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Development and Characterization of Antimicrobial Dental Gel for Oral Health from Herbal Source

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Abstract

Background The development of natural antimicrobial dental gels has gained significant attention in recent years due to the growing concern about the use of synthetic chemicals in oral care products. Neem extract, clove oil, honey, and vitamin E are some of the natural ingredients that have been traditionally used for their antimicrobial and anti-inflammatory properties. These ingredients have been shown to exhibit potent activity against various microorganisms, including Streptococcus mutans, a primary causative agent of dental caries. **Objectives** The objective of this study was to develop and characterize an antimicrobial dental gel using natural ingredients, including neem extract, clove oil, honey, and vitamin E. The specific aim was to evaluate the antimicrobial activity, pH, viscosity, spreadability, and skin irritation potential of three different gel formulations (F1, F2, and F3) prepared with varying concentrations of these ingredients. **Methods** Three gel formulations (F1, F2, and F3) were prepared using different concentrations of neem extract, clove oil, honey, and vitamin E. The formulations were evaluated for their antimicrobial activity against Streptococcus mutans using standard microbiological techniques. The pH, viscosity, and spreadability of the formulations were also determined using standard methods. **Results** The results of the study showed that all three formulations exhibited antimicrobial activity against Streptococcus mutans, with F3 demonstrating the most significant activity. The pH of F3 was found to be 7.0, which is suitable for oral application. The viscosity of F3 was 9632 cps, indicating a suitable consistency for application in the oral cavity. The spreadability of F3 was found to be 26.08, indicating good spreadability. **Conclusion** The study suggests that the F3 formulation holds potential for development as a natural, plant-based oral gel for managing and preventing oral infections. The use of natural ingredients like neem extract, clove oil, honey, and vitamin E in the formulation provides a safer and more effective alternative to synthetic chemicals. Further studies are needed to evaluate the efficacy and safety of the F3 formulation in clinical settings.

Keywords: neem extract; clove oil; antimicrobial dental gel; natural oral care; *Streptococcus mutans*; oral infections; herbal formulation

1. Introduction

Oral health remains a critical component of general well-being, yet a significant portion of the global population continues to suffer from common dental conditions such as dental caries, gingivitis, and the accumulation of hard calculus. These conditions, if left untreated, can lead to more serious systemic health issues and a decline in quality of life. Conventional treatment options, while effective, may be limited by issues such as patient compliance, side effects, or lack of accessibility in underserved areas [1]. In recent years, there has been growing interest in the use of natural agents for the prevention and treatment of oral diseases. Among these, **neem (*Azadirachta indica*)** and **clove oil**, which contains the active compound **eugenol**, have demonstrated notable antibacterial, anti-inflammatory, and analgesic properties. Neem, traditionally used in Ayurvedic medicine, has been

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shown to inhibit the growth of various oral pathogens and reduce plaque formation. Similarly, clove oil is well-documented for its effectiveness against oral bacteria and its ability to reduce pain and inflammation [2] Additionally, natural substances such as **honey** and **vitamin E** have garnered attention for their roles in wound healing and tissue regeneration. Honey exhibits antimicrobial properties and promotes the healing of mucosal tissues, while vitamin E acts as a powerful antioxidant, protecting oral tissues from oxidative stress and supporting epithelial regeneration. The use of **Carbopol**, a mucoadhesive polymer, offers a promising delivery system for these active ingredients. Carbopol-based gels can adhere to the mucosal surfaces of the oral cavity, allowing for prolonged contact time, sustained release of therapeutic agents, and improved patient compliance. This research aims to formulate and evaluate three mucoadhesive gel prototypes, each containing varying concentrations of neem extract, clove oil, honey, and vitamin E. By analyzing parameters such as antibacterial efficacy, mucoadhesive strength, pH stability, and user acceptability, the study seeks to identify the most effective formulation for potential use in the prevention and management of common oral health conditions [3].

1.1. Key Herbal Ingredients

1.1.1. Neem (*Azadirachta indica*)

- **Common Names**: Neem, Indian Lilac, Margosa Tree.
- **Botanical Source**: Obtained from dried leaves and bark of *Azadirachta indica*.
- **Family**: Meliaceae.
- **Ecology and Habitat**:
  - **Climate**: Tropical to subtropical; drought-resistant.
  - **Soil**: Well-drained sandy or loamy soils.
  - **Tolerance**: Poor, dry, and rocky soils.
- **Plant Description**:
  - **Type**: Evergreen tree.
  - **Height**: Typically grows 15–20 meters tall, can reach up to 35–40 meters in ideal conditions.
  - **Leaves**: Pinnate leaves with 8–19 leaflets, bright green, serrated edges.
  - **Flowers**: Small, white, fragrant flowers in clusters.
  - **Fruit**: Smooth, olive-like drupe; green when unripe and yellow when mature [4].
- **Preparation and Dental Application**: *Azadirachta indica* was collected in Kale plant center, Nagpur and identified by Prof. Acharya Botany Dep., Wardha. RTMNU, Nagpur. Neem bark extract is used in dental gels due to its strong antibacterial and anti-inflammatory effects. The process begins by cleaning, drying, and grinding the bark into a fine powder. This powder is then subjected to solvent extraction—commonly with ethanol, methanol, or water—to draw out beneficial compounds such as nimbin and nimbidin. Once filtered and concentrated by removing the solvent, the extract is incorporated into dental gel formulations. Its inclusion helps fight harmful oral microbes, soothe gum irritation, and promote better oral health.

1.1.2. Clove Oil (*Eugenia caryophyllata*)

- **Common Name**: Clove Oil.
- **Botanical Source**: Obtained from dried flower buds of *Eugenia caryophyllata*.
- **Family**: Myrtaceae.
- **Oil Extraction Method**: Steam distillation.
- **Part Used for Oil Extraction**: Dried flower buds, leaves, and stems are used.

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- **Major Constituents of Clove Oil**: Eugenol (70–90%) – primary active compound, Eugenyl acetate, Caryophyllene, Humulene.
- **Aroma**: Strong, spicy, warm, and woody scent.
- **Plant Description**: Clove is a medium-sized evergreen tree that grows up to 8–12 meters tall. It has large green leaves and clusters of aromatic flower buds that start pale, then turn green, and finally develop into a bright red when ready for harvesting [5].
- **Medicinal Properties and Dental Application**: Clove oil, primarily composed of the active compound eugenol, is widely used in dental care due to its potent analgesic, anti-inflammatory, and antimicrobial properties. When incorporated into dental gels, clove oil offers several therapeutic benefits including pain relief, antibacterial effects, and anti-inflammatory effects [6].

2. Materials and Methods

2.1. Preparation of Gel Formulations

Carbopol 940 gel was prepared by soaking Carbopol 940 in water and neutralizing with triethanolamine to pH 6.0. Weighed amounts of methyl and propyl paraben were added to the water prior to the addition of Carbopol 940. In another beaker, the required quantity of propylene glycol was taken, to which accurately measured amounts of neem extract and clove extract, corresponding to their Minimum Inhibitory Concentrations (MICs), was incorporated. This mixture was then added to the beaker containing Carbopol 940 with continuous stirring until a homogenous product was formed. The volume was made up with distilled water and stirring was done vigorously. All the prepared gels were then subjected to evaluation tests in order to select the best formulation. The composition of different gel formulations is listed in Table 1 [7].

Table 1. Composition of Different Gel Formulations.

Sr. No	Ingredients	Functions	F1	F2	F3
1.	Carbopol 940	Gelling agent	4.0	4.0	4.0
2.	Neem extract	Antibacterial	2.0	3.5	5.0
3.	Clove oil	Analgesic, Antiseptic	0.5	0.75	1.0
4.	Peppermint oil	Flavour, mild antiseptic	0.25	0.30	0.50
5.	Honey	Healing, Humectant	2.0	3.5	5.0
6.	Vitamin E oil	Antioxidant	0.5	0.5	0.5
7.	Propylene glycol	Humectant, Solvent	10.0	10.0	10.0
8.	Triethanolamine	pH adjuster	-	-	-
9.	Methyl Paraben	Preservative	0.1 g	0.1 g	0.1 g

2.2. Evaluation of Formulated Gel

Gels were evaluated for their clarity, pH, viscosity, spreadability, skin irritation, and antimicrobial studies using standard procedures. All studies were carried out in triplicate, and average values were reported [8].

2.2.1. Physical Stability

Gels were inspected visually for their color, homogeneity, consistency, and spreadability [9].

2.2.2. pH Measurement

pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter [10].

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2.2.3. Spreadability

For the determination of spreadability, an excess of sample was applied between two glass slides and compressed to uniform thickness by placing a 1 kg weight for 5 minutes. A weight (50 g) was then added to the pan. The time in which the upper glass slide moves over the lower plate was taken as a measure of spreadability [11].

The spreadability (S) was calculated using the formula: S = (M \times L) / T Where:

- S = Spreadability
- M = Mass attached with the slide
- L = Length moved by the glass slide
- T = Time required to travel a distance by the slid

2.2.4. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels had set in the container for their appearance and presence of any aggregate [12].

2.2.5. Extrudability Study

The force required to extrude the material from the tube was measured. Extrudability was based upon the quantity in percentage of gel and gel extruded from a lacquered aluminium collapsible tube on application of weight in grams required to extrude at least a 0.5 cm ribbon of gel in 10 seconds [13].

2.2.6. Content Uniformity

Drug content of the gel was determined by dissolving accurately weighed 1 gm of gels in Methanol. After suitable dilution, absorbance was recorded using a UV-visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 222 nm. Drug content was determined using the slope of a standard curve [14].

2.2.7. Viscosity Measurement

The viscosity of the different gel formulations was determined at 25^\circ \text{C} using a cone and plate viscometer [15].

3. Antimicrobial Assay

3.1. Bacterial Strain and Maintenance

The bacterial strain used in this study was the Gram-positive bacterium *Streptococcus mutans*. Bacterial strains were grown and maintained on nutrient agar slants, then stored at 4^\circ \text{C}. Test strains were prepared and tested against the extracts for estimating the minimum inhibitory concentration (MIC) [16].

3.2. Well Diffusion Method

An agar well diffusion method was used to evaluate antimicrobial activity against *Streptococcus mutans*. Wells measuring 6 mm in diameter were created, and the plates were incubated at 37^\circ \text{C} for 24 hours. The diameters of the resulting **inhibition zones** were recorded in millimeters to assess antibacterial effectiveness [17,18].

Zone of Inhibition

The zone of inhibition is a clear, circular area that appears around an antimicrobial agent (such as an antibiotic disc) placed on an agar plate inoculated with bacteria. It indicates how effectively the

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agent inhibits bacterial growth. Antimicrobial activity of the formulations was evaluated by measuring the zones of inhibition against *Streptococcus mutans* [19].

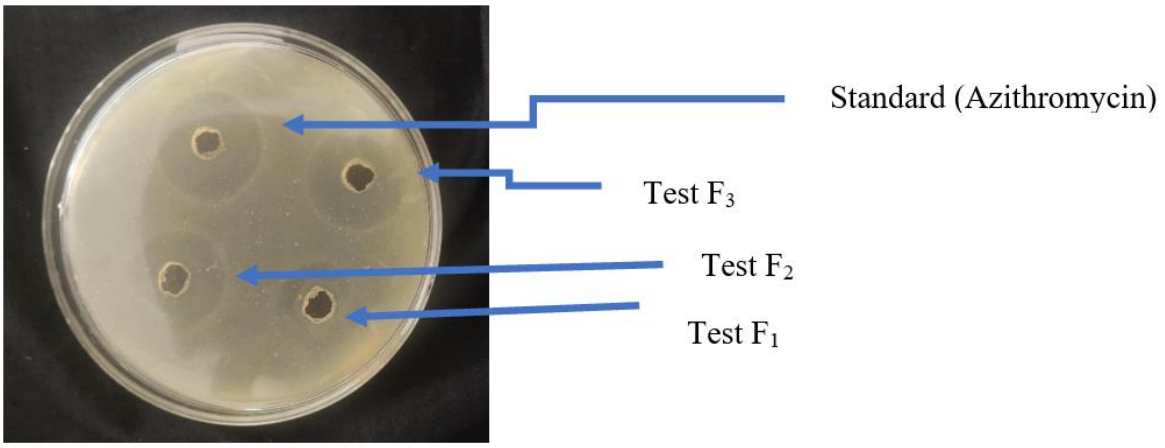

Figure 1. Zone of Inhibition.

3.3. Determination of MIC (Minimum Inhibitory Concentration)

A cup plate method was used to calculate the MIC. Serial dilutions (0.25, 0.5, 1, 2, 3 mg/ml) of extracts were prepared directly in a plate containing MH agar. The bacterial inoculum was added to get a final concentration of 5 \times 10^5 \text {CFU/ML} in each well. Azithromycin was utilized as a standard drug at final concentrations of 0.126-129 mg/ml. The petri dish was incubated for 24 hours at room temperature for 37^\circ \text{C}. The MIC was shown as the minimum concentration of gel that completely inhibited bacterial growth after 24-48 hours [20].

Table 2. Minimum Inhibitory Concentration (MIC) of Formulations against *Streptococcus mutans.*

Formulation	*Different Concentrations (µg/ml)*				
Concentration	0.25 (µg/ml)	0.5 (µg/ml)	1 (µg/ml)	2 (µg/ml)	3 (µg/ml)
F1	2.00±0.22	6.21±0.56	8.25±0.46	9.12±0.98	12.10±0.50
F2	4.52±0.25	7.85±1.85	10.50±1.52	12.11±0.82	15.18±0.45
F3	7.23±0.55	9.63±0.61	13.82±0.44	18.42±0.66	19.28±1.75
Standard	7.25± 0.21	8.93±0.50	14.58±0.71	17.33±0.51	18.96±0.84

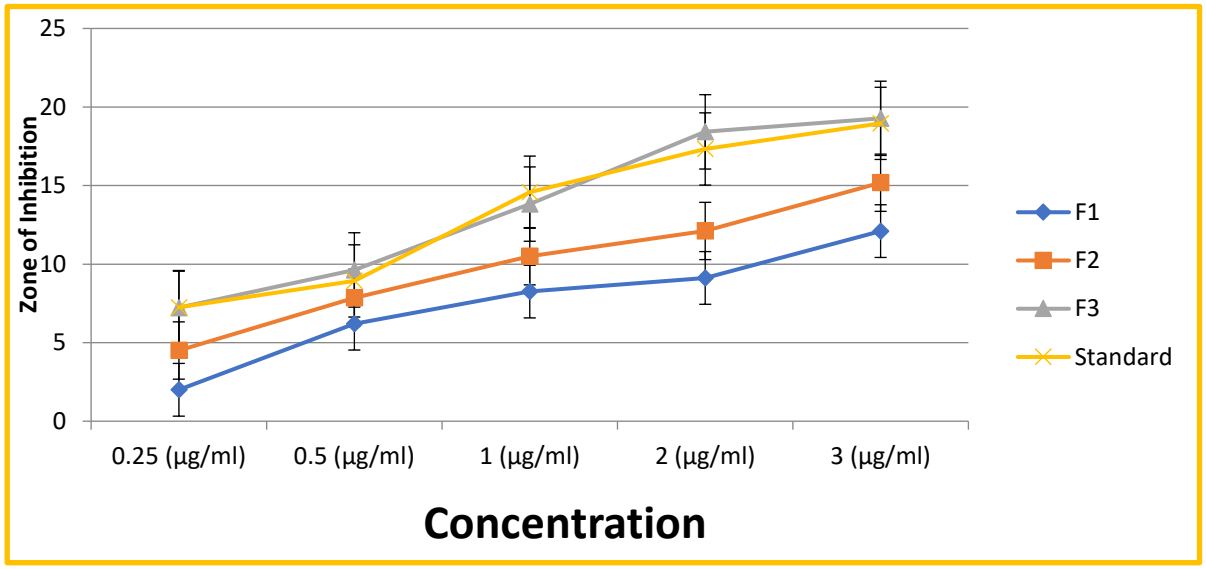

Figure 2. Graph for Zone of Inhibition/Concentration.

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4. Results & Discussion

The evaluation of the formulated gels yielded the following results, as summarized in Table 3.

Table 3. Evaluation Parameters of Formulated Gels.

FORMULATION	CLARITY	pH	SPREADABILITY (gms.cm/sec)*	VISCOSITY (cps)	SKIN IRRITATION	EXTRDURABILITY	ANTIMICROBIAL ACTIVITY
F1	++	6.0	20.66 ±0.1159	7954	NI	++	Shows Antimicrobial Activity
F2	++	7.0	20.01 ±0.0152	8122	NI	++	Shows Antimicrobial Activity
F3	+++	7.0	26.08 ±0.0152	9632	NI	+++	Shows Most Significant Antimicrobial activity

5. Discussion

- **pH:** Formulations F2 and F3 showed a neutral pH of 7.0, which is generally considered suitable for oral application, minimizing irritation to oral tissues. F1 had a slightly acidic pH of 6.0.
- **Appearance and Odor:** F1 and F2 were slightly opaque, while F3 had a brownish appearance, likely due to the higher concentration of herbal extracts. The odor increased from mild in F1 to strong in F3, correlating with the increased herbal content.
- **Spreadability:** F3 demonstrated significantly higher spreadability (26.08 gms.cm/sec) compared to F1 and F2, indicating better applicability and ease of use. This is crucial for a dental gel to ensure proper coverage of oral surfaces.
- **Viscosity:** F3 exhibited the highest viscosity (9632 cps), which is consistent with its enhanced spreadability. An optimal viscosity is important for retaining the gel at the site of application and allowing for sustained release of active ingredients.
- **Skin Irritation:** All three formulations were found to be non-irritant, suggesting they are safe for topical application on oral mucosal membranes.
- **Extrudability:** F3 showed the best Extrudability (+++), indicating it can be easily dispensed from a tube, which is a key factor for user convenience.
- **Antimicrobial Activity:** All formulations exhibited antimicrobial activity against *Streptococcus mutans*. Crucially, **F3 demonstrated the most significant antimicrobial activity**, aligning with its higher concentrations of neem extract and clove oil. This superior efficacy is further supported by the MIC data presented in Table 2, where F3 showed the lowest MIC values across various concentrations, indicating its greater potency in inhibiting bacterial growth compared to F1, F2, and even the standard drug at some concentrations. The synergistic effects of neem, clove oil, honey, and vitamin E likely contribute to the enhanced performance of F3.

6. Summary

This study developed and evaluated three antimicrobial dental gel formulations (F1, F2, and F3) using natural ingredients like neem extract, clove oil, honey, and vitamin E. The formulations were tested for their antimicrobial activity, pH, viscosity, spreadability, and skin irritation potential. The

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results showed that all formulations exhibited antimicrobial activity, with **F3 demonstrating the most significant activity against *Streptococcus mutans***. The study suggests that F3 has potential as a natural oral gel for managing and preventing oral infections, warranting further research and development.

7. Conclusions

The **F3 formulation**, containing the highest concentration of neem extract, clove oil, and honey, exhibited the most potent antimicrobial activity without compromising physicochemical properties. The synergistic effects of the natural ingredients likely contributed to its improved performance against common oral pathogens. The promising results suggest that **F3 holds significant potential for development as a natural oral gel for managing and preventing oral infections**. Further studies, including *in vivo* evaluations and long-term stability assessments, are warranted to explore its therapeutic effectiveness and safety profile.

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