

Evaluation of the efficacy of a novel fluoridated Lemon Grass oil tooth paste in maintaining oral hygiene - An In-vitro study.

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Abstract:

Dental plaque is a mixed culture microbial bio-film which forms naturally on teeth. It can be of benefit to the host by acting as a barrier to subsequent colonization by exogenous and possible pathogenic microorganisms although fewer studies are done on lemongrass oil as a mouth wash and as a local drug delivery system less studies are available on use of lemongrass oil as a tooth pastes. Thus in the present study assessment of oral hygiene efficacy of a novel fluoridated herbal lemongrass oil toothpaste was prepared and used in vitro against oral pathogens.

Aim and objective:

To evaluate the antibacterial activity of lemongrass essential oil with ciprofloxacin against *Staphylococcus aureus* and *Aggregatibacter actinomycetemcomitans* and to determine the minimal inhibitory Concentration and minimal Bactericidal concentration of lemongrass essential oil.

Materials and Method:

The study was aimed to evaluate an antimicrobial efficacy of herbal lemongrass oil toothpaste which demonstrated favorable physical properties, stability, and effectiveness against common oral pathogens. The study highlights this formulation as a viable natural alternative for preventive oral healthcare, confirmed through testing methods like the Resazurin dye test, streaking method and agar well diffusion assay technique. Tooth paste was formulated using the standard ingredients and quality control tests were performed after the formulation.

Results:

Lemon grass oil toothpaste was found to be effective against the oral pathogens *staphylococcus aureus* and *Aggregatibacter actinomycetemcomitans*.

Conclusion:

Novel formulated lemon grass oil toothpaste acts as an effective, natural alternative to conventional, chemical-based toothpastes for prevention of caries and maintenance of oral health.

Key words: lemongrass oil, herbal toothpaste, antimicrobial, antibacterial, novel toothpaste

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Introduction:

Lemongrass (*Cymbopogon citratus*) is a tropical, aromatic grass from the Poaceae family, which includes about 55 species. Widely used as a traditional medicine, it helps treat respiratory issues like coughs and pneumonia, as well as infections like malaria and flu, headaches, gingivitis. Beyond internal health, it is also a

common remedy for skin conditions like acne and scalp issues such as dandruff and lice. (1) Maintaining good oral hygiene is essential for stopping dental issues like cavities, gum disease, and bad breath. Beyond just your mouth, poor dental care is linked to serious health problems like heart disease and diabetes. Because of this, the World Health Organization views oral health as a vital part of your general well-being.

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While most people use standard toothpastes, many are now switching to natural options due to concerns about the synthetic chemicals found in traditional brands. Regular toothpastes usually rely on fluoride and chemical abrasives to fight plaque and tartar. In contrast, herbal toothpastes use plant-based ingredients that have been trusted in traditional medicine for centuries. [2]

Plants like neem, clove, and turmeric are popular because they naturally fight bacteria and reduce inflammation. Specifically, lemongrass oil has shown strong potential in killing the germs that cause oral infections while keeping gums healthy and teeth clean. [3]

As more people look for eco-friendly, chemical-free products, the market for herbal toothpaste is booming. Because of this growth, experts are focused on testing these natural products to ensure they are safe, effective, and shelf-stable. This particular study focuses on creating a new herbal toothpaste featuring lemongrass oil to see how well it handles common dental issues and if it can truly compete with conventional toothpaste.

Types of Toothpastes available(4):

T yp e	K ey In gr ed ie nt s	P r i m a r y U s e / P u r p o s e	C o m m o n E x a m p l e s
Fl uo ri de T oo th pa st	S od iu m fl uo ri de ,	P r e v e n t s	C o l g a t e ,

e	st an no us fl uo ri de	c a v i t i e s a n d s t r e n g t h e n s e n a m e l	S e n s i t i v e D a i l y P r o t e c t i o n
W hi te ni ng T oo th pa st e	H yd ro ge n pe ro xi de , ba ki ng so da , sil ic a	R e m o v e s s u r f a c e s t a i n s a n	C o l g a t e O p t i c W h i t e , C r e s

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		d w h i t e n s i t y t e e t h	t 3 D W h i t e
T a r t a r C o n t r o l	P y r o p h o s p h a t e s, z i n c i t r a t e	P r e v e n t s t a r t a r (c a l c u l u s) b u i l d u p	C r e s t T a r t a r P r o t e c t i o n , C o l - g a t e T a r t a r

			C o n t r o l
S e n s i t i v i t y T o o t h p a s t e	P o t a s i u m n i t r a t e, s t r o n t i u m c h l o r i d e , a r g i n i n e	R e d u c e s t o o t h s e n s i t i v i t y t o h o t / c o l d	S e n s o d y n e , C o l g a t e S e n s i t i v e
H e r b a l/ N a t u r a l	N e e m, c l o v e, m i s w a k , m	N a t u r a l c l e a	D a b u r R e d , H

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	in t, ch ar co al	n s i n g a n d b r e a t h f r e s h e n i n g , f e w e r c h e m i c a l s	i m a l a y a H e r b a l , P a t a n j a l i D a n t K a n t i
C h i l d r e n ' s T o o t h p a s t e	L o w e r f l u o r i d e , m i l d	S a f e f o r k i d	C o l g a t e K i d s
	fl av o r s	s , p r e v e n t s e a r l y c a v - i t i e s	, P i g e o n , C h i c c o
S m o k e r ' s T o o t h p a s t e	St ro n g a b r a s i v e s, str on g m i n t	R e m o v e s t o b a c c o s t a i n s a n d n	V i c c o V a j r a d a n t i , C l o s e u p R e

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		eutralizes odors	dent
Characteristical Tooth paste	Activated charcoal	Claims to detoxify and whiten teeth	Hello Activated Charcoal, Colgate Charcoal

			al Clean
Antibacterial Tooth paste	Triclosan, zinc, chlorhexidine	Reduces plaque and gingivitis	Colgate Total, Parodontax
Enzyme Tooth paste	Papain, bromelain	Breaks down plaque	Biotene, Zendium

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		b i o l o g i c a l l y	
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Herbs and their oral benefits(4):

Herb	Scientific name	Oral health benefits
Neem	<i>Azadirachta indica</i>	Antibacterial, plaque control, anti-inflammatory
Clove	<i>Syzygium aromaticum</i>	Antiseptic, analgesic, fights toothache and gum pain
Turmeric	<i>Curcuma longa</i>	Anti-inflammatory, antimicrobial, whitens teeth
Aloe vera	<i>Aloe barbadensis</i>	Soothing, healing for gums, anti-inflammatory
Babool	<i>Acacia arabica</i>	Strengthens gums, antimicrobial

		obial
Mint	<i>Mentha spp.</i>	Refreshes breath, cooling effect
Holy Basil	<i>Ocimum sanctum</i>	Antibacterial, strengthens gums
Licorice	<i>Glycyrrhiza glabra</i>	Prevents cavities, reduces plaque

Materials and methods:

The present study was conducted from Department of Periodontics KAHER's KLE VK Institute of dental sciences Belagavi. Permissions to conduct the study was obtained from principal and Institutional review ethical board committee. The antimicrobial effect was assessed in KLEs Dr. Prabhakar Kore Basic science Research Centre Belagavi and formulation of toothpaste was done in KAHERs college of pharmacy Belagavi.

The commercially available Lemon grass oil was used for the formulation of toothpaste. The toothpaste was formulated based on minimum inhibitory concentration and minimal bactericidal concentration by culturing the common oral pathogens staphylococcus aureus and Aggregatibacter actinomycetem comitans by using Agar well diffusion method.

Extract concentration used was: Stock:100mg/ml working:50mg/ml, 25mg/ml.

Agar well diffusion method: Muller Hinton agar plates inoculated with the test organism and drug taken as sample and loaded into the wells of the agar. All plates were incubated (37°C) for 24 hrs. After incubation, the diameters of any clear zones around the antimicrobial containing discs or wells were measured using scale.

TOOTHPASTE FORMULATION PREPARATION PROTOCOL

1. OBJECTIVE

To formulate and develop a semi-solid dentifrice (toothpaste) using suitable excipients, abrasives, humectants, binding agents, surfactants, preservatives, sweeteners, and active

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ingredients, aimed at achieving optimal cleaning efficiency, user acceptability, and antimicrobial activity.

2. INGREDIENTS AND THEIR PHARMACEUTICAL ROLES

S.No.	Ingredient	Function
1	Calcium carbonate (CaCO ₃)	Abrasive – mechanical cleaning of teeth
2	Silica gel	Abrasive and thickening agent
3	Sodium CMC	Binding agent – provides viscosity and texture
4	Glycerine	Humectant – prevents drying and retains moisture
5	Propylene glycol	Co-solvent, humectant, mild surfactant
6	Sodium lauryl sulfate (SLS)	Surfactant – foaming and cleaning agent
7	Methylparaben	Preservative – prevents microbial growth
8	Sodium benzoate	Preservative – enhances microbial

		stability
9	Sodium saccharin	Sweetening agent – improves palatability
10	Peppermint oil	Flavoring agent – provides fresh sensation
11	Lemongrass oil	Active ingredient – antimicrobial and refreshing
12	Distilled Water	Vehicle – facilitates dispersion and solubilization

3. EQUIPMENT USED

- Analytical balance / Digital weighing scale
- Glass beakers (100 mL, 250 mL, 500 mL)
- Mechanical stirrer or overhead stirrer
- Magnetic stirrer with hot plate (optional)
- pH meter
- Homogenizer or high-speed blender
- Heating plate (if required)
- Clean, sterilized collapsible tubes or glass jars
- Spatulas and funnel
- Vacuum deaerator (optional)

4. METHODOLOGY

Step 1: Preparation of the Gel Base

a. Hydration of the Binder:

- Weigh the required amount of **Sodium Carboxymethyl Cellulose (Sodium CMC)** (typically 0.5–1.0% w/w).

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- Sprinkle slowly into approximately 30–40% of the required volume of **warm distilled water** (40–50°C) under continuous gentle stirring.
- Allow the binder to hydrate and swell completely for **1–2 hours**, ensuring no lumps or air entrapment.
- Avoid high-speed stirring at this stage to prevent foam formation.

b. Addition of Humectants:

- Weigh and add **Glycerine** (20–30% w/w) to the hydrated gel.
- Optionally, add **Propylene glycol** (1–5% w/w) as a co-solvent and mild surfactant.
- Stir continuously until a homogeneous, viscous gel base is formed.

Step 2: Incorporation of Abrasives

- Weigh the **Calcium carbonate** and **Silica gel** in appropriate proportions (total abrasives ~40–60% w/w depending on abrasivity required).
- Slowly incorporate abrasives into the gel base in small portions under mechanical stirring.
- Continue stirring to achieve a **smooth, lump-free dispersion**.
- Ensure abrasives are evenly distributed without sedimentation.

Step 3: Addition of Surfactants

- Gradually add **Sodium lauryl sulfate (SLS)** (1–2% w/w) to the formulation while stirring.
- Add **slowly** to prevent excessive foaming.
- Ensure complete dispersion of SLS in the matrix to form a stable emulsion-like system.

Step 4: Addition of Preservatives and Sweetening Agents

- Accurately weigh **Methyl paraben** and **Sodium benzoate** (0.1–0.2% each).
- If necessary, **pre-dissolve** them in a small amount of warm distilled water or propylene glycol to enhance solubility.
- Add these to the main formulation with thorough mixing.
- Incorporate **Sodium saccharin** (0.1–0.5%) to improve taste acceptability.

Step 5: Addition of Flavoring and Active Ingredients

- Measure and add **Peppermint oil** (0.5–1.0%) as the primary flavoring agent.
- Add **Lemongrass oil** (0.2–0.5%) as the **active antimicrobial agent**.

- Stir gently but thoroughly to ensure uniform distribution and prevent volatilization of essential oils.

Step 6: Final Adjustments and Homogenization

a. pH Adjustment:

- Use a calibrated **pH meter** to determine the pH of the formulation.
- Adjust to the optimal range of **6.5 to 7.5** using:

- **Citric acid** solution (for lowering pH)
- **Sodium hydroxide** solution (for increasing pH)

- Make adjustments slowly under stirring, and verify after each addition.

b. Homogenization and Deaeration:

- Use a **homogenizer or hand blender** to ensure a fine, consistent, and smooth texture.
- Eliminate entrapped air by:
 - Resting the formulation in a closed container for 24 hours, or
 - Applying **vacuum deaeration** if equipment is available.

Step 7: Packaging

- Transfer the final formulation into **sterilized collapsible aluminum/plastic tubes** or airtight glass jars using a clean funnel or filling apparatus.
- Seal the tubes using a crimping machine or appropriate method.

5. QUALITY CONTROL TESTS (Post-formulation)

Test	Standard
pH	6.5–7.5
Appearance	Smooth, homogeneous, no phase separation
Texture	Non-gritty, spreadable, uniform
Foaming capacity	Moderate, consistent
Viscosity	Consistent flow from tube, no leakage
Stability (optional)	No change in color, odor, or texture for 30 days
Microbial testing	Absence of pathogenic organisms

1. pH

Trial 1	6.7
Trial 2	6.9

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Trial 3	6.5
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2. Appearance: smooth, No-phase separation

3. Texture : Non gritty, spread ability

4. Foam capacity:

T r i a l		F o r 5 m i n	F o r 1 0 m i n	F o r 1 5 m i n
1	1 0 m l	6 . 1 c m	4 c m	3 . 3 c m
2	1 0 m l	5 . 9 c m	3 . 8 c m	2 . 7 c m
3	1 0 m l	6 c m	5 . 1 c m	4 c m

5. Viscosity: Consistent flow without leakage

6. Microbial testing: Assessed Minimum inhibitory concentration and minimal bactericidal concentration of sample.

Results: The formulated tooth-paste was evaluated based on several key parameters, including organoleptic properties, pH, foam ability, spread ability and stability. The results showed that the herbal toothpaste had a pleasant aroma and taste, acceptable consistency, and a neutral to slightly alkaline pH suitable for oral use. The foaming ability was moderate, indicating gentle cleansing without harsh surfactants. The paste remained physically stable over time with no evidence of phase separation, microbial contamination, or color change.

The anti bacterial activity results. Table no 1 shows HF1 exhibits a strong inhibitory effect against the Staphylococcus aureus producing 5mm Zone of inhibition at 25% lemongrass oil tooth paste and 10mm at 50% lemongrass oil toothpaste. HF2 does not produce any Zone of inhibition against Aggregatibacter

actinomycetem comitans at 25% and 50% lemongrass oil toothpaste.

Table 2, Graph 1 shows Comparison of three groups with MIC values of Staphylococcus aureus by Kruskal Wallis ANOVA test where the mean score of lemongrass essential oil tooth paste 25% and 50% against

Streptococcus aureus showed 5.33mm and 10.33 mm values which is lesser than the standard group (ciprofloxacin) with mean value of 20 mm. Of the two concentrations of the formulated herbal toothpaste, 50% lemongrass essential oil tooth paste has better antimicrobial activity against Staphylococcus aureus. This indicates that lemon grass oil toothpaste is effective in reducing the oral bacteria and has shown highly statistically significant result with p value 0.0234* (p<0.05).

Table 3 and Graph 2 shows Comparison of three groups with MIC values of Aa by Kruskal Wallis ANOVA test where the of lemongrass essential oil tooth paste 25% and 50% against Aggregatibacter actinomycetemcomitans showed no Zone of inhibition and no antibacterial activity against Aggregatibacter actinomycetemcomitans when compared to standard (ciprofloxacin) with mean value of 35.67.

Discussion:

The present study focused on the formulation and evaluation of a herbal toothpaste using key natural ingredient: lemongrass oil (*Cymbopogon citratus*). This component was selected due to its well-documented pharmacological activities and their traditional use in oral care. Lemongrass oil is rich in citral and limonene, compounds known for their potent antimicrobial, anti-inflammatory and antioxidant properties. In toothpaste formulation, lemongrass oil contributes to bacterial control in the oral cavity, freshens breath, and helps prevent gingivitis and plaque buildup.[5]

In the present study, strong inhibitory effect against the Staphylococcus aureus was found at 25% and 50% lemongrass oil toothpaste and did not find any Zone of inhibition against Aggregatibacter actinomycetem comitans. Since the lemon grass oil was used in the form of tooth paste it has shown its anti plaque and anti microbial effect against gram positive bacteria. This is in accordance with study conducted by Mourya et al.in 2025.(4)

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In the present study, Of the two concentrations of the formulated herbal toothpaste, 50% lemongrass essential oil tooth paste has better antimicrobial activity against *Staphylococcus aureus*. This indicates that lemon grass oil toothpaste is effective in reducing the oral bacteria, lesser the concentration there would be less antimicrobial effect against oral pathogens. This is in accordance with study done by Paavai I, et al in 2019.(6)

In this study lemongrass essential oil tooth paste against *Aggregatibacter actinomycetemcomitans* showed no Zone of inhibition and found lesser than the standard group indicating lemongrass essential oil showed no antibacterial activity against *Aggregatibacter actinomycetemcomitans* when compared to standard (ciprofloxacin). It indicates that lemongrass oil toothpaste is effective against *Staphylococcus aureus* but no antimicrobial activity was observed against *Aggregatibacter actinomycetemcomitans*. This is in accordance with study conducted by Ambade et al. in 2022(7).

This could be due to the tissue invasive nature of *Aggregatibacter actinomycetemcomitans*.

Also, since in the present study we have formulated a toothpaste, it has not shown significant activity against the periodontal pathogen. Within the limitations of the present study, the antimicrobial efficacy of the lemongrass oil may be evaluated in future studies by using it in other forms such mouthwashes, chips and gels.

Conclusion: within the limitations of the present study it can be concluded that So far various studies have also been conducted to assess the antimicrobial effect of lemongrass by preparing mouth washes and pastes along with other herbal concentrations. Thus present in-vitro study was initiated using only lemon grass concentration by formulating toothpaste where the antimicrobial effect of lemongrass was tested against *S. aureus* and *Aa comitans* mainly using lemongrass oil.

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Figure 1:Composition for the formulation

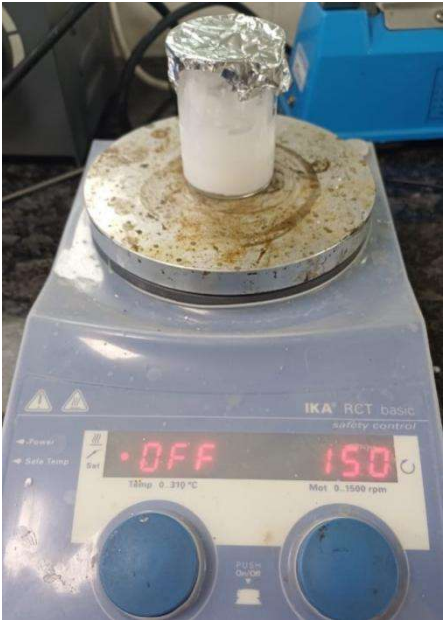


Figure 2: Formulation of tooth paste

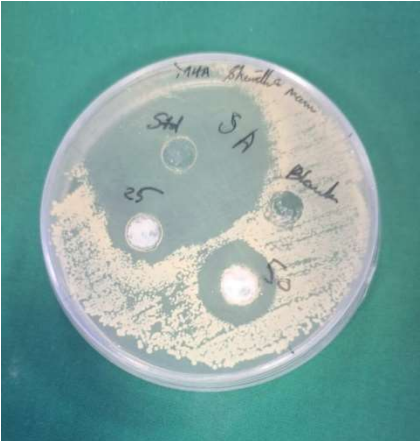


Figure 3: Zone of Inhibition for pathogens Staphylococcus aureus and Aggregatibacter actinomycetemcomitans by using Agar well diffusion method.

Table 1.: Comparison of three groups with MIC values of Staphylococcus aureus by Kruskal Wallis ANOVA

Grou p	M e a n	M e d i a n	S D	M e a n r a

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				n k
Cipro floxacin	2 0 . 0 0	2 0 . 0 0	0 . 0 0	8 . 0 0
25% Lem on grass tooth paste	5 . 3 3	5 . 0 0	0 . 5 8	2 . 0 0
50% Lem on grass tooth paste	1 0 . 3 3	1 0 . 0 0	1 . 5 3	5 . 0 0
H- value	7.5130			
P- value	0.0234*			

*p<0.05

Graph 1.: Comparison of three groups with MIC values of Staphylococcus aureus

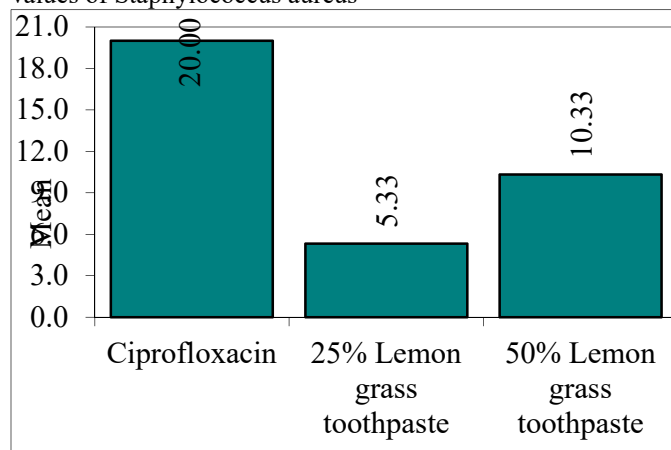


Table 2.: Comparison of three groups with MIC values of Aa by Kruskal Wallis ANOVA

Grou p	M e a n	M e d i a n	S D	M e a n r a n k
Cipro floxacin	3 5 .	3 6 .	0 . 5	8 . 0

	6 7	0 0	8 0	0 0
25% Lem on grass tooth paste	0 . 0 0	0 . 0 0	0 . 0 0	2 . 0 0
50% Lem on grass tooth paste	0 . 0 0	0 . 0 0	0 . 0 0	5 . 0 0
H- value	7.7143			
P- value	0.0211*			

*p<0.05

Graph 2 : Comparison of three groups with MIC values of Aa

