Original Article

Comparative Evaluation of the Antimicrobial Efficacy of *Anethum Graveolens* Gel with Chlorhexidine Gel against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum*- an in vitro study

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ABSTRACT

Periodontitis is an infection of the periodontium caused by a group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone.Initial treatment for periodontitis is mechanical scaling and root planing, but this does not cause sufficient reduction of the bacterial load due to lack of accessibility to microorganisms. The incorporation of an adjunctive chemotherapeutic agent enhances the outcome at sites which are not responsive to conventional therapy. Chlorhexidine is regarded as the gold standard for local drug delivery systems; however, it has been associated with undesirable side effects, including tooth staining, xerostomia, and calculus formation. This has given rise to an increased demand for herbal medicine, as it is associated with fewer side effects and is cost-effective. Anethum graveolens, which contains natural phytochemicals, is a well-known herbal remedy with therapeutic properties. The present in-vitro microbiological study was therefore undertaken to evaluate and compare the antimicrobial activity of Anethum graveolens gel with Chlorhexidine gel for Aa,Pg and Fn.The MIC and MBC of the ethanolic extract of Anethum graveolens against standard ATCC bacterial strains of A.a, P.g and F.n were determined using the broth dilution method and streaking on blood agar plates. The antimicrobial activity of the prepared Anethum graveolens gel was evaluated and compared with Chlorhexidine gel using the agar well diffusion assay. The zone of inhibition for Chlorhexidine gel was found to be 15.6 mm, 17 mm and 15.3 mm for A.a, P.g and F.n, respectively, whereas for A. graveolens gel, it was 12.6 mm, 13 mm and 12 mm for 24. A.a, P.g and F.n, respectively. The results obtained suggested that Chlorhexidine gel exhibited a marginally superior antimicrobial activity in comparison to the Anethum graveolens gel against Aa, Pg and Fn.

Keywords: Anethum Graveolens, Dental Plaque, Chlorhexidine, Herbal Extract, Periodontal Disease.

1. Introduction

Periodontitis is an inflammatory condition affecting the tissues surrounding the teeth. It is characterized by the gradual deterioration of the support of the affected teeth, resulting in clinical attachment loss, bone loss and the formation of pockets (1). This condition can potentially result in tooth loss and disability, impacting the chewing ability, appearance and overall quality of life (2). It is widely accepted that bacterial colonization of the oral environment is the primary cause of periodontal disease. Secondary factors contributing to its etiology include dental plaque, calculus build up, anatomical factors such as developmental grooves, short root trunk, cervical enamel projections, overhanging restorations, as well as lifestyle factors like stress and smoking (3). The following organisms have been strongly linked to periodontitis: Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Eikenella corrodens and Fusobacterium nucleatum (4). It is particularly noteworthy that P. gingivalis and A. actinomycetemcomitans have been identified as significant contributors to the progression of disease, underscoring their pathogenic potential. The association with A. actinomycetemcomitans has been linked to accelerated degeneration in the pocket epithelium, characterised by the presence of micro clefts and necrotic areas. Porphyromonas gingivalis is recognised as one of the primary periodontal pathogens and is considered one of the most virulent microorganisms contributing to the pathogenesis of periodontal disease (5). Fusobacterium nucleatum is extensively studied and is regarded as a key bacterium associated with periodontal diseases. It is a Gram-negative anaerobic bacterium belonging to the Bacteroidaceae family within the phylum Fusobacteria, and it is particularly abundant in dental plaque biofilms (6). Periodontal therapy is a multifaceted approach encompassing both mechanical and chemical methodologies, which are aimed at reducing or eradicating microbial biofilm. The initial component of periodontal treatment is traditional plaque control, which is vital, albeit somewhat limited in its effectiveness, as it fails to reach microorganisms in the subgingival environment. Therefore, adjunctive chemotherapies are employed to enhance outcomes, particularly at sites unresponsive to conventional mechanical therapy (7). The utilisation of systemic antibiotics for the treatment of periodontitis is constrained by numerous factors, including the necessity for elevated dosages to attain the desired concentrations in the gingival crevicular fluid (GCF), the emergence of bacterial resistance, and the potential for adverse effects. Consequently, the concept of controlled local drug delivery was introduced with the objective of delivering the drug to the base of the periodontal pocket and maintaining its presence for an adequate duration to exert its antimicrobial effects (8). Antimicrobial agents suitable for local administration include metronidazole, chlorhexidine, doxycycline and tetracycline, which can be delivered through various controlled drug delivery systems, such as gels, strips, fibres, films and injectable systems.CHX is recognised as a cationic bisbiguanide possessing broad-spectrum antibacterial properties against both gram-positive and gram-negative bacteria, yeasts, dermatophytes and certain lipophilic viruses (9). In the contemporary era, there is a discernible trend towards the consumption of organic products, which are increasingly regarded as effective remedies for a wide range of common ailments. These products are prized for their antibacterial, antioxidant, immune-regulatory, and anti-inflammatory properties, rendering them potent antidotes for various health concerns. Additionally, they are favoured for their cost-effectiveness, relative safety, and association with reduced development of resistance, toxicity, and fewer side effects, including hypersensitivity reactions and staining of teeth, when compared to conventional antimicrobial agents. Anethum graveolens, commonly known as Dill, is an annual medicinal plant found in the Mediterranean region, as well as in Central and Southern Asia. It belongs to the Umbelliferae family. Dill is widely utilised in Ayurvedic medicine to alleviate abdominal discomfort, aid digestion, and address rheumatism. Anethum graveolens is characterised by a high flavonoid content, which confers a range of beneficial properties, including antimicrobial, anti-inflammatory, analgesic, gastric mucosal protection, antisecretory effects, smooth muscle relaxation, and hyperlipidaemic effects (10). The essential oils found in Anethum graveolens seeds typically range from 1% to 4%, with major compounds including "carvone (30–60%), limonene (33%), α-phellandrene (20.61%), pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiole" (11). It is evident that the herbal drug exhibits advantageous properties; consequently, this in vitro study was conducted to evaluate and compare the antimicrobial effectiveness of Anethum graveolens gel with Chlorhexidine gel against Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Fusobacterium nucleatum.

2. Materials and Methods

All experimental procedures were approved by the Research and Ethical Committee of "KAHER's KLE V K Institute of Dental Sciences, Belagavi."The seeds of Anethum graveolens were collected and authenticated from "KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi." The laboratory procedure and the preparation of hydroethanolic extract of Anethum graveolens was undertaken at the Dr. Prabhakar Kore Basic Science Research Centre (BSRC), Belagavi. The Anethum graveolens gel was prepared and collected from the KLE College of Pharmacy, Belagavi. The study used commercially available 1% Chlorhexidine gel (Hexigel). The experiment was conducted in three groups:

Group1: Control (saline), Chlorhexidine gel (1%), Anethum graveolens gel against Aggregatibacter actinomycetemcomitans.
Group 2: Control (saline), Chlorhexidine gel (1%), Anethum graveolens gel against Porphyromonas gingivalis.
Group 3: Control (saline), Chlorhexidine gel (1%), Anethum graveolens gel against Fusobacterium nucleatum. (Saline was used as a negative control and 1% Chlorhexidine gel was used as a positive control).

2.1 Extract Preparation

Anethum graveolens seeds were collected and authenticated from KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi, and subsequently stored in an airtight container. Subsequently, the seeds underwent drying using a hot air oven at 70°C for 2 hours before being powdered. Thereafter, approximately 40 g of Anethum graveolens powder was immersed in a solution comprising 160 ml of 90% ethanol and 40 ml of water. The mixture was left to soak for 72 hours at room temperature. Subsequently, the filtrate was concentrated by evaporation using the "New Brunswick Scientific Excella E24 Incubator Shaker Series" until it reached the desired concentration. The extract was then filtered through Whatman No.1 filter paper. The extract was then subjected to a process of evaporation, which was carried out using a hot water bath. Following this, the extract underwent a sterilization process that was conducted overnight using UV irradiation. Thereafter, the extract was stored at a temperature of 4°C. In order to prepare the stock solution, 200 mg of crude extract was dissolved in 10 ml of DMSO at a pH of 7.0, which resulted in a concentration of 20 mg/ml. The stock solution was then stored at a temperature of 4°C in conditions of darkness in order to prevent oxidation until it could be used further.

2.2. Inoculum Preparation

BHI broth and ATCC strains of Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Fusobacterium nucleatum were utilized in the preparation of the inoculum. Colonies were picked using a sterile loop and transferred into a tube containing 5 mL of BHI broth. This stock culture was then incubated at 37°C for 8–14 hours. The turbidity of the actively growing bacterial culture with broth was then adjusted to match the 0.5 McFarland standard guidelines.

2.3. Broth Dilution Method With Resazurin Test for Determining Minimum Inhibitory Concentration

The preparation of the broth commenced with the dissolution of 5.5 grams of BHI powder in 150 ml of water, which was subsequently subjected to thorough stirring. Following this, the solution was autoclaved at a temperature of 120°C and a pressure of 15 psi. Thereafter, the broth was subjected to cooling at room temperature in an aseptic environment under laminar air flow. Subsequently, the broth was supplemented with 20 mg/ml of erythromycin. The broth dilution procedure was conducted in a sterilized 96-well plate, with the experiment being performed in triplicate. Initially, 10 wells were selected, with 100 µl of broth added to each well. In the first well, 100 µl of Anethum graveolens extract was added and serially diluted to the required concentrations up to the tenth well. A similar procedure was carried out in the other two rows of the well plates. Subsequently, 20 µl of bacterial inoculum was added to all ten wells. Separate wells were used for positive and negative controls. The 96-well plates were then placed for incubation in a McIntosh and Fildes' anaerobic jar for 48 hours. Following this, 30 µl of Resazurin reagent per 100 µl of extract was added to the wells, and the plates were observed after 4 hours for any potential color change. The color change from blue/violet to slight pink/pink/magenta was noted as the MIC of the emulsion. The results were recorded by capturing high-quality photographs (Figure 1 & Table1).

2. 4. Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) values of Anethum graveolens extracts were determined using agar plates. BHI agar plates were prepared for A. actinomycetemcomitans and F. nucleatum by dissolving 52 grams of BHI powder in 1000 ml of distilled water, followed by autoclaving at 120 ºC and 15 psi pressure. The plates were then allowed to cool to room temperature in an aseptic condition under laminar air flow for a period of 10-15 minutes. Following this, 20 mg/ml of erythromycin was added to the agar, which was then poured and allowed to solidify. For P. gingivalis, agar plates were prepared by first dissolving 3.12 grams of BHI powder in 60 ml of distilled water. This was followed by autoclaving at 120 ºC and 15 psi pressure. The plates were then allowed to cool to room temperature in an aseptic environment under laminar air flow for a period of 10-15 minutes.Following this, 3 ml of blood, 60 µl of Vitamin K, and 0.6 ml of horse serum were added to the mixture, which was subsequently poured and allowed to solidify. Subsequently, streaks were made on the agar plates using an inoculating loop, and the plates were sealed with paraffin film before being incubated in a bacteriological incubator for 12 hours.The minimum concentration at which the bacteria showed no growth was considered to be the MBC value (Figure 2).The results are listed in Table 2.

2. 5. Gel Preparation

The Anethum graveolens gel was prepared at KAHER's KLE College of Pharmacy, Belagavi. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Anethum graveolens extract was used to prepare the gel. The composition of Anethum graveolens is given in (Table 3).

2.5.1. Preparation of Carbopol 940 Gel Base

The quantity of 1% Carbopol 940 was measured out and added to approximately 50 ml of distilled water. This was done gradually to prevent clumping and promote uniform distribution.

a) Subsequently, the mixture was subjected to continuous stirring on a magnetic stirrer for a period of three hours. Thereafter, it was left to hydrate for a duration of 24 hours to ensure complete hydration.

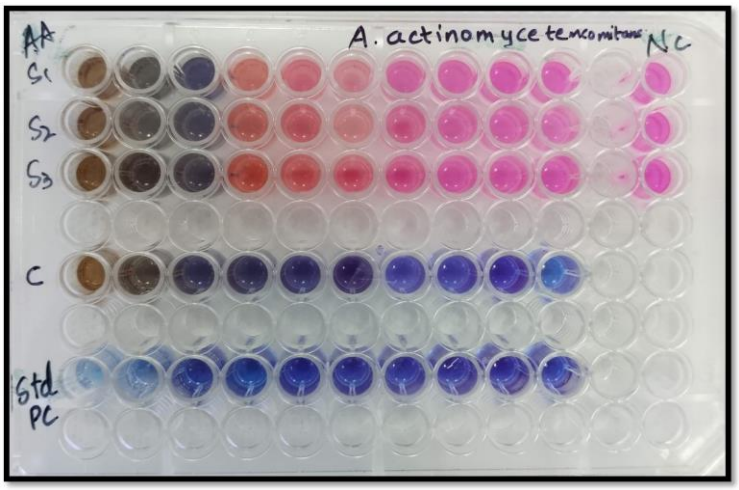

Figure 1: Broth dilution method with resazurin test showing MIC of *Anethum graveolens* extract against *Aggregatibacter actinomycetemcomitans.*

Note: Separate 96 well plates were used for each organism i.e *A.a*, *P.g* and *F.n* and results are listed in (Table 1).

Table. 1. Minimum inhibitory concentration (MIC) of *Anethum graveolens* extract in (mg/ml).

Extract Name	*A.a*		*P.g*		*F.n*	
Anethum graveolens	1.25	1.25	0.625	0.625	2.5	2.08
	1.25		0.625		2.5	
	1.25		0.625		1.25	

All values are expressed in mg/ml against tested organism.

Figure 2: MBC of *Anethum graveolens* extract against *Aggregatibacter actinomycetemcomitans.*

Table. 2. Minimum bactericidal concentration (MBC) of *Anethum graveolens* extract in (mg/ml).

Extract Name	*A.a*		*P.g*		*F.n*	
Anethum graveolens	1.25	1.25	2.5	2.5	2.5	2.5
	1.25		2.5		2.5	
	1.25		2.5		2.5	

All values are expressed in mg/ml against tested organism.

Table 3. Composition of *Anethum graveolens* gel.

SL No.	Ingredients	Formulation	Function
1.	*Anethum graveolens*	20% w/w	Natural active ingredient
2.	Carbopol 940	1% w/w	Gelling agent
3.	Tween 80	0.06% w/w	Dispersing agent
4.	Propylene glycol	2% w/w	Plasticizer and Humectant
5.	Sodium methyl paraben	0.033% w/w	Bactericidal agent
6.	Sodium propyl paraben	0.066% w/w	Bactericidal agent
7.	Sodium benzoate	0.03% w/w	Bacteriostatic agent
8.	Triethanolamine	0.5% w/w	pH adjuster and stabilizer
9.	Distilled water	q.s	Solvent

2.5.2. Preparation of Extract Dispersion

a) 20% w/w of Anethum graveolens extract was subjected to trituration in a mortar and pestle.

b) 0.06% of Tween 80, a dispersing agent, and 2% of Propylene glycol, a plasticiser and humectant, were added to the mixture. The triturated extract was then subjected to a process of uniform dispersion, involving the addition of 0.06% Tween 80 (a dispersing agent) and 2% propylene glycol (a plasticiser and humectant).

c) Subsequently, 30 ml of distilled water was added to the aforementioned triturated extract, along with preservatives in the form of 0.033% sodium methyl paraben, 0.066% sodium propyl paraben and 0.03% sodium benzoate. The solution was then stirred with a magnetic stirrer for 30 minutes at 700 rpm.

2.5.3. Gel Formation

d) The extract dispersion was added to the Carbopol 940 gel base, and the volume was adjusted with distilled water to achieve a final weight of 100 g of gel.

e) 0.5% of triethanolamine was added dropwise. The mixture was then added to the previously prepared triethanolamine solution, which had been prepared dropwise, and stirred using a high-speed propeller stirrer at 1200 rpm for 30 minutes. The gel was then exposed to UV irradiation for 20-30 minutes. It was then transferred into an airtight container (Figure 3). The gel was stored at ambient temperature for future use.

Figure 3. Anethum graveolens gel.

2. 6. Agar Well Diffusion Assay

The agar well diffusion assay was conducted on bacteriological agar plates for the following organisms: A. actinomycetemcomitans, P. gingivalis, and F. nucleatum. Mueller Hinton agar plates were prepared by adding 38 grams of Mueller Hinton agar powder to 1000 ml of distilled water, followed by sterilization in a steam sterilizer. The plates were then left to cool at room temperature for 10-15 minutes before being poured and allowed to solidify. Bacterial broth cultures (100 µL) of A. actinomycetemcomitans (Figure 4), P. gingivalis, and F. nucleatum with a turbidity equivalent to 0.5 McFarland's standard were distributed uniformly across the prepared agar plates using a sterile cotton spreader. Aseptic wells were then created in a uniform manner using a cork borer. The creation of these wells was followed by the addition of sample reagents (100 µL saline, 100 µL Anethum graveolens gel, and 100 µL Chlorhexidine gel) and the placement of the plates in an anaerobic incubator set at 37°C.The plates were then observed for diffusion over a period of 24-72 hours of incubation. Growth patterns were observed, and the zone of inhibition was measured for each sample reagent on the plates. The results were then compared against Chlorhexidine as the standard.The diffusion assay was performed in triplicates for all three micro-organisms, and the results are listed in Table 4.

Statistical Analysis

1. Comparison of the three groups (saline, 1% chlorhexidine gel and Anethum graveolens gel) against A.a, P.g and F.n was done by Kruskal Wallis ANOVA.

2. Comparison of three organisms (A.a, P.g and F.n) against saline, 1% chlorhexidine gel and Anethum graveolens gel was done using Kruskal Wallis ANOVA.

3. Paired comparisons between groups were made using Mann-Whitney U test.

4. A probability value of less than 0.05 was considered statistically significant. SPSS software version 22 was used for statistical analysis.

3. Results

The mean and standard deviation for the 1% chlorhexidine gel was found to be 15.67±0.58, and the mean and standard deviation for the Anethum graveolens gel was found to be

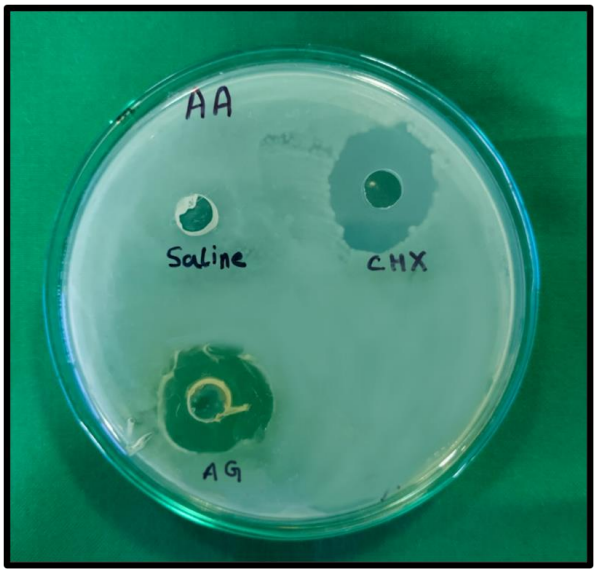

Figure 4. Agar well diffusion test for prepared *Anethum graveolens* gel and commercially available Chlorhexidine gel against *Aggregatibacter actinomycetemcomitans.*

12.67±1.53. The intergroup comparison of the saline, chlorhexidine gel and Anethum graveolens gel for A.a. showed a statistically significant difference (p=0.0230) (Table 5). The mean and standard deviation for 1% chlorhexidine gel was 17.00±1.00, and the mean and standard deviation for Anethum graveolens gel was 13.00±1.00. The intergroup comparison of saline, chlorhexidine gel and A. graveolens gel for P. g. showed a statistically significant difference (p=0.0240) (Table 5). The mean and standard deviation for the 1% chlorhexidine gel was 15.33 ± 1.53, and the mean and standard deviation for the Anethum graveolens gel was 12.00±1.00. The intergroup comparison of the saline, chlorhexidine gel and Anethum graveolens gel for F.n. showed a statistically significant difference (p = 0.0240) (Table 5).

4. Discussion

Dental plaque constitutes a microbial community that adheres to tooth surfaces, forming a biofilm within a matrix of host and bacterial polymers. This process follows a sequential order, resulting in a structured and diverse microbial community. (12) Scaling and root planning, a process that entails the mechanical removal of plaque and calculus from the affected teeth, is widely regarded as the primary treatment for periodontitis. However, its efficacy in the complete debridement of the subgingival area is frequently diminished (13).

The utilisation of locally delivered anti-infective pharmacological agents through sustained-release delivery systems offers several clinical, pharmacological and toxicological advantages over conventional treatment for periodontal diseases. Chlorhexidine, an antiseptic drug with poor gastrointestinal absorption, would not effectively reach the periodontal pocket if administered orally (14). The mean and standard deviation of the zone of inhibition for A.a with Chlorhexidine gel was 15.67 ± 0.58, and for A. graveolens gel was 12.67 ± 1.53. For P.g with Chlorhexidine gel was 17.00 ± 1.00, and for A. graveolens gel was 13.00 ± 1.00. For F.n, the mean values were 15.33 and 12.00, respectively. It is notable that no zone of inhibition was observed in the saline control group. Pattnaik et al. and Lecic et al. also reported superior outcomes with Chlorhexidine, including an increase in "clinical attachment level (CAL), reduction in probing pocket depth" (PPD), and decreased bleeding on probing. (7) Nonetheless, the utilisation of Chlorhexidine gel may result in adverse effects such as xerostomia, hypogeusia and tongue discolouration. Furthermore, prolonged use may lead to the formation of calculus and extrinsic staining of teeth, and there is a possibility of cross-resistance to antibiotics developing as a result of extended exposure to Chlorhexidine (15). In order to address the limitations of conventional chemotherapeutic agents, researchers are exploring alternative approaches for treating oral diseases. Medicinal herbs offer a distinct advantage in this regard due to their lower likelihood of adverse reactions such as hypersensitivity and the development of bacterial resistance. Among these herbal remedies is Anethum graveolens, which contains natural phytochemicals known for their therapeutic properties. A hydroethanolic extract of A. graveolens has been shown to possess broad-spectrum antibacterial activity against pathogens such as "S. aureus, E. coli, and P. aeruginosa." This efficacy can be attributed to the chemical composition of its major constituents, such as dillapiole and anethole (16). A study was conducted by Safoura Derakhshan to examine the antibacterial efficacy of Anethum graveolens (dill) essential oil, with the results indicating a satisfactory to moderate level of activity against the strains tested (17). The antibacterial effectiveness of A. graveolens oil was evaluated through the agar well diffusion method. The outcomes of this investigation demonstrated substantial to moderate levels of antibacterial activity, with a zone of inhibition ranging from 10.0 to 15.0 mm (Dahiya and Purkayastha (2012).This activity was observed against both Gram-positive bacteria, including "S. aureus and Enterococcus species, and Gram-negative bacteria such as E. coli, Klebsiella pneumoniae, and P. aeruginosa".

Table 4. Agar well diffusion assay of *Anethum graveolens* gel, Chlorhexidine gel and saline against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

Groups	***A.a***			***P.g***			***F.n***		
Saline	NG	NG	NG	NG	NG	NG	NG	NG	NG
1% Chlorhexidine gel	16mm	16mm	15mm	17mm	18mm	16mm	15mm	17mm	14mm
Anethum graveolens	14mm	13mm	11mm	13mm	14mm	12mm	11mm	12mm	13mm

NG: No growth, mm: millimeter

Table 5: Summary of Agar well diffusion (growth in mm) among three groups (Saline, 1% Chlorhexidine gel, *Anethum graveolens* gel) and three organisms (*A.a*, *P.g* and *F.n).*

Factors	n	Mean	SD	SE
Groups				
Saline	9	0.00	0.00	0.00
1%Chlorhexidine gel	9	16.00	1.22	0.41
Anethum graveolens	9	12.56	1.13	0.38
Organisms				
A.a	9	9.44	7.25	2.42
P.g	9	10.00	7.73	2.58
F.n	9	9.11	7.04	2.35
Interactions (Groups x organisms)				
Saline with *A.a*	3	0.00	0.00	0.00
Saline with *P.g*	3	0.00	0.00	0.00
Saline with *F.n*	3	0.00	0.00	0.00
1%Chlorhexidine gel with *A.a*	3	15.67	0.58	0.33
1%Chlorhexidine gel with *P.g*	3	17.00	1.00	0.58
1%Chlorhexidine gel with *F.n*	3	15.33	1.53	0.88
Anethum graveolens with *A.a*	3	12.67	1.53	0.88
Anethum graveolens with *P.g*	3	13.00	1.00	0.58
Anethum graveolens with *F.n*	3	12.00	1.00	0.58

However, contradictory findings were noted in certain microorganisms. Dill oil exhibited weak effectiveness against Aspergillus niger, according to Elgayyar et al. (2001). However, no inhibitory effect on the growth of "Lactobacillus plantarum, Listeria monocytogenes and Pseudomonas aeruginosa" was observed with dill oil in the same study. In a randomized clinical trial conducted by Shruti Eshwar et al., the effectiveness of dill seed oil mouthrinse was compared to that of Chlorhexidine mouth rinse, with the aim of assessing plaque levels and gingivitis. The study concluded that both mouthrinses showed similar efficacy in reducing plaque and gingivitis, along with significant improvements in clinical parameters (19). In a separate study by Nazish Badar et al., the antimicrobial efficacy of A. graveolens seed oil was evaluated at various dilutions. The findings demonstrated that at dilutions of 1:10, 1:50, and 1:100, the oil produced zones of inhibition measuring 7 mm, 6 mm, and 4 mm, respectively. However, at a dilution of 1:200, the antimicrobial activity against E. coli was found to be negative. (20) The current study identified a statistically significant difference between the two groups ($p<0.05$). Chlorhexidine gel displayed a broader zone of inhibition compared to A. graveolens gel against A.a, P.g and F.n. In consideration of the study's limitations, it can be deduced that the antimicrobial efficacy of Chlorhexidine gel exceeds that of Anethum graveolens gel. Nevertheless, further research at the biomolecular level is required to identify the active phytochemical components responsible for the antimicrobial properties and clinical applications of Anethum graveolens gel.

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Authors' Contribution
Conducted experimental work: R. P. and R. N.
Analysed the data: A. P. and S. D.
Designed and reviewed the manuscript: R. M. and R. P.
All authors commented on the manuscript and approved the final manuscript.

Ethics
The present article contains no studies with human participants or animals performed by any of the authors.

Conflict of Interest
Ruchi Patel, Renuka Metgud, Suneel Dodamani, Rubeen Nadaf, and Archana Patil hereby declare that they have no conflicts of interest.

Financial disclosure
It is asserted that there are no financial interests related to the material in the manuscript.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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