Title

1. 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. (Original research)

Abstract

2. Periodontitis is an infection of the periodontium caused by 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 it does not cause sufficient reduction of the bacterial load due to lack of accessibility to microorganisms. Hence, the incorporation of adjunctive chemotherapeutic agent enhances the outcome at sites which are not responsive to conventional therapy. Chlorhexidine is considered the gold standard for local drug delivery system but it has side effects like tooth staining, xerostomia and calculus formation. This has led to increasing demand for herbal medicine as they show fewer side effects and are cost effective. Among these herbal remedies Anethum graveolens, which contains natural phytochemicals is known for its therapeutic properties. Hence the present in-vitro microbiological study was undertaken to evaluate and compare the antimicrobial activity of Anethum graveolens gel with Chlorhexidine gel for Aa,Pg and Fn. 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 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

15.6 mm, 17mm and 15.3mm for A.a, P.g and F.n respectively, whereas for A. graveolens gel it was 12.6mm 13mm and 12mm for 24. A.a, P.g and F.n respectively.

The results obtained suggested that Chlorhexidine gel showed a slightly better antimicrobial activity as compared 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, marked by the gradual deterioration of 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).

Bacterial colonization in the oral environment is widely regarded as the primary cause of periodontal disease. Secondary factors contributing to its etiology include dental plaque, calculus buildup, anatomical factors such as developmental grooves, short root trunk, cervical enamel projections, overhanging restorations, as well as lifestyle factors like stress and smoking (3). Organisms strongly linked to periodontitis include "*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola*, *Eikenella corrodens*, and *Fusobacterium nucleatum*" (4).

Particularly, *P.gingivalis* and *A. actinomycetemcomitans* are highlighted for their significant role in disease progression due to their pathogenic potential. Association with *A. actinomycetemcomitans* is linked to accelerated degeneration in the pocket epithelium, characterized by micro clefts and necrotic areas. *Porphyromonas gingivalis* stands out as one of the primary periodontal pathogens and is recognized as one of the most virulent microorganisms contributing to the pathogenesis of periodontal disease (5). *Fusobacterium nucleatum* is extensively studied and considered a key bacterium associated with periodontal diseases. It is a Gram-negative anaerobic bacterium belonging to the Bacteroidaceae family within the phylum Fusobacteria. This bacterium is particularly abundant in dental plaque biofilms (6).

Periodontal therapy encompasses both mechanical and chemical approaches aimed at reducing or eradicating microbial biofilm. Traditional plaque control serves as the initial and vital component of periodontal treatment, albeit its effectiveness is somewhat limited 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).

Systemic antibiotics are limited in their application for treating periodontitis due to several factors, including the necessity for higher dosages to reach desired concentrations in the gingival crevicular fluid (GCF), the emergence of bacterial resistance, potential side effects Hence, the concept of controlled local drug delivery was introduced with the aim of delivering the drug to the base of the periodontal pocket and sustaining its presence for a sufficient duration to exert its antimicrobial effects (8).

Antimicrobial agents suitable for local administration mainly include Metronidazole, Chlorhexidine, Doxycycline and Tetracycline. These agents can be delivered through various controlled drug delivery systems such as gels, strips, fibers, films, injectable systems. CHX is recognized 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 today's modern age, there's a growing preference for organic products, because of their antibacterial, antioxidant, immune-regulatory and anti-inflammatory potentials, making them effective antidotes for various common ailments. Moreover, they are favoured for being cost-effective, relatively safe and associated with reduced development of resistance, toxicity and fewer side effects, including hypersensitivity reactions and staining of teeth, 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 Dill is widely utilized in Ayurvedic medicine to alleviate abdominal discomfort, aid digestion,

and address rheumatism. *Anethum graveolens* is rich in flavonoids, which possess 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).

Therefore, recognizing the advantageous properties of the herbal drug, 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 "KAHER's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi." The Anethum graveolens gel was prepared and collected from "KAHER's KLE College of Pharmacy, Belagavi." Commercially available 1% Chlorhexidine gel (Hexigel) was used.

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. Following this, the seeds underwent drying using hot air oven at 70ºC for 2 hours before being powdered. About 40g of *Anethum graveolens* powder was then immersed in a solution containing 160 ml of 90% ethanol and 40 ml of water, left to soak for 72 hours at room

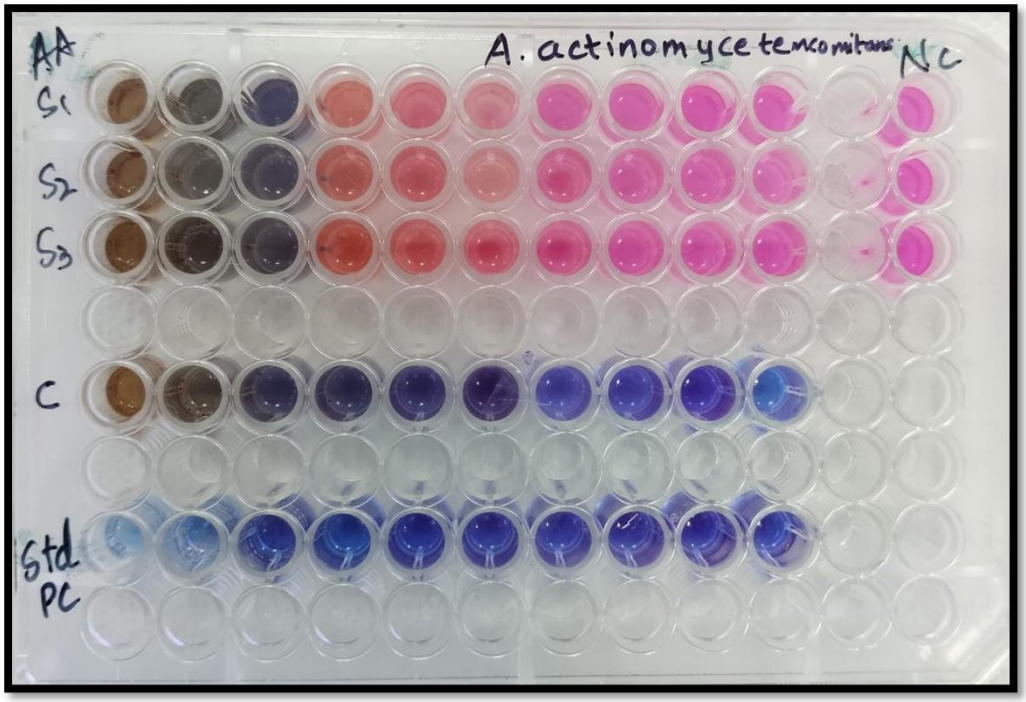
temperature. Subsequently, the filtrate was concentrated by evaporation using the "New Brunswick Scientific Excella E24 Incubator Shaker Series" until it reached the desired concentration. The extract was then filtered through Whatman No.1 filter paper. The extract was then evaporated using hot water bath. The extract then underwent sterilization overnight through UV irradiation and was stored at 4ºC. To prepare the stock solution, 200mg of crude extract was dissolved in 10 ml of DMSO at pH 7.0, resulting in a concentration of 20 mg/ml. The stock solution was then kept at 4°C in the dark to prevent oxidation till further use.

2.2. Inoculum preparation: BHI broth and ATCC strains of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* were utilized to prepare 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 adjusted to match the 0.5 McFarland standard guidelines.

2.3. Broth dilution method with Resazurin test for determining Minimum Inhibitory Concentration: To prepare the broth, 5.5 grams of BHI powder was dissolved in 150 ml of water and thoroughly stirred. Subsequently, it was autoclaved at 120 ºC and 15 psi pressure. The broth was then cooled at room temperature in an aseptic condition under laminar air flow. Then 20 mg/ml of erythromycin was added to the broth. Broth dilution was performed in a sterilized 96-well plate, with the procedure being conducted in triplicates. Initially 10 wells were selected. A total of 100 µl of broth was added to all 10 wells in triplicates. 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. Further, 20 µl of bacterial inoculum was added to all the 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 incubation, 30 µl of Resazurin reagent per 100 µl of extract was added to the wells and 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)

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.

2. 4. Minimum Bactericidal Concentration (MBC)

MBC was determined using the MIC values of *Anethum graveolens* extracts with the help of agar plates. BHI agar plates for *A. actinomycetemcomitans* and *F. nucleatum* were prepared by dissolving 52 grams of BHI powder in 1000 ml of distilled water, followed by autoclaving at 120 ºC and 15 psi pressure. It was then cooled to room temperature in an aseptic condition under Laminar air flow for 10-15 minutes, 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 dissolving 3.12 grams of BHI powder in 60 ml of distilled water, followed by autoclaving at 120 ºC and 15 psi pressure. It was then cooled to room temperature in an aseptic condition under laminar air flow for 10-15 minutes, 3 ml of blood, 60 µl of Vitamin K, and 0.6 ml of horse serum were added to the mixture, which was then poured and allowed to solidify. 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 as the MBC value (Figure 2). The results are listed in (Table 2).

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

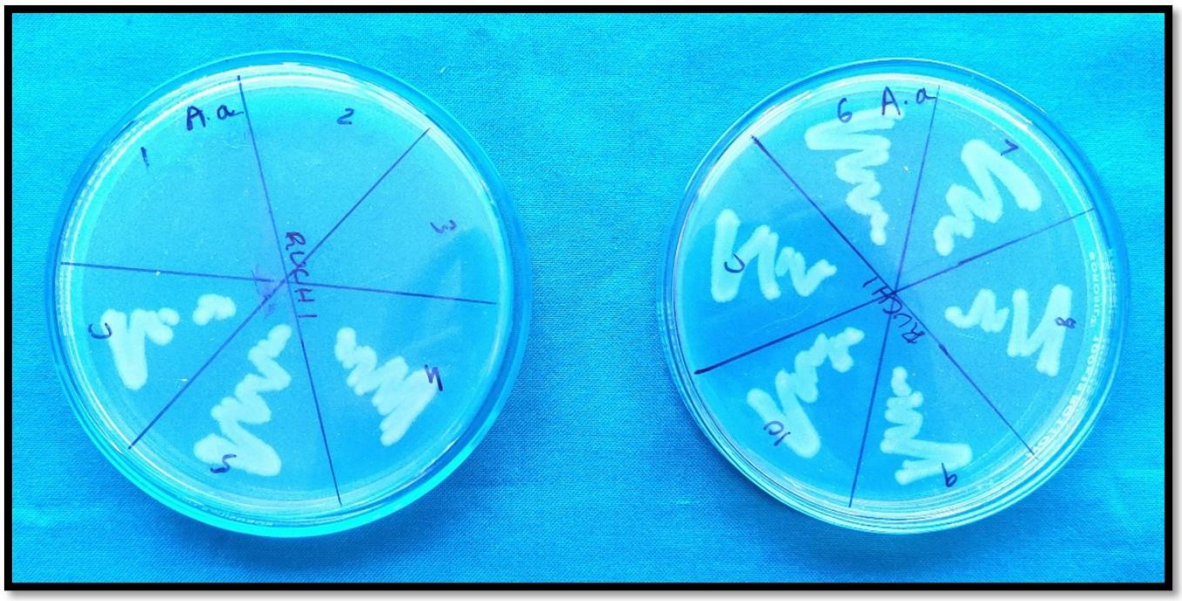


Table. 2. Minimum bactericidal concentration (MBC) of *Anethum graveolens* extract in (mg/ml).

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.

2. 5. Gel preparation:

The *Anethum graveolens* gel was prepared at the 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 listed in (Table 3)

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

Preparation of Carbopol 940 gel base:

a) Weighed quantity of 1% Carbopol 940 was added in about 50ml of distilled water ensuring Carbopol 940 is added gradually to prevent clumping and promote uniform distribution.

b) Then, it was stirred continuously on a magnetic stirrer for three hours and kept 24 hours for complete hydration.

Preparation of Extract Dispersion:

a) 20% w/w of *Anethum graveolens* extract was triturated in a mortar and pestle.
b) 0.06% of Tween 80 which is a dispersing agent and 2% of Propylene glycol which is a plasticizer and humectant was added to the triturated extract and ensured uniform dispersion.
c) 30 ml of distilled water was added to the above triturated extract along with preservatives like 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 mins at 700 rpm.

Gel Formation:

d) The extract dispersion was added to the Carbopol 940 gel base and the volume was adjusted with distilled water to achieve the final weight of 100 gm of gel.
e) 0.5% of triethanolamine was added dropwise to the above mixture and stirred using high speed propeller stirrer at 1200rpm for 30 mins.
f) The gel was then passed through UV irradiation for 20-30 minutes.
g) Then was 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 *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 and sterilized in a steam sterilizer. After cooling at room temperature for 10-15 minutes, the agar plates were 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 spread evenly over the prepared agar plates using a sterile cotton spreader. Aseptic wells were then created uniformly using a cork borer. Sample reagents (100 µL saline, 100 µL Anethum graveolens gel, and 100 µL Chlorhexidine gel) were added to these wells and placed in a anaerobic incubator at 37℃. The plates were observed for diffusion over 24-72 hours of incubation. Growth patterns were observed, and the zone of inhibition was measured for each sample reagent on the plates, with results compared against Chlorhexidine as the standard. The diffusion assay was performed in triplicates for all the three micro-organisms. The results are listed in (Table 4).

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

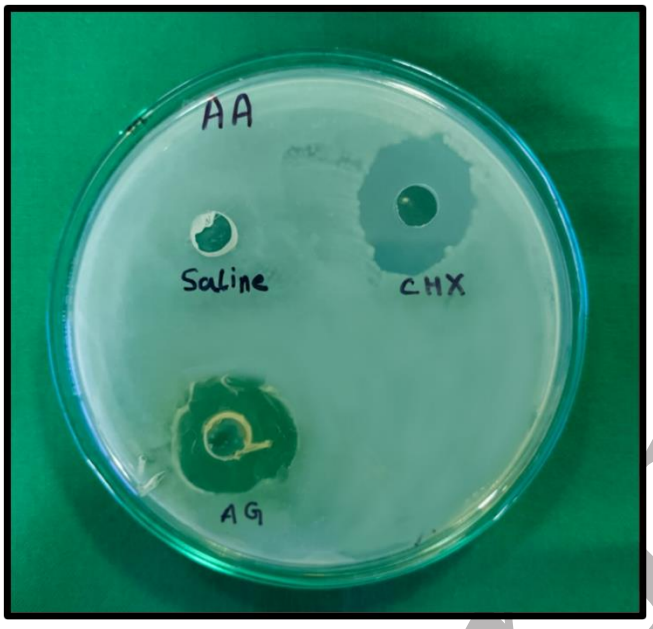


Table 4. Agar well diffusion assay of *Anethum graveolens* gel, Chlorhexidine gel and saline against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

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

Statistical analysis

1. Comparison of the three groups (Saline, 1% Chlorhexidine gel and *Anethum graveole*ns gel) against *A.a, P.g and F.n* was done using Kruskal Wallis ANOVA.

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

3. Pair wise comparisons between the groups was done using Mann-Whitney U test.

4. Probability value of less than 0.05 was considered as statistically significant.

SPSS software version 22 was used to carry out the statistical analysis

3. Results

3.1 The mean and standard deviation for 1% Chlorhexidine gel was 15.67±0.58 and the mean and standard deviation for *Anethum graveolens* gel was 12.67±1.53. The intergroup comparison of Saline, Chlorhexidine gel and *A. graveolens* gel for *A.a* showed statistically significant difference. (p=0.0230) (Table5)

3.2 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 statistically significant difference. (p=0.0240) (Table 5)

3.3 The mean and standard deviation for 1% Chlorhexidine gel was 15.33 ±1.53 and the mean and standard deviation for Anethum graveolens gel was 12.00 ±1.00. The intergroup comparison of Saline, Chlorhexidine gel and *A.graveolens* gel for F.n showed statistically significant difference. (p=0.0240) (Table 5)

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

4. Discussion

Dental plaque constitutes a microbial community adhering to the tooth surfaces, forming a biofilm within a matrix of host and bacterial polymers. Its formation follows a sequential process, leading to a structured and diverse microbial community. (12) Scaling and root planing, 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 utilization of locally delivered anti-infective pharmacological agents through sustained-release delivery systems offer several clinical, pharmacological and toxicological advantages over conventional treatment for periodontal diseases. Chlorhexidine, is an antiseptic drug with poor gastrointestinal absorption, which 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 A. graveolens gel was 12.67±1.53. For P.g with Chlorhexidine gel was 17.00±1.00 and A. graveolens gel was 13.00±1.00. For F.n with Chlorhexidine gel was 15.33±1.53 and A. graveolens gel was 12.00±1.00. Zone of inhibition was not noted with the (Saline) control group.

Pattnaik et al. and Lecic et al. also reported superior outcomes with Chlorhexidine, including gain in “clinical attachment level (CAL), reduction in probing pocket depth” (PPD), and decreased bleeding on probing. (7)

Nonetheless, the utilization of Chlorhexidine gel may result in adverse effects such as xerostomia, hypogeusia and tongue discoloration. Prolonged use may also lead to calculus formation and extrinsic staining of teeth. Furthermore, extended exposure to Chlorhexidine could potentially induce cross-resistance to antibiotics. (15)

To address these limitations, researchers are exploring alternative approaches for treating oral diseases. Medicinal herbs offer a distinct advantage over conventional chemotherapeutic agents 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. Hydroethanolic extract of A. graveolens has demonstrated 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)

Safoura Derakhshan undertook a study to examine the antibacterial efficacy of Anethum graveolens (Dill) essential oil. The results indicated 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 results indicated significant to moderate 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". 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. (18)

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 assessing plaque levels and gingivitis. The study concluded that both the mouthrinse showed similar efficacy in reducing plaque and gingivitis, along with significant improvements in clinical parameters. (19)

In another study conducted by Nazish Badar et al., the antimicrobial efficacy of A. graveolens seed oil was assessed at various dilutions. Results indicated that at dilutions of 1:10, 1:50, and 1:100, the oil provided 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*.

Given the constraints of the study, it can be inferred that the antimicrobial efficacy of Chlorhexidine gel surpassed that of *Anethum graveolens* gel. However, additional research at the biomolecular level is necessary to pinpoint the active phytochemical components accountable for the antimicrobial properties and clinical uses of *Anethum graveolens* gel.

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Author's contribution

Ruchi Patel and Rubeen Nadaf conducted experimental work. Archana Patil and Suneel Dodamani analysed the data. Renuka Metgud and Ruchi Patel designed and reviewed the manuscript. All authors commented on the manuscript and approved the final manuscript.

Ethics

This 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 declare that they have no conflicts of interest.

Data availability

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

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List of abbreviations

A.a	*Aggregatibacter actinomycetemcomitans*

A.graveolens	*Anethum graveolens*
ATCC	American type culture collection
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
F.n	*Fusobacterium nucleatum*
GI	Gingival Index
MBC	Mean bactericidal concentration
MIC	Mean inhibitory concentration
mg	Milligram
ml	Millilitre
P.g	*Porphyromonas gingivalis*
PI	Plaque Index
PPD	Pocket Probing Depth
µL	Microlitre
%	Percentage