases, herbal mouthwashes are an effective remedy as they do not have any chemicals which might cause adverse effects, hardly elicit an allergic reaction, and is easy to make and pocket friendly for the masses. Herbal plants like Tulsi, Neem, Turmeric, etc., have been known for their antimicrobial effects since thousands of years. A formulation of their bio-active compounds in proper concentrations can help in reducing oral pathogens effectively.

Keywords: Herbal, Antimicrobial, Mouthwash, S. mutans, Bioactive Materials.

1. Introduction:

The importance of oral hygiene has been of utmost importance, back from the earliest civilizations to the modern times. The use of toothpicks by the early Sumerians, the mention of toothbrushes and mouthwashes found in early scriptures of Chinese and Babylonian civilizations and the medication regimes for gingivitis found from early Egyptian civilization epitaphs suggest that oral hygiene was much emphasized since the early ages [1]. The use of herbal products like Neem (Azadirachta indica), Tulsi (Ocimum tenuiflorum) and Peppermint (Mentha piperita) have found multiple mentions in scriptures stating their antibacterial properties and hence in aiding oral hygiene[2]. The use of such natural ingredients has minimal side effects and are suitable for people of all age groups and most health conditions. The rise of chemical substituents in hygiene products have seen dwindling use of such natural ingredients and indiscriminate use of chemical products. This can sometimes be harmful as synthetic compounds can only be used in certain concentrations, the aberration from which can cause adverse effects on individuals. Furthermore, the exposure to chemicals is not recommended for young children and people with certain health conditions[3]. The use of toothpaste is often not satisfactorily enough to ensure a well rounded oral health regime. The use of mouthwashes have become indispensable to ensure thorough cleansing of the oral cavity. As such the use of all herbal

mouthwash can be a safe and effective oral health routine.

1.1 **Microbial activity in dental problems**: The major bacteria responsible for dental caries and gingivitis, the leading oral problems, are Streptococcus mutans and Porphyromonas gingivalis [4]. The ability of the bacteria to form a biofilm that eventually forms a dental plaque leads to weaking of teeth and gums. Certain proteins like glucosyltransferases, multiple glucan-binding proteins, protein antigen c, and collagen-binding protein, surface proteins coordinate to produce dental plaque [5]. The active or catalytic domain of such proteins can be inhibited by masking the active site with certain ligands. S. mutans produce 3 types of Glucosyltransferase, and they altogether coordinate the adherence of bacterial cells. The simultaneous synthesis of glucans also form a matrix that increases coordination of the bacteria and form a highly dense biofilm [6]

1.2 **Bioactive compounds as a remedy**: Plants are a reservoir of a rich source of bioactive compounds which protect them from pathogens such as bacteria, fungi and viruses. These bioactive compounds when tested against a wide variety of pathogens show antimicrobial effects [7]. Hence, plants have been utilized since early ages by humans for concocting home remedies to combat diseases and infections. Even animals show the use of certain plants for covering wounds, in case of stomach distress, etc[8]. Some examples of plants and their bioactive compounds are:

Plant	Scientific name	Bioactive compound
Neem	Azadirachta indica	Nimbin
Tulsi	Ocimum tenuiflorum	Oleanolic acid
Turmeric	Curcuma longa	Curcumin
Cinnamon	Cinnamomum verum	cinnamaldehyde
Cloves	Syzygium aromaticum	Eugenol

Table 1: List of herbal plants and their Bioactive compounds

1.3 **Molecular Docking of target and ligand**: Certain proteins like glucosyltransferases and glucan binding proteins play a major role in dental plaques and gingivitis by the formation of biofilms on the surface of teeth [9]. Certain bioactive compounds found in plants act as ligand and bind to their proteins and prevent them from reacting with other proteins to form dental plaque. Furthermore, some ligands bind to the proteins or genes necessary for the biochemical or physiological processes of the pathogens and hence are crucial to their survival. The binding disrupts the functioning of the pathogens and either kill them or inhibit their growth. The bindings which have negative score have hinging spontaneous binding and hence higher

inhibition. These ligands and compounds can be used effectively as an antimicrobial compound.

2. Formulation of the Mouthwash

The bioactive compounds in plants are the secondary metabolites. Some of them act as antimicrobial compounds to protect the plant. These compounds can be extracted from the plant by mixing crushed plant parts in steeping water. The separate compounds can be mixed to formulate an effective concoction for a mouthwash. It must be tested against oral pathogens and must have a long shelf life.

2.1 **Molecular docking of bioactive compounds**: The formulation of the mouthwash was decided by the ability of the bioactive compounds of the plants to bind with the glucosyltransferase and glucan binding proteins and DNA segments of the pathogens, S. aureus and S. mutans. It helps to inhibit the growth or kill the pathogen. Furthermore, it can prevent the activity of the plaque forming proteins. The list of proteins and their bioactive compounds along with their binding affinity is given below:

Plant	Bioactive compound	vina score
Neem	Nimbin	-8.4
Tulsi	Oleanolic acid	-9
Turmeric	Curcumin	-7.9
Cinnamon	cinnamaldehyde	-5.3
Cloves	Eugenol	-5.7
Guava leaves	Rutin	-6.7
Cardamom	alpha-Pinene	-5.2
Mint	dl Menthol	-5.3
Ginger	Raffinose	-6.3

Table 2: Vina scores for docking of bioactive compounds with glucosyltransferase of S.mutans

The Vina score shows the affinity of bonding, the more negative the score, higher the antimicrobial activity of the compounds. The vina score here is calculated for different bioactive compounds against Truncated Catalytic Domain of Glucosyltransferase of S. mutans which is mainly responsible for the formation of dental plaques.

2.2 **Extraction of Bioactive compounds**: The plant parts contain bioactive compounds which might not be obtained by simply boiling. Hence crushing and grinding of the plant parts in appropriate solvent is crucial before boiling the crushed parts. The weight

taken of the individual plants and their anatomical parts are given below:

Plant	Plant part	amount in grams/L
Neem	Leaves	60
Tulsi	Leaves	60
Turmeric	Root	25
Cinnamon	Bark	25
Cloves	Flower buds	25
Guava	Leaves	60
Cardamom	Dried fruit	25
Mint	Leaves	60
Ginger	Stem	25

Table 3: List of plants, anatomical parts and amount in g/L used.

Each of these components were first crushed in water. Then each component was separately steeped in 50ml of water at 80 degrees Celsius. Steeping the solution instead of boiling is crucial as it does not degrade the bioactive compounds, many of which are heat liable. The solutions were filtered through a Whatmann filter paper to remove the particles and small plant parts. The filtrate of the separate components were then mixed together to form the mouthwash solution.

3. Physical and Biochemical Properties:

The crucial properties that a mouthwash must have apart from being effectively antimicrobial are long shelf life and a near to physiological pH value. It is not ideal if the mouthwash degrades in room temperature very fast. Furthermore, the antimicrobial activity should not reduce for a considerable amount of time. The pH should be in a range of 6.5 to 7, as the pH of the buccal cavity is 6.7. Any variation from the range can lead to changes in the enzymatic reactions and growth of natural flora in the mouth.

3.1 **pH of the mouthwash**: The pH of the mouthwash should be as close to the physiological pH of the buccal cavity as possible. The pH of mouth is 6.4-7.4 which is ideal for the enzymatic activities of the oral enzymes. If the pH becomes more acidic, it can lead to enamel decay of the teeth. If the pH increases, it can cause decay of organic substances and leave the teeth vulnerable. Hence it is imperative to check if the pH of the mouthwash stays in the range over an extended period of time. The pH of the mouthwash was checked over a period of 21 days.

3.2 **Physical properties of the mouthwash:** The precipitation or

phase separation of a mouthwash is an indicator of degradation of inactivation of the constituents of the mouthwash. Hence there must be no precipitate or phase separation between the constituents. Discoloration of the solution or large variation in OD can be an indication of microbial or chemical degradation and hence is unsuitable for use. The mouthwash must be able to retain its physical properties for long and must not vary hugely in its optical properties.

3.3 **Antimicrobial effect of Mouthwash**: The mouthwash must be able to significantly reduce the growth of oral pathogens. To compete with other widely available chemical mouthwashes, it must be able to reduce growth of bacteria and fungi in the mouth. To test the antimicrobial activity, two swabs tests were performed, one taken from the buccal cavity after normal brushing and one taken after brushing and rinsing with the mouthwash. The swabs were streaked on LB agar plates and incubated for 24 hours to notice the growth of pathogens.

4. Results and discussion

The mouthwash in fairly effective in reducing the growth of pathogens in the buccal cavity. It does not degrade over time and the pH stays fairly consistent over days. These properties, combines with the fact that it is made with readily available ingredients and budget friendly make it an ideal product for everyone.

4.1 **pH variation of mouthwash**: The variation in pH gives an ideal of its shelf life and feasibility of use. If the pH lies outside of the physiological range, it cannot be used. The pH of the mouthwash was found to be within the pH range over a period of 21 days.

Figure 1: Plot representing variation of pH over days

As we can see, the variation in pH remains quite low and the pH lies in the desired range of 6.6 to 6.9 over a period of 21 days. Hence, we can deduce that the pH of the mouthwash is suitable for use.

4.2 Variation in physical properties: The physical properties and the optical properties are important in determining the shelf life of the mouthwash. There must be no phase separation and less variation in the optical properties of the mouthwash. The phase separation and precipitation was noticed for 21 days at an interval of 5 days

Day	Temperature	Phase Separation	Precipitation
0	4	None	None
0	37	None	None
5	4	None	None
5	37	None	None
10	4	None	None
10	37	None	None
15	4	None	None
15	37	None	None
21	4	None	None
21	37	None	None

Table 4: Observation of physical properties over days

There is no phase separation or precipitation which shows that the mouthwash remains stable for a long time, i.e., 21 days without the use of preservatives. This property is conferred by the use of Cinnamon which contains a chemical, coumarin, which is a powerful anticoagulant. Hence, it prevents phase separation and precipitation.

4.3 Variation in Optical properties: The variation in color and optical properties should be minimal. Discoloration of the mouthwash suggest an underlying chemical reaction between the components and degradation of its constituents. The Optical density of the mouthwash was measured at Room temperature and kept in a refrigerator separately at 650nm absorbance. Water was used as a blank.

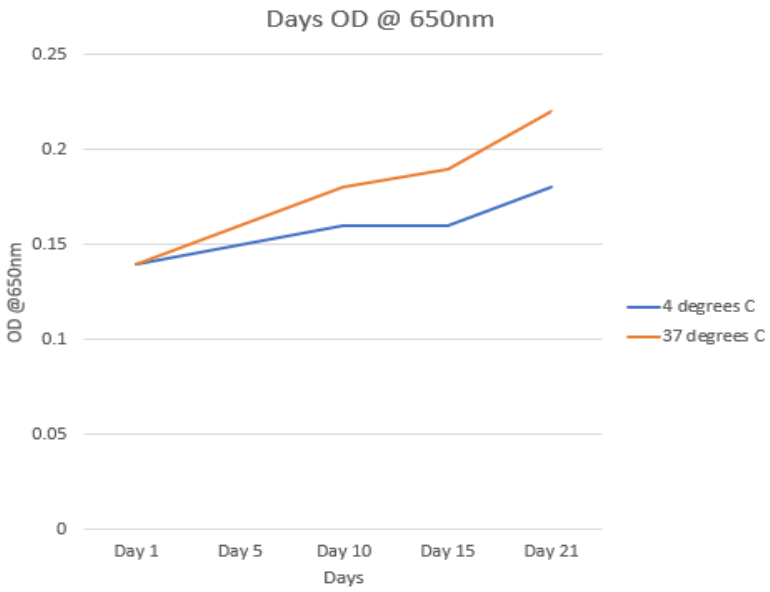

Figure 2: Plot representing variation of OD with days

The plot shows that there is not a large variation between the optical densities of the two samples kept at refrigerated and at room temperature. Furthermore, the OD does not change significantly over the days and hence does not show any signs of degradation or contamination. Hence its properties as a mouthwash is appropriate.

4.4 Antimicrobial properties: The agar plates streaked with swabs were analysed after 24 hours. There was a significant reduction of microbial growth on the plate streaked with swabs after rinsing with mouthwash. On the plate, streaked with swab before using mouthwash, there was significant growth of bacteria. Gram's staining was performed on the colonies. It was noticed that majority of the bacteria were Gram positive due to their purple-bluish appearance, whereas some bacteria were Gram negative owing to their reddish appearance due to retention of safranin dye. There was also a growth of filamentous fungus in between. However, in the agar plate with swab after the use of mouthwash, there was a 90 percent reduction in bacterial growth, all of them being Gram positive. There was no Gram negative bacterial growth and furthermore no growth of fungus was seen. This proves that the mouthwash is significantly effective in reducing microbial growth and is a effective mouthwash for use.

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References

The list of references are given below:

1. Gurudath, G., Vijayakumar, K. and Arun, R. (2012) 'Oral Hygiene Practices: Ancient Historical Review', Journal of Orofacial Research, 2, pp. 225–227. doi:10.5005/jp-journals10026-1047.

2. Chandrappa PM, Dupper A, Tripathi P, Arroju R, Sharma P, Sulochana K. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against Enterococcus faecalis in Endodontics: An in vitro study. J Int Soc Prev Community Dent. 2015;5(Suppl 2):S89-S92. doi:10.4103/2231-0762.172952

3. Acute ethanol toxicity from ingesting mouthwash in children younger ... Available at: https://www.aapd.org/globalassets/media/publications/archives/shulman19-06.pdf (Accessed: 01 June 2023).

4. Chapter 99 Microbiology of Dental Decay and Periodontal Disease Walter J. Loesche.

5. Matsumoto-Nakano M. Role of Streptococcus mutans surface proteins for biofilm formation. Jpn Dent Sci Rev. 2018;54(1):22- 29. doi:10.1016/j.jdsr.2017.08.002

6. Zayed SM, Aboulwafa MM, Hashem AM, Saleh SE. Biofilm formation by Streptococcus mutans and its inhibition by green tea extracts. AMB Express. 2021;11(1):73. Published 2021 May 25. doi:10.1186/s13568-021-01232-6

7. Shurkin J. News feature: Animals that self-medicate. Proc Natl Acad Sci U S A. 2014;111(49):17339-17341. doi:10.1073/pnas.1419966111 8. 8. Devulapalle KS, Mooser G. Glucosyltransferase inactiva- tion reduces dental caries. J Dent Res. 2001;80(2):466-469. doi:10.1177/00220345010800021301