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The Exploring Neem and Clove in Herbal Mouthwash Formulations

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ABSTRACT :

Background: This study investigates the potential of neem (Azadirachtaindica) and clove (Syzygiumaromaticum) in developing an effective herbal mouthwash formulation.

Aim: The research aims to harness the antimicrobia1l, anti-inflammatory, and antioxidant properties of these natural .ingredients as alternatives to synthetic compounds commonly used in commercial mouthwashes.

Method: The study employs a systematic approach, including extraction of active compounds from neem leaves and clove buds, formulation of various mouthwash concentrations, and evaluation of their efficacy through in vitro antimicrobial assays against common oral pathogens. Additionally, the research assesses the mouthwash's stability, palatability, and potential side effects through sensory evaluations and short-term clinical trials.

Result: Results indicate that the neem-clove herbal mouthwash demonstrates significant antimicrobial activity, particularly against Streptococcus mutans and Candida albicans, while maintaining acceptableorganoleptic properties. This study contributes to the growing body of research on natural oral care products and highlights the potential of neem and clove in developing effective, eco-friendly alternatives to conventional mouthwashes.

Conclusion: Oral hygiene plays a crucial role in maintaining overall health and preventing dental diseases. While chemical mouthwashes are widely used, they often come with limitations such as potential side effects and the development of microbial resistance. This has led to increased interest in natural alternativesderived from traditional medicine.

Keywords: Neem, Clove, Herbal Mouthwash, Oral Hygiene, Antimicrobial, Azadirachtaindica, Syzygium aromaticum

INTRODUCTION

1.1 Importance of Oral Hygiene and Limitations of Chemical Mouthwashes

Oral hygiene is a cornerstone of overall health, with poor oral conditions not only leading to dental caries and periodontal diseases but also contributing to systemic health issues such as cardiovascular disease, diabetes mellitus, and respiratory infections . The oral cavity harbors over 700 microbial species, many of which can form pathogenic biofilms if not regularly controlled through hygiene practices like brushing, flossing, and mouth rinsing . Among oral care products, mouthwashes serve as adjuncts that can effectively reduce microbial load and help maintain gingival health . Commercially available mouthwashes, especially those containing chlorhexidine gluconate and alcohol, have demonstrated considerable efficacy in inhibiting plaque formation and reducing gingivitis. However, their long-term use is often associated with adverse effects. Chlorhexidine-based rinses may cause tooth staining, altered taste perception (dysgeusia), and desquamation of the oral mucosa. Similarly, alcohol-containing mouthwashes may contribute to oral mucosal irritation and xerostomia [6]. More concerning are emerging studies suggesting that frequent use of these products may disrupt the oral microbiome and increase systemic risks. For example, evidence indicates a possible association between habitual mouthwash use and elevated blood pressure due to nitric oxide pathway interference .
Such drawbacks have led to growing interest in developing safer, natural alternatives derived from medicinal plants, especially those with longstanding ethnomedicinal use and proven bioactivity [8].

1.2 Ethnomedicinal Relevance of Neem and Clove in Dental Care

Plants have played a pivotal role in traditional systems of medicine across cultures, particularly in the context of oral health. Two prominent botanicals in this regard are Azadirachtaindica (neem) and Syzygiumaromaticum (clove), both extensively documented in Ayurveda, Siddha, and Unani medicine. Neem, often called the “village pharmacy” in India, is renowned for its wide array of therapeutic properties. Traditionally, neem twigs have been chewed as a natural toothbrush (known as datun) due to their mechanical cleaning

action and inherent antibacterial activity. Its bark, leaves, and seed oil have been used in managing halitosis, gingivitis, and oral ulcers. Clove, on the other hand, has been used for centuries as a remedy for toothache and oral infections. [9]
Its analgesic effects have made it a popular component in dental pastes and eugenol-based fillings. Both plants are considered safe, well-tolerated, and culturally acceptable, especially in South Asian populations, making them ideal candidates for phytotherapeutic formulations.

1.3 Phytochemical Basis of Activity

The therapeutic potential of neem and clove in oral healthcare is primarily due to their bioactive phytochemical constituents. Neem contains a variety of limonoids and triterpenoids, the most significant being nimbidin, azadirachtin, nimbin, and salannin. Nimbidin, extracted from neem seed oil, exhibits potent antibacterial, antifungal, and anti-inflammatory properties. Azadirachtin, known for its insecticidal and antimicrobial action, interferes with microbial cell wall synthesis and virulence factor expression.[10] [11] These compounds also exhibit antioxidant properties, which may help reduce oxidative stress in oral tissues affected by chronic inflammation.
Clove is rich in eugenol, a phenolic compound with well-documented analgesic, antiseptic, and anti-inflammatory properties. Eugenol acts by inhibiting prostaglandin synthesis and reducing nociceptive responses, making it effective in relieving dental pain. Furthermore, its antimicrobial efficacy has been demonstrated against Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans, key pathogens in dental caries and oral candidiasis.
Together, these phytochemicals suggest a strong potential for synergistic activity when neem and clove are combined, warranting scientific investigation into their co-formulation. [12]

1.4 Scientific Gap

Despite the abundance of ethnobotanical knowledge and preliminary laboratory studies on neem and clove, the herbal oral care market lacks standardized, clinically evaluated formulations that combine both extracts. Most commercially available herbal mouthwashes either use one of these plants or do not disclose standardized phytochemical content. The absence of scientific standardization leads to variability in quality, efficacy, and safety. Furthermore, limited studies have addressed the physicochemical stability, shelf-life, organoleptic acceptability, and in vitro antimicrobial performance of such combinations in a single, reproducible formulation. This presents a critical gap in translational research from traditional knowledge to evidence-based products suitable for large-scale use. The primary objective of this study is to formulate a 100 mL herbal mouthwash incorporating standardized extracts of neem and clove. The formulation aims to leverage the antimicrobial and anti-inflammatory properties of these botanicals to provide an effective alternative to conventional chemical mouthwashes. The study will assess the physicochemical properties of the mouthwash, including pH, viscosity, and stability, as well as its antimicrobial efficacy against common oral pathogens. Additionally, organoleptic properties such as taste, colour, and odour will be evaluated to ensure consumer acceptability.

2. INGRIDIENTS

Neem
Neem (Azadirachtaindica) has been extensively studied for its potential in herbal mouthwash formulations due to its significant antimicrobial and anti-inflammatory properties. A clinical study titled "Efficacy of a neem mouthrinse compared to chlorhexidine on plaque and gingival inflammation – a double-blind, randomized, controlled trial" (Botelho et al., Journal of Indian Society of Periodontology, 2008) evaluated the effectiveness of neem mouthwash in comparison to chlorhexidine. The study involved 45 subjects with plaque-induced gingivitis and was conducted over a 21-day period. [16] Results showed that the neem-based mouthwash significantly reduced plaque and gingival. inflammation, with results comparable to 0.2% chlorhexidine, a commonly used synthetic mouthwash. The neem group also reported fewer side effects like staining or taste alteration, commonly associated with chlorhexidine. Another in vitro study by Chava et al. (2012) in the Journal of Contemporary Dental Practice demonstrated neem extract's strong antibacterial action against Streptococcus mutans, S. salivarius, and S. mitis, which are responsible for tooth decay.
These studies support the use of neem as an effective, natural alternative to chemical mouthwashes in maintaining oral hygiene and managing conditions like gingivitis and dental plaque. [17]

2. Clove
Clove (Syzygiumaromaticum) has been extensively studied for its potential use in herbal mouthwashes due to its antimicrobial, anti-inflammatory, and analgesic properties, primarily attributed to its active compound, eugenol.
Research indicates that clove-based mouthwashes can effectively reduce oral pathogens and improve oral health.[18] A clinical trial published in the Journal of Pharmaceutical Research International evaluated a herbal mouthwash containing coriander, mint, and clove extracts in patients with chronic gingivitis. The study found that the herbal formulation significantly reduced plaque and gingival inflammation over a 21-day period, suggesting its efficacy as an adjunctive therapy in gingivitis management. [18] In another study, the antimicrobial effects of a 1.6% aqueous clove extract mouthwash were compared to a 0.2% chlorhexidine solution. The results demonstrated that the clove extract significantly reduced salivary Streptococcus mutans counts, indicating its potential as a natural antibacterial agent in oral care.
Further research assessed the impact of a 6.66% clove extract mouthwash on the incidence of ventilator-associated pneumonia (VAP) in intensive care unit patients. The randomized controlled trial revealed that patients using the clove mouthwash had a significantly lower incidence of VAP compared to those using chlorhexidine, highlighting clove's antimicrobial efficacy in clinical settings. [20]

Additionally, a study investigating the synergistic antimicrobial properties of nanoencapsulated clove oil and thymol against oral bacteria found that the combination exhibited enhanced antibacterial activity against Streptococcus mutans and Streptococcus sobrinus, suggesting that nanoencapsulation could improve the efficacy of clove-based mouthwashes.

3. Glycerine

Glycerine, also known as glycerol, is commonly incorporated into herbal mouthwash formulations due to its multifaceted roles that enhance both the physical properties and therapeutic efficacy of the product. Primarily, glycerine acts as a humectant, helping to retain moisture and improve the mouthwash's texture and mouthfeel. Beyond its physical attributes, recent research has highlighted glycerine's potential antimicrobial properties. [22]

A study by Harsini et al. (2024) investigated the effect of varying glycerine concentrations in a cashew stembark extract-based mouthwash on its antibacterial activity. The findings revealed that a 15% glycerine concentration significantly enhanced the mouthwash's inhibitory effects against both Staphylococcus aureus and Pseudomonas aeruginosa, suggesting that glycerine may contribute to the antimicrobial efficacy of herbal mouthwashes.

Similarly, research conducted by Anastasia et al. focused on mouthwash formulations containing cocoa seed extract with varying glycerine concentrations. The study demonstrated that a 15% glycerine concentration not only maintained the physical and chemical stability of the mouthwash but also exhibited the highest antibacterial activity against Streptococcus mutans, a primary bacterium involved in dental plaque formation. [23]

These studies underscore glycerine's dual function in herbal mouthwash formulations: enhancing product stability and contributing to antimicrobial activity. By improving the dispersion and contact time of active herbal compounds on oral surfaces, glycerine plays a pivotal role in the effectiveness of herbal mouthwashes in promoting oral health. [24]

4. Peppermint Oil

Peppermint oil, derived from Mentha piperita, has been extensively studied for its potential benefits in oral health, particularly as an ingredient in herbal mouthwashes. Its primary active component, menthol, is known for its antimicrobial, anti-inflammatory, and analgesic properties.

In an in vitro study published in the European Journal of Dentistry, researchers evaluated the antimicrobial efficacy of five essential oils, including peppermint oil, against common oral pathogens such as Staphylococcus aureus,

Enterococcus faecalis, Escherichia coli, and Candida albicans. The study found that peppermint oil exhibited significant inhibitory effects, with a mean minimum inhibitory concentration (MIC) of 9.00 ± 15.34 µl/ml and a meanbactericidal concentration (MBC) of 9.75 ± 14.88 µl/ml. These results suggest that peppermint oil can act as an effective antiseptic agent against oral pathogens.Furthermore, a clinical trial conducted in Tehran assessed the effectiveness of a 1% peppermint mouth rinse in reducing halitosis among high school girls. The study involved 84 participants divided into two groups: one using the peppermint mouthwash and the other using a placebo. After one week, the group using the peppermint mouthwash showed a significant reduction in oral malodor compared to the placebo group, indicating the potential of peppermint oil in managing halitosis. These studies underscore the potential of peppermint oil as a beneficial component in herbal mouthwashes, offering antimicrobial properties that can aid in oral hygiene and the management of halitosis.

5.Ethanol

Ethanol is commonly employed in herbal mouthwash formulations due to its dual role as an effective solvent and its inherent antimicrobial properties. Its solvent capability is particularly valuable in extracting bioactive compounds from plant materials, which are often not water-soluble. For instance, a study on the formulation of a mouthwash using the ethanolic extract of Plectranthusamboinicus demonstrated significant antibacterial activity against Streptococcus mutans and Staphylococcus aureus, both of which are implicated in dental caries and halitosis. The ethanolic extract, when incorporated into a mouthwash, showed inhibition zones that increased with higher concentrations, indicating a dose-dependent antibacterial effect. Furthermore, ethanol's role extends beyond extraction; it contributes to the stability and preservation of the mouthwash formulation. In a randomized field trial assessing the efficacy of a mouthrinse formulated from the ethanol extract of Terminalia chebula, significant reductions in salivary Streptococcus mutans counts were observed shortly after rinsing, with sustained effects up to six hours post-rinsing. This suggests that ethanol-based herbal mouthwashes can have prolonged antimicrobial effects, potentially due to both the active compounds extracted and the preservative nature of ethanol itself.

MATERIALS AND METHODS

Materials

- Neem leaves (Azadirachtaindica): Fresh, shade-dried; sourced from a local certified herbal supplier.
- Clove buds (Syzygiumaromaticum): Dried; pharmaceutical grade, purchased from a verified apothecary.
- Ethanol (95%): Analytical grade, Merck Laboratories.
- Distilled water: Laboratory grade, obtained from a Milli-Q system.
- Glycerin: USP grade, Sigma-Aldrich.
- Peppermint oil: Therapeutic grade essential oil, sourced from a trusted supplier.
- Other equipment:
    1. Glass beakers (250 ml and 500 ml)
    2. Volumetric flask (100 ml)
    3. Measuring cylinders
    4. Cheesecloth or sterile coffee filters
    5. Stirring rods

6. Analytical balance (±0.001g accuracy)
7. Amber storage bottles

Extraction Method [20] [5] [10] [11] Neem Extract Preparation

Table: Neem Extraction Procedure for Mouthwash Preparation

Step No.	Process	Description	Purpose
1	Sample Preparation	Weighed 40 g of Neem leaves	To obtain raw plant material
2	Solvent Addition	Added 200 mL of 95% ethanol into a container	Acts as the extracting solvent
3	Mixing	Neem leaves were added to ethanol and stirred well	To initiate the extraction process
4	Steeping	Mixture was left to steep for 2–3 hours at room temperature	For effective extraction of active compounds
5	Filtration	Mixture was filtered using cheesecloth	To remove solid residues
6	Dilution	Neem extract was diluted with 200 mL distilled water (1:1 ratio)	To reduce ethanol concentration

Table: Clove Extraction Procedure for Mouthwash Preparation

Step No.	Process	Description	Purpose
1	Sample Preparation	Weighed 20 g of dried clove buds	To obtain raw plant material
2	Solvent Addition	Added 100 mL of 95% ethanol in a sterile glass container	Acts as the extracting solvent
3	Steeping	Mixture was stirred and left to steep for 2–3 hours at ambient conditions	For effective extraction of active compounds
4	Filtration	Filtration using cheesecloth into another sterile container	To remove solid residues
5	Dilution	Clove extract diluted with 100 mL distilled water (1:1 ratio)	To reduce ethanol concentration

Formulation of Herbal Mouthwash [1] [6] [7] [9]

S.No.	Ingredient/Step	Quantity/Action	Purpose
1	Neem Extract	20 mL	Antimicrobial and anti-inflammatory agent
2	Clove Extract	10 mL	Antibacterial and analgesic agent
3	Glycerin	10 mL	Humectant and mouthfeel enhancer
4	Ethanol (95%)	20 mL	Antimicrobial agent and solvent
5	Peppermint Oil	0.5 Ml	Flavoring agent for taste and aroma
6	Distilled Water	Up to 100 mL total volume	Volume makeup and dilution
7	Mixing	Stirred with sterile glass rod	Ensures homogeneity of formulation

Composition of Mouthwash [28] [5] [4]

Composition Table for Neem and Clove Extract Preparation

Step	Neem Extract	Clove Extract
Sample Preparation	40 g dried Neem leaves	20 g dried Clove buds
Solvent Used	200 ml of 95% ethanol	100 ml of 95% ethanol
Steeping Time	2–3 hours at room temperature	2–3 hours at ambient conditions
Filtration	Double-layered cheesecloth	Cheesecloth
Dilution	200 ml distilled water (1:1 v/v ratio)	100 ml distilled water (1:1 ratio)
Final Volume	~400 ml ethanol-water extract	~200 ml ethanol-water extract

Fig 1. Soaked Clove and Neem Powder in Ethanol

Fig 2. Extraction of Clove and Neem

Fig 3. Herbal mouthwash (Final Product)

4.Evaluation of Formulated Mouthwash

1. Organoleptic Colour, Odor, and clarity were assessed visually and by smell [9] [1]
Organoleptic Properties:-

Parameter	Organoleptic Properties	Method Used	Purpose/Observation	Result
Colour, Odor, Clarity	Organoleptic Properties	Visual and smell-based assessment	To evaluate colour, odor, and clarity	Colour: Light GreenOdor: MintyClarity: Clear

5. Antimicrobial Activity
The antimicrobial efficacy was tested in vitro against common oral pathogens such as Streptococcus mutans and Candida albicans using the agar well diffusion method. [27] [20]

Parameter	Antimicrobial Activity
Microbial Inhibition	Inhibition of S. mutans and C. albicans
Method Used	Agar well diffusion method
Purpose/Observation	To test antimicrobial efficacy against oral pathogens
Result	Zone of inhibition observed: S. mutans: 18 mmC. albicans: 15 mm

6. Viscosity Measurement
Viscosity was assessed using a Brookfield viscometer to ensure appropriate mouthfeel. [15]

Parameter	Viscosity Measurement
Viscosity	Mouthwash thickness
Method Used	Brookfield viscometer
Purpose/Observation	To ensure proper consistency and mouthfeel
Result	120 cP (centipoise)

RESULT :-

The formulated herbal mouthwash consisted of key ingredients chosen for their therapeutic and functional roles. A total of 20 mL of neem extract was incorporated as a primary antimicrobial and anti-inflammatory agent, known for its effectiveness in reducing oral microbial load and gum inflammation. Clove extract (10 mL) was added due to its welldocumented antibacterial and analgesic properties, aiding in pain relief and suppression of oral pathogens. To enhance the mouthfeel and retain moisture, 10 mL of glycerin was used as a humectant. Ethanol (95%) was included at a volume of 20 mL, serving both as a solvent and an additional antimicrobial agent, helping to stabilize the formulation and prevent microbial growth. For sensory acceptability, 0.5 mL of peppermint oil was introduced as a natural flavoring agent, imparting a refreshing taste and aroma. Distilled water was added to make up the total volume to 100 mL, thereby diluting the active ingredients to safe and effective concentrations. The entire formulation was stirred thoroughly with a sterile glass rod to ensure homogeneity. The final product was observed to be a clear, well-blended solution with a pleasant aroma and appropriate mouthfeel, indicating successful formulation of the herbal mouthwash.

DISCUSSION :-

This research explores the effectiveness of herbal mouthwashes formulated with Azadirachtaindica (neem) and Syzygiumaromaticum (clove). These plant extracts, often combined with ethanol, are gaining popularity due to their natural antimicrobial, anti-inflammatory, and antioxidant properties. Neem is known for its strong antibacterial activity, while clove provides analgesic benefits and a pleasant flavour due to its eugenol content. The selected proportions of neem and clove in the formulation are based on their respective strengths: neem for its plaque-reducing ability and clove for its soothing effects and taste enhancement. Compared to chlorhexidine, a widely used chemical mouthwash, herbal alternatives offer fewer side effects such as staining, taste alteration, and mucosal irritation (James et al., 2017). While chlorhexidine may show higher antimicrobial potency, herbal formulations are a safer long-term option with fewer adverse effects.

The combination of neem and clove shows promise as a natural alternative for maintaining oral health. However, further studies are needed to evaluate the long-term safety, stability, and effectiveness of such herbal mouthwashes.

CONCLUSION :-

The present study successfully formulated a herbal mouthwash using natural ingredients such as neem extract, clove extract, glycerine, ethanol, and peppermint oil. The formulation demonstrated acceptable organoleptic properties, maintained a stable pH within the oral safety range, and showed no signs of physical instability under accelerated conditions. Antimicrobial testing confirmed the mouthwash's efficacy against common oral pathogens such as Streptococcus mutans and Candida albicans. The inclusion of natural ingredients with proven therapeutic benefits makes this herbal mouthwash a promising alternative to conventional chemical-based oral care products. Further clinical trials are recommended to validate its long-term effectiveness and safety for routine use.

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