“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.”

By

REG NO. IK0221003

Dissertation

Submitted to KLE Academy of Higher Education and Research (KAHER), Belagavi

In Partial Fulfillment of the Requirements for the Degree Of

MASTER OF DENTAL SURGERY
In
PERIODONTICS
(Branch-II)

DEPARTMENT OF PERIODONTICS
KAHER’S KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES,
NEHRU NAGAR, BELAGAVI-590010,
KARNATAKA.

2021 - 2024

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,

BELAGAVI, KARNATAKA

Endorsement by the Head of Department & Principal / Head of the Institution

*This is to certify that the dissertation **"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."** is a bonafied research work done by*

post graduate student under the guidance of

Professor, Department of Periodontics, KAHER's KLE V. K. Institute of Dental Sciences Belagavi.

Dr. VINAYAK KUMBHOJKAR ***M.D. S***
Professor & Head,
Department of Periodontics,
KAHER's KLE V.K.Institute of Dental Sciences, Belagavi

Professor and Head
Department of Periodontics
KLE V. K. Institute of Dental Sciences
Belagavi

Date: 12/04/24
Place: Belagavi

Dr. ALKA KALE ***M.D.S, Ph.D.***
Principal,
KAHER's KLE V.K. Institute of Dental Sciences, Belagavi

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar BELAGAVI-590010

Date: 15/4/24
Place: Belagavi

UNDERTAKING

I, ***REG NO. IK0221003*** post graduate student in Department of Periodontics, have completed my research work on the topic ***"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"*** in the year 2021-2024.

I have been given to understand that any research work I undertake for the purpose of dissertation, oral presentation or publication during my study course shall be the property of KAHER's KLE Vishwanath Katti Institute of Dental Sciences, Belagavi. Hence, I hereby declare that the name of the Department, Institute and University shall be mentioned in my publication. The authorship shall be according to the guidelines informed to me.

Date:

Place: Belagavi

UNDERTAKING

I, ***REG NO. IK0221003*** hereby declare that the information and the data mentioned in my thesis titled, ***"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"*** belongs to me and is original.

I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts, or ideas as one's own without attribution in connection with the submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is an original one and does not involve plagiarism anywhere. In case at a later stage, it is found that I have indulged in plagiarism, then I am solely responsible for the same and the institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the university.

Date:

Place: Belagavi

LIST OF ABBREVIATIONS

A.a	*Aggregatibacter actinomycetemcomitans*
A.graveolens	*Anethum graveolens*
BHI	Brain Heart Infusion
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
Cdt	Cytolethal distending toxin
Conc	Concentration
DMSO	Dimethyl Sulfoxide
DO	Doxycycline
F.n	*Fusobacterium nucleatum*
GCF	Gingival crevicular fluid
GI	Gingival Index
Hrs	Hours
LDD	Local Drug Delivery
Ltx	Leukotoxin
MBC	Mean bactericidal concentration
MHA	Mueller-Hinton agar
MIC	Mean inhibitory concentration
Mg	Milligram

Ml	Millilitre
Mm	Millimeter
MMP	Matrix Metalloproteinases
PCR	Polymerase Chain Reaction
P.g	*Porphyromonas gingivalis*
PI	Plaque Index
P.i	*Prevotella intermedia*
PPD	Pocket Probing Depth
RAL	Relative Attachment Level
RANKL	Receptor activator of nuclear factor kappa-B ligand
SD	Standard Deviation
SRP	Scaling and root planing
Mg	Microgram
μL	Microlitre
%	Percentage

ABSTRACT

INTRODUCTION

Periodontitis is an infection of the periodontium caused by group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation.

Therapeutic approaches which include mechanical scaling and root planing, is the first recommended periodontal therapy but it prevents sufficient reduction of the bacterial load due to lack of accessibility to microorganisms. Hence, incorporation of adjunctive chemotherapeutic agent enhances the outcome at sites 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 in the demand for herbal medicine as they show fewer side effects and are cost effective. Among these herbal remedies is *Anethum graveolens*, which contains natural phytochemicals known for their therapeutic properties.

AIM

To compare and evaluate the antimicrobial efficacy of *Anethum graveolens* gel with Chlorhexidine gel against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Fusobacterium nucleatum*.

MATERIALS AND METHODS

The study was an in-vitro microbiological study. The hydroethanolic extract of *Anethum graveolens* was prepared through maceration and was then filtered and concentrated. 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 intergroup comparison was done using Kruskal Wallis ANOVA. Pair wise comparisons between the groups was done using Mann-Whitney U test.

RESULTS

The Minimum Inhibitory Concentration (MIC) of hydroethanolic extract of *A. graveolens was* 1.25 mg/ml, 0.625mg/ml and 2.08mg/ml for *A.a, P.g* and *F.n* respectively. The Minimum Bactericidal Concentration (MBC) of hydroethanolic extract of *A.graveolens was* 1.25 mg/ml, 2.5mg/ml and 2.5mg/ml for *A.a, P.g* and *F.n* respectively. 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 *A.a, P.g* and *F.n* respectively.

CONCLUSION

In light of the observations drawn from our study we conclude that *Anethum graveolens* gel showed reduced diffusion activity. This implies that the prepared *Anethum graveolens* gel showed potent anti-microbial activity but Chlorhexidine gel showed superior antimicrobial activity.

KEYWORDS: *Anethum graveolens,* Dental plaque, Chlorhexidine, Herbal extract, Periodontal disease.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF ANNEXURES

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]

The transition from a state of periodontal health to disease involves a significant change in the composition of the oral microbial community. Initially, a balanced community dominated by facultative bacterial genera like Actinomyces and Streptococci undergoes a shift towards a dysbiotic microbiota characterized by an abundance of anaerobic genera primarily from the phyla Proteobacteria, Spirochaetes, and Bacteroidetes. The dysbiotic oral microbiota exhibits an increased presence of virulence factors and has adapted to flourish in an inflamed environment. [3]

The prevalence of periodontal disease varies, ranging from 24.4% in adults aged 30 to 34 years to 70.1% in adults aged 65 years and older. [4]

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. [5]

Organisms strongly linked to periodontitis include "*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *Eikenella corrodens*, and *Fusobacterium nucleatum*". [6]

In the advanced stages of the disease, inflammation penetrates deeper into the tissues, resulting in the breakdown of periodontal fibers and the migration of the gingival epithelium towards the root, forming periodontal pockets.[7] Bacteria, including *P. gingivalis, A. actinomycetemcomitans*, and *F. nucleatum* have been found to invade the apical and lateral regions of these periodontal pocket walls. [8,9] These bacteria are known to produce various virulence factors that contribute to the degradation of periodontal tissues.

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.[10] This bacterium tends to be more prevalent in deeper pockets compared to shallow periodontal pockets.[11] It is characterized as a non-motile, asaccharolytic, gram- negative obligate anaerobic rod and forms black-pigmented colonies on blood agar plates.

Aggregatibacter actinomycetemcomitans, is the second most prevalent and frequently isolated bacterium in infected periodontal pockets. It is characterized as a gram-negative, non-sporing, non-motile, facultative anaerobic coccobacillus.[12] Cytolethal distending toxin (Cdt) and leukotoxin (LtxA) are unique for *A. actinomycetemcomitans* among bacterial species colonizing the oral biofilm. The LtxA selectively impacts human cells of hematopoietic origin through its interaction with the lymphocyte function-associated receptor 1 leading to membrane integrity disruption. Cdt is expressed by a several Gram-negative bacteria and induces host cells death by inhibiting proliferation and upregulating the expression of receptor activator of nuclear factor kappa-B ligand (RANKL), a crucial factor in osteoclastogenesis.[13]

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.[14] *Fusobacterium nucleatum* is implicated in various manifestations of periodontal diseases, ranging from mild forms of gingivitis to more severe conditions such as chronic periodontitis, localized aggressive periodontitis and generalized aggressive periodontitis.[15]

Periodontal pockets create an optimal environment for the proliferation of gram-negative, facultative anaerobic bacteria. If left untreated, the condition can escalate to a more severe stage, resulting in the formation of alveolar bone defects accompanied by eventual tooth loss. Hence, the removal of local factors and subgingival microbial flora is crucial in the treatment of periodontal disease.

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.[16]

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 and concerns regarding cost-effectiveness.[17] Consequently, to address these limitations, the development of drug delivery systems in the form of direct subgingival administration has been utilized for three decades. [18,19]

The concept of controlled drug delivery for treating periodontal diseases 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.[20] This approach is particularly beneficial for patients who are medically compromised or for whom periodontal surgery is not recommended. 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, nanoparticle systems and microspheres.

Topical antiseptics, with chlorhexidine (CHX) being notably effective, have been widely utilized for treating plaque-related gingivitis and periodontitis. 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.[21]

Chlorhexidine digluconate 1% exhibits rapid release within the initial 24 hours, releasing approximately 34% of the total CHX content from the gel at a consistent rate. This release continues over an average period of 6–9 days, reaching approximately 85% of the total CHX content within the gel.[16] Studies have demonstrated its effective antibacterial action against *A. actinomycetemcomitans*, *F. nucleatum*, and *P. gingivalis.*[22]

In today's modern age, there's a growing preference for organic products, and researchers are increasingly exploring herbal alternatives due to their numerous medicinal and beneficial properties. These herbal remedies are known for their antibacterial, antioxidant, immune-regulatory and anti-inflammatory potentials, making them effective antidotes for various common ailments. Moreover, they are favored 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 family and features tiny yellow flowers.[23] Dill has a rich history in Ayurvedic medicine, dating back to ancient times, and is renowned for its aromatic properties. The genus name Anethum is derived from the Greek word "aneeson" or "aneeton," meaning strong smelling. Dill is widely utilized in Ayurvedic medicine to alleviate abdominal discomfort, aid digestion, and address rheumatism. Additionally, it serves as a popular culinary herb, often yielding essential oil. Dill typically reaches heights of up to 90 cm, featuring slender stems and alternate leaves that are intricately divided into three or four pinnate sections. These leaves are slightly broader compared to those of fennel, a similar herb.[24]

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. The hydroalcoholic extract of *Anethum graveolens* seeds has demonstrated significant anti-inflammatory and antimicrobial properties.[25] 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”.[26]

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*.

AIM OF THE STUDY

To evaluate and compare the antimicrobial efficacy of *Anethum graveolens* gel with Chlorhexidine gel against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Fusobacterium nucleatum.*

OBJECTIVES OF THE STUDY:

1. To determine the Minimum Inhibitory concentration (MIC) of hydroethanolic extract of *Anethum graveolens* against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*
2. To determine the Minimum Bactericidal concentration (MBC) of hydroethanolic extract of *Anethum graveolens* against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*
3. To assess and compare the antimicrobial efficacy of *Anethum graveolens* gel with Chlorhexidine gel against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.

REVIEW OF LITERATURE

Dill, scientifically known as *Anethum graveolens*, is an annual herbaceous plant characterized by its small yellow flowers. It has a rich history in Ayurvedic medicine, where it has been utilized for centuries. Beyond its medicinal applications, dill is widely embraced as a culinary spice and a source of essential oil. In Ayurveda, it is commonly employed to alleviate abdominal discomfort, aid digestion, and address rheumatism.[24] Notably, research has revealed that the hydroalcoholic extract of dill seeds exhibits notable anti-inflammatory and antimicrobial properties.

The diverse array of properties exhibited by *Anethum graveolens* renders it an effective agent in combating periodontal diseases.

1. **Faiza Hasan et al 2021;** This study aimed to evaluate the efficacy of a local drug delivery system utilizing 1% CHX gel in patients with periodontal diseases. The 1% CHX gel was formulated and its physicochemical properties were assessed. The preparation involved the combination of 5 ml of chlorhexidine gluconate solution with carbopol 940 gel to produce 500 gm of 1% CHX gel. Preservatives such as methyl paraben, propyl paraben, and EDTA were incorporated, while triethanolamine was utilized to adjust the pH of the solution. Distilled water was added to achieve the final formulation of the 1% CHX gel. Clinical parameters including "pocket depth, clinical attachment level, tooth mobility, plaque index, gingival index, and bleeding on probing" were evaluated as part of the assessment process. Salivary biomarkers, including tumor necrosis factor-α, prostaglandin E2,

and nitric oxide levels, were measured using ELISA kits. Clinical parameters were assessed both before treatment initiation and after 4 weeks of treatment. The study's author concluded that the utilization of 1% CHX gel as a local drug delivery system exhibited high efficacy in reducing both gingivitis and periodontitis. This efficacy was attributed to the gel's ability to effectively retain the drug within the periodontal pocket for an extended duration.[27]

2. **Marcela Popa et al 2020;** The antimicrobial properties of essential oils extracted from "*Salvia officinalis* (sage), *Satureja hortensis* (summer savory), and *Anethum graveolens"* (dill) were analyzed. These evaluations targeted both gram-positive and gram-negative bacterial strains isolated from the oral cavity of individuals with periodontitis. The study examined the efficacy of the essential oils against bacterial growth in both planktonic and biofilm states, utilizing culture-based methodologies. Results indicated that certain essential oils demonstrated potent bactericidal and antibiofilm effects, with activity observed at concentrations ranging from 0.08 to 1.36 mg/mL. Flow cytometry was employed to delve into the potential mechanisms underlying the antibacterial activity of the tested essential oils (EOs). The findings confirmed that these EOs operate by inhibiting the activity of efflux pumps. Furthermore, the immunomodulatory effects of the three EOs were examined by analyzing gene expression profiles for pro- and anti-inflammatory cytokines in THP-1 cells. Overall, these results suggest that the EO from summer savory, and to a lesser extent, sage and dill EOs, may serve to inhibit bacteria implicated in oral plaque formation and reduce the expression of genes associated with inflammatory responses, as determined through cell culture assessments. [28]

3. **Randhir kumar et al 2019;** The objective of this study was to compare the effectiveness of an experimental local drug delivery system containing 1.5% chlorhexidine (comprising chlorhexidine digluconate 0.5% and chlorhexidine dihydrochloride 1%) with two other treatment modalities: scaling and root planing alone, and scaling and root planing combined with the experimental local drug delivery system. A total of 30 subjects diagnosed with chronic localized or generalized periodontitis were included in the study. Assessment parameters included "plaque index, gingival index, and pocket depth" measurements using William's graduated probe with an acrylic stent. Additionally, plaque samples were collected for microbiological analysis, specifically targeting coccoids, rods, and spirochetes under dark field microscopy. These clinical and microbiological parameters were documented at three time points: baseline (0 day), 30 days after treatment initiation, and 45 days post-treatment. Results revealed that the experimental material, when administered alongside scaling and root planing, exhibited greater efficacy compared to scaling and root planing alone, as assessed both clinically and microbiologically. This enhanced efficacy may be attributed to the antimicrobial and anti-plaque properties of the experimental material.[29]

4. **Sulthana A et al 2019;** The author highlighted a review article discussing local drug delivery (LDD) in periodontal disease treatment. Periodontitis, characterized as a multifactorial, immunomodulatory condition, primarily affects the supportive tissues around teeth. Traditional treatment methods include mechanical debridement and the administration of antimicrobial drugs.

The introduction of LDD systems represents a promising approach in periodontal disease management, showcasing improved clinical outcomes when employed alongside scaling and root planning. However, it's crucial to note that LDD alone is not sufficient as a standalone therapy. Consequently, research endeavors have concentrated on developing novel agents for utilization within the LDD system. Advancements in the medical field continually introduce new therapeutic agents and innovative modes of controlled drug delivery systems. A persistent challenge involves achieving adequate drug concentrations at specific target sites. Consequently, the introduction of local drug delivery (LDD) systems has emerged, offering the advantage of attaining sufficient drug concentrations at the target site. Moreover, LDD presents several advantages over systemic drug administration, including reduced dosage requirements, minimized side effects, and mitigated drug resistance. Consequently, LDD is commonly utilized as an adjunct to scaling and root planing in the treatment of periodontal diseases.[30]

5. **Derakhshan S. et al 2017;** The antibacterial activity of dill seed essential oil was assessed by measuring the inhibition zone diameter and determining the minimum inhibitory concentration (MIC) against key pathogenic bacteria, including “Vibrio cholerae, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus”. Additionally, the impact of sub-inhibitory concentrations of cumin seed alcoholic extract on the biofilm formation ability of K. pneumoniae was examined. Biofilms were cultivated on semi-glass lamellas and observed using a scanning electron microscope. Dill essential oil exhibited a noteworthy level of activity ranging from good to moderate against the strains tested. Particularly, it demonstrated the highest antibacterial efficacy against *S. aureus*, with an inhibition zone of 15 mm and an MIC of 0.62 mg/ml, as well as against *V. cholerae*, with an inhibition zone

of 14 mm and an MIC of 0.7 mg/ml. These findings underscore the presence of antimicrobial compounds within dill extract. Given the medicinal properties associated with this plant, there is significance in exploring promising herbs and novel chemical compounds for potential therapeutic applications.[31]

6. **K K Chahal et al 2017;** The literature review focused on evaluating the chemistry and biological activities of *Anethum graveolens* (dill) essential oil. It demonstrated strong efficacy against pathogens such as Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, Geotrichum candidum, and Rhodotorula glutinis, with inhibition zones ranging from 36 to 69 mm. It exhibited moderate effectiveness against Salmonella typhimurium with an inhibition zone of 26 mm. [32]

7. **Arunachalam R et al 2017;** The article evaluates different locally administered perioceutic agents in terms of their intended purpose, efficacy as a standalone treatment compared to scaling and root planing, and their potential to improve traditional therapy methods. Until now, the primary approach to periodontal treatment has been the mechanical removal of plaque and calculus deposits from both above and below the gumline. However, achieving complete elimination of these harmful agents becomes increasingly challenging as pocket depth increases. The use of intra-pocket drug administration through local drug delivery systems has demonstrated improved clinical outcomes when employed alongside traditional non-surgical periodontal therapy. This is because periodontal pockets retain gingival crevicular fluid, facilitating the controlled release of antimicrobials directly to the site. This advancement has propelled the field of perioceutics, which involves the utilization of antimicrobial and host-modulating agents for the betterment of the periodontium. In response to concerns about antibiotic resistance and side effects, researchers are now striving to minimize antibiotic usage. Instead, the current focus lies in the development of new local drug delivery systems, host-

modulating agents, antibodies, and biofilms, all aimed at achieving faster and safer results.[33]

8. **Eshwar et al. 2016;** The objective of this study was to assess and compare the efficacy of two mouth rinses—commercially available 0.2% chlorhexidine gluconate mouthrinse and dill seed oil mouthrinse—in reducing plaque levels and gingivitis. A randomized controlled, double-blind parallel-arm study was conducted over a period of 90 days involving 90 subjects. Participants were randomly assigned to two groups, and baseline data were collected using the Loe and Silness gingival index and Quigley Hein plaque index. All subjects underwent oral prophylaxis prior to the intervention. Participants were instructed to use the assigned mouth rinse, and follow-up assessments were conducted at 45 and 90days post-intervention to evaluate changes using the respective indices. The study revealed that there was no significant difference in gingival and plaque scores between the two mouth rinses from baseline to 45 days and 90 days. The conclusion drawn from these findings is that dill seed oil and Hexodent (0.2% chlorhexidine gluconate) mouthrinse exhibit similar effectiveness in reducing plaque and gingival inflammation.[34]

9. **Varga A 2016 et al;** The objective of this study was to examine the chemical makeup, antibacterial properties, and time-kill effects of essential oil extracted from dill seeds (Anethum graveolens). The chemical composition of the oil was identified using gas chromatography coupled with mass spectrometer detector. The antibacterial efficacy against Escherichia coli was assessed using the broth microdilution method, determining a minimum inhibitory concentration (MIC) of 56.81 µl/ml and a minimum bactericidal concentration (MBC) of 113.62 µl/ml. Additionally, a time-kill assay was conducted, revealing that the antimicrobial effectiveness of the essential oil was concentration-dependent over time.[35]

10. **Ruangamnart A et al 2015;** The aim of this study was to explore the chemical compositions and antibacterial properties of dill fruit essential oil cultivated in Thailand, specifically in the Thani province. Essential oils were extracted through hydro distillation and steam distillation methods, with a higher yield observed in hydro distillation. Gas chromatography coupled with mass spectrometry (GC-MS) revealed "dillapiole, D-carvone, and D-limonene as the primary constituents, alongside minor components such as β-pinene, β-myrcene, and myristicin." Results suggested that steam distillation is preferable due to its higher content of active compounds and lower levels of dillapiole, which is reported to be toxic to insects. The antibacterial activity against eleven microorganisms was assessed through micro-broth dilution assay, indicating that both types of dill oils exhibited activity against five bacteria with an average minimum inhibitory concentration (MIC) of 10 mg/ml. Furthermore, the major constituents, D-limonene and D-carvone, showed significant to moderate antibacterial activity against the tested microorganisms.[36]

11. **Ali et al 2014;** A comprehensive review on the pharmacological importance of Anethum graveolens, commonly known as dill, was conducted. Anethum graveolens is rich in various biologically active constituents. A long history of traditional use across various cultures. Pharmacological studies have demonstrated several beneficial effects of Anethum graveolens. These include antimicrobial properties, anti-inflammatory activity, analgesic effects, gastric mucosal protection, inhibition of gastric acid secretion, smooth muscle relaxation, hypolipidemic effects, and enhancement of progesterone concentration, among others. Both the essential oils and acetone extracts of Anethum graveolens have demonstrated significant antimicrobial activity against a broad spectrum of pathogens, including Staphylococcus aureus, Bacillus cereus, Enterococcus

faecalis, Listeria monocytogenes, Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis, Salmonella typhimurium, Shigella flexneri, Salmonella typhii, Pseudomonas aeruginosa, and Mycobacterium. Additionally, Anethum graveolens seed extracts have exhibited anti-ulcer activity and displayed moderate efficacy against Helicobacter pylori. Both aqueous and organic extracts of the seeds have shown potent antibacterial activity. [26]

12. **S. Jana et al 2010;** The review aimed to evaluate the properties of Anethum graveolens, commonly known as dill. With a history rooted in Ayurvedic medicine dating back to ancient times, dill has maintained popularity as both a culinary herb and a source of essential oil. Belonging to the aromatic and annual herb family Apiaceae, dill seeds are traditionally utilized in Ayurveda for their carminative, stomachic, and diuretic properties. Dill seeds and herbs contain various volatile components, with carvone being the predominant odorant in dill seeds and "α-phellandrene, limonene, dill ether, myristicin, coumarins, flavonoids, phenolic acids, and steroids" have also been isolated from dill seeds.[24]

13. **Badar et al 2008**; Essential oils were extracted from Anethum graveolens seeds using steam distillation. The seeds were placed in a retort, and pressurized steam was passed through them. The distillation flask temperature was maintained above 100ºC using a sand bath to prevent vapor condensation inside the flask. The steam and oil vapors mixture were then condensed and collected in a receiver, which was kept in ice water to prevent the evaporation of low-boiling constituents of the oil. The essential oil was subsequently extracted from the condensed distillate in a separator. Dill seeds essential oil and its various dilutions (1:10, 1:50, 1:100, and 1:200) were prepared in distilled water. The zones of inhibition for the 1:10, 1:50, and 1:100 dilutions were measured at 7 mm, 6 mm, and 4 mm, respectively, indicating antimicrobial activity. However, the antimicrobial activity was negative

against E. coli for the 1:200 dilution.[37]

14. **Cosyn et al 2005**; The systematic review focused on investigating the effects of subgingival chlorhexidine gel administration in the treatment of chronic periodontitis. An extensive search, including electronic databases and manual searches, was conducted to identify relevant studies examining the use of chlorhexidine gels either as a standalone treatment or as an adjunct to scaling and root planing. Eight studies meeting the inclusion criteria were ultimately selected for analysis. Given the considerable heterogeneity in study design and outcome variables measured across the selected studies, a qualitative data analysis was performed. The review found evidence indicating that subgingival chlorhexidine gel administration, when used as a monotherapy, leads to a temporary reduction in bleeding tendency upon probing. This clinical effect was found to coincide with relevant microbiological changes described in one study. The extent to which chemical effects contribute to these changes appears to be correlated with the frequency of gel administration. However, there is limited to no data indicating that the treatment outcome of scaling and root planing will benefit from the adjunctive subgingival administration of a chlorhexidine (CHX) gel.[38]

15. **Daneshmand et al 2002**; The present study aimed to compare the subgingival microbiota of periodontitis sites undergoing treatment with the chlorhexidine chip plus scaling and root planing versus scaling and root planing alone. Thirteen participants, consisting of seven males and six females with a mean age of 49 years, all diagnosed with moderate to advanced periodontitis, were enrolled in the study. In each patient, two bilateral pockets probing 6–7 mm in depth were randomly assigned to receive either treatment with the chlorhexidine chip in addition to scaling and root planing, or scaling and root planing alone. The subgingival placement of chlorhexidine chips followed the manufacturer's

instructions. Scaling and root planing procedures were performed using hand instruments for a minimum of 10 minutes per study tooth. Subgingival samples were collected using paper points at baseline, as well as at 2 weeks and 4 weeks post-treatment, for microbiological analysis. [39]

MATERIALS AND METHODS

ARMAMENTARIUM:

For extract preparation	***For MIC & MBC***
• *Anethum graveolens* seeds • 90% Ethanol • Distilled water • Grinder • Sieve • Weighing Scale • Beaker • Conical Flask • Mortar & Pestle • Whatman No. 1 filter paper	• Hydroethanolic extract of *Anethum graveolens* • *Aggregatibacter actinomycetemcomitans* ATCC 29522 (01175P) • *Porphyromonas gingivalis* ATCC 33277 (0912P) • *Fusobacterium nucleatum* ATCC 25586 (0328P) • Weighing Scale • DMSO • Eppendorf Tubes • Micropipettes (10 µL, 100 µL, 1000 µL) • BHI broth • Erythromycin • Vitamin K • Horse serum • Blood • BHI agar • Anaerobic incubator Jar • Platinum Inoculum loops • Resazurin reagent • Electric loop sterilizer

For gel preparation	***For antimicrobial activity of Anethum graveolens gel and Chlorhexidine gel.***
• Hydroethanolic extract of *Anethum graveolens* • Carbopol 940 • Tween 80 • Sodium Methyl paraben • Sodium Propyl paraben • Distilled water • Glycerin • Digital weighing scale • Glass funnel • Glass measuring cylinder • Pipette • Propylene glycol • Sodium benzoate • Triethanolamine	• Mueller Hinton agar • 90mm diameter petri plates • Sterile cork borer • *Anethum graveolens* gel • Chlorhexidine gel (Hexigel 1%) • Saline

SOURCE OF DATA

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.)

METHODOLOGY

1) **Extract preparation:** *Anethum graveolens* seeds (Figure 1) 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 (Figure 2) at 70ºC for 2 hours before being powdered. About 40g of *Anethum graveolens* powder (Figure 3) 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 (Figure 4). Subsequently, the filtrate was concentrated by evaporation using the New Brunswick Scientific Excella E24 Incubator Shaker Series until it reached the desired concentration (Figure 5). The extract was then filtered through Whatman No.1 filter paper (Figure 6). The extract was then evaporated using hot water bath (Figure 7). 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) **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. Subsequently, the turbidity of the actively growing bacterial culture in the broth was adjusted to meet the 0.5 McFarland standard guidelines.

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 (Figure 11). 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 (Figure 12). 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 13-15).

   Note: Separate 96 well plates were used for each organism i.e *A.a, P.g* and *F.n*

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 (Figure 10) for 12 hours. The minimum concentration at which the bacteria showed no growth was considered as the MBC value (Figure 16-18).

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.

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. (Figure 19)

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:

a) 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.

b) 0.5% of triethanolamine was added dropwise to the above mixture and stirred using high speed propeller stirrer at 1200rpm for 30 mins. (Figure 20)

c) The gel was then passed through UV irradiation for 20-30 minutes.

d) Then was transferred into an airtight container. (Figure 21)

The gel was stored at ambient temperature for future use.

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

6) **Agar well diffusion assay:** It 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*, *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°C. 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 (Figure 22-24). The diffusion assay was performed in triplicates for all the three micro-organisms.

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.

Figure 1: ***Anethum graveolens*** **seeds**

Figure 2: Hot air oven used for drying of ***Anethum graveolens*** **seeds**

Figure 3: ***Anethum graveolens*** **powder**

Figure 4: Powdered ***Anethum graveolens*** **seeds undergoing maceration**

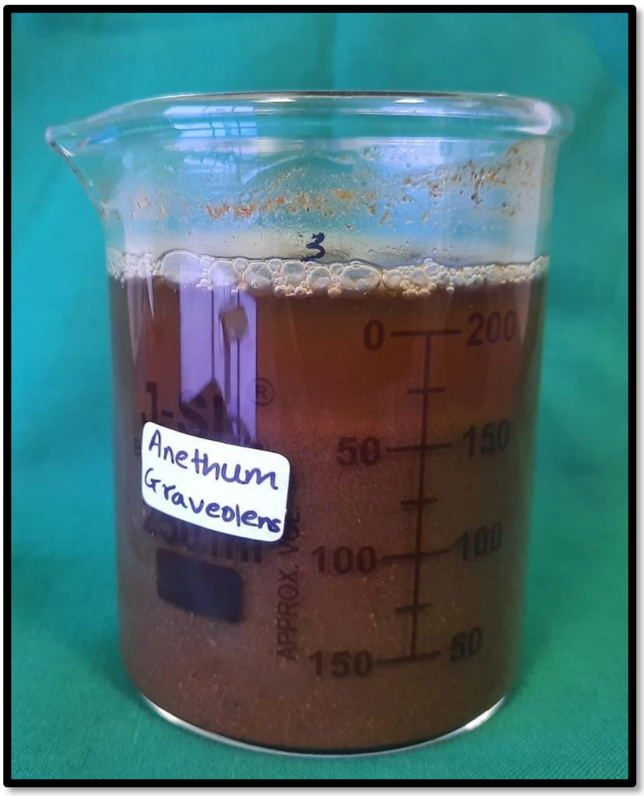


Figure 5: New Brunswick scientific Excella E24 Incubator shaker series

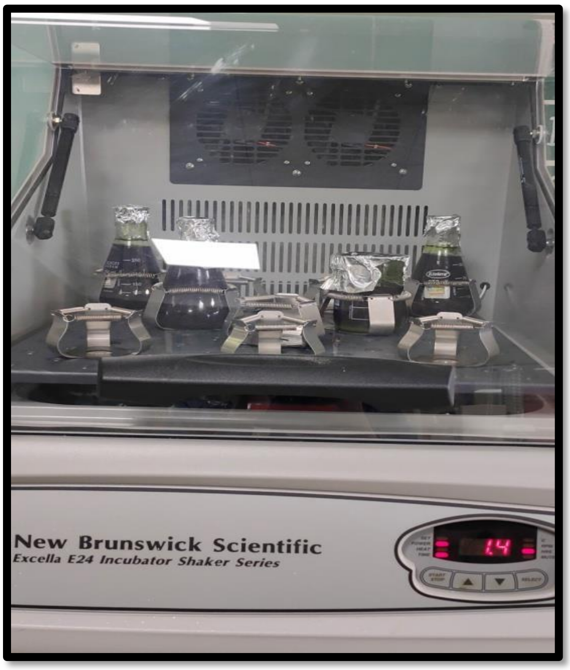


Figure 6: Crude extract filtration using Whatman No 1 filter paper

Figure 7: Water bath used for evaporation of *Anethum graveolens* extract.

Figure 8: Chemical reagents

Figure 9: Clinical armamentarium

Figure 10: Labotech Bacteriological Incubator

Figure 11: Laminar air flow

Figure 12: McIntosh Fildes anaerobic jar

Figure 13: Broth dilution method with resazurin test showing MIC of *Anethum graveolens* extract against *Aggregatibacter actinomycetemcomitans.*

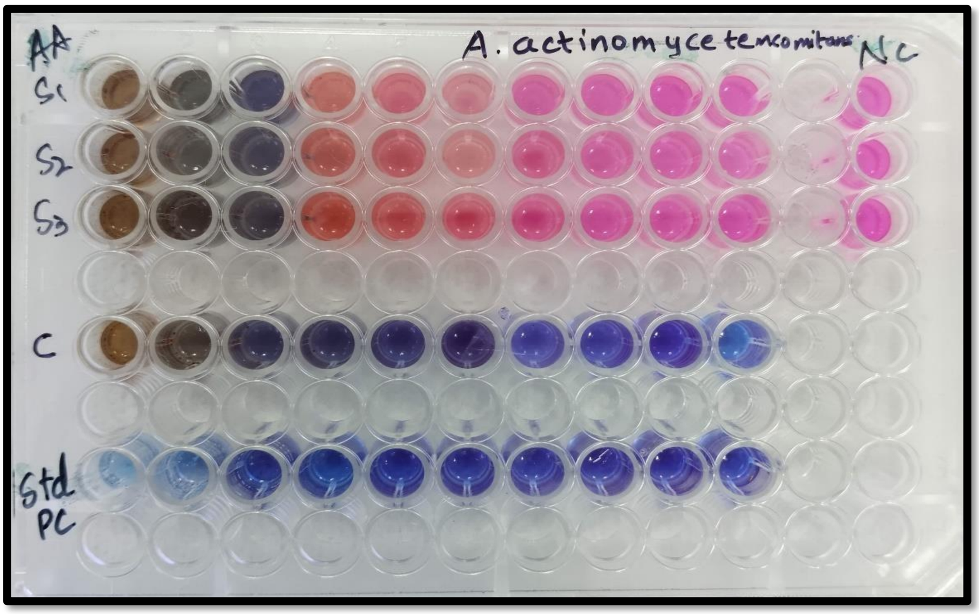


Figure 14: Broth dilution method with resazurin test showing MIC of *Anethum graveolens* extract against *Porphyromonas gingivalis.*

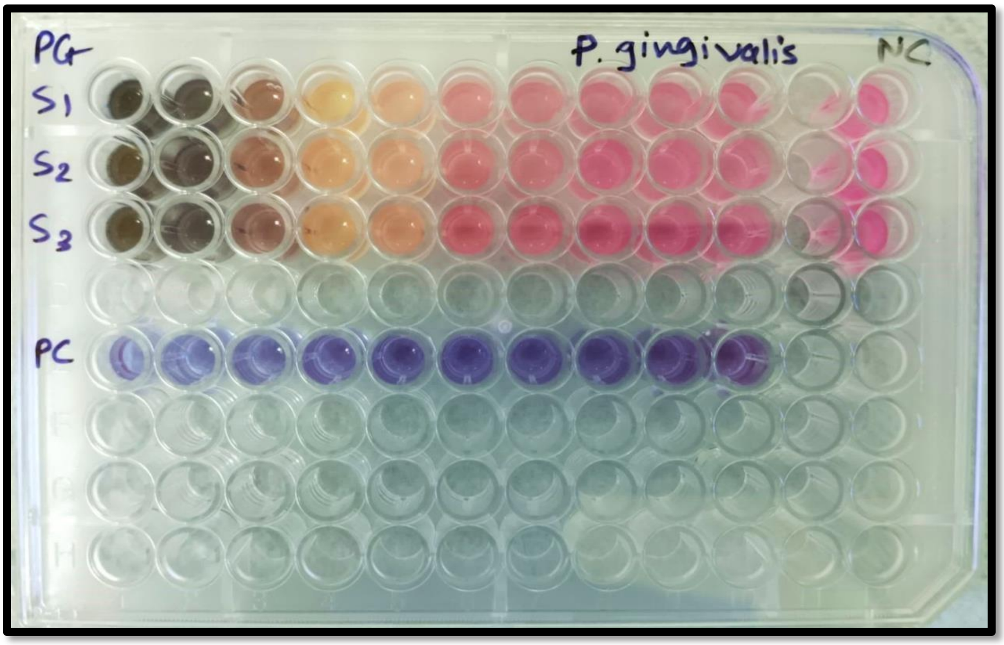


Figure 15: Broth dilution method with resazurin test showing MIC of *Anethum graveolens* extract against *Fusobacterium nucleatum.*

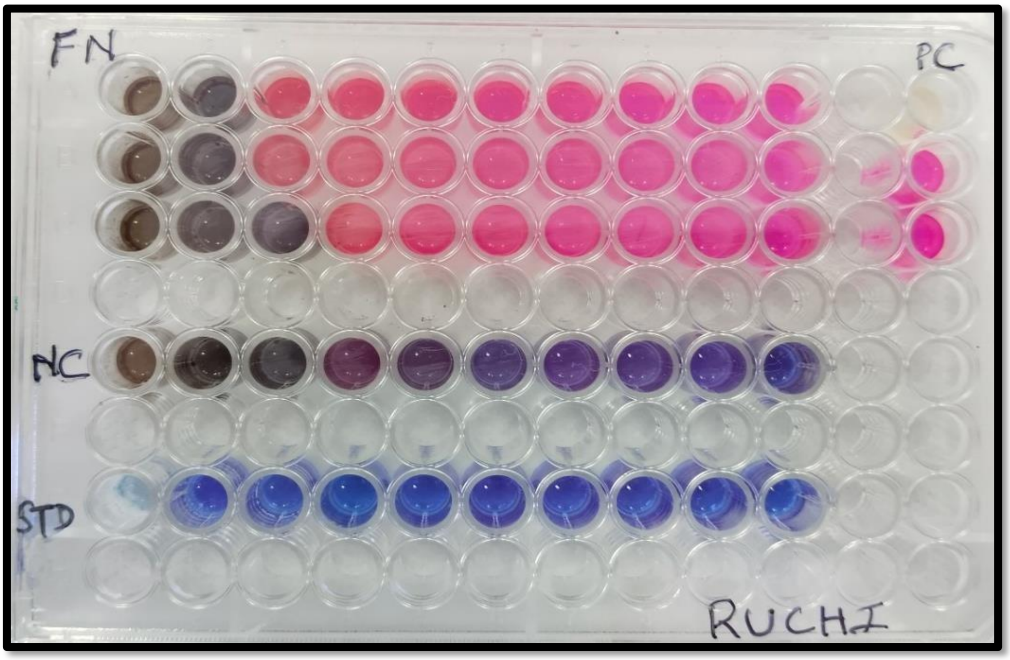


Figure 16: MBC of *Anethum graveolens* extract against *Aggregatibacter actinomycetemcomitans*

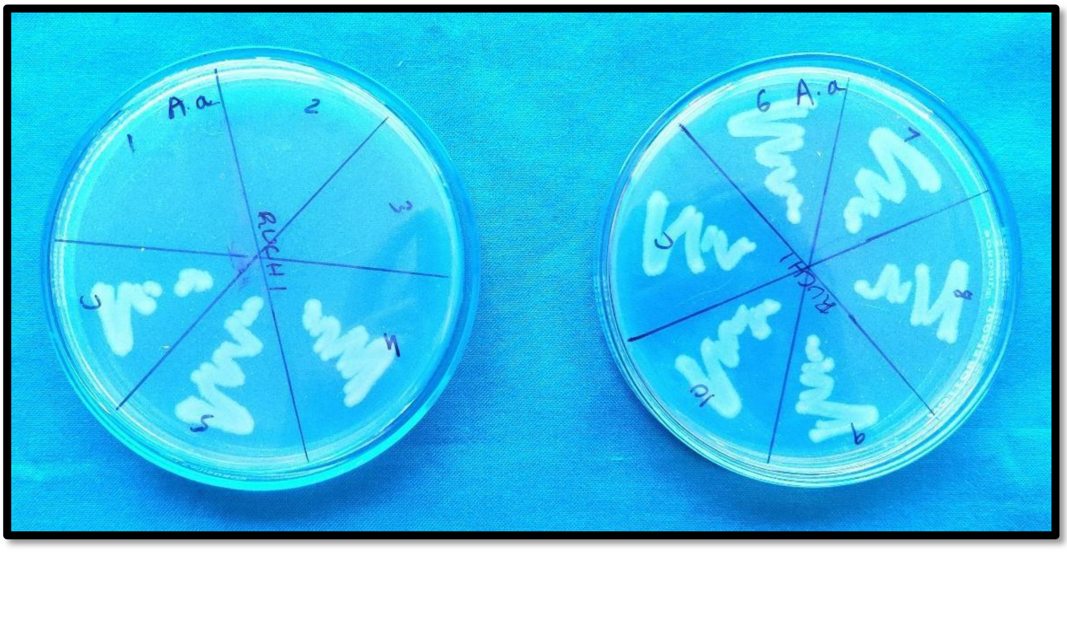


Figure 17: MBC of *Anethum graveolens* extract against *Porphyromonas gingivalis*

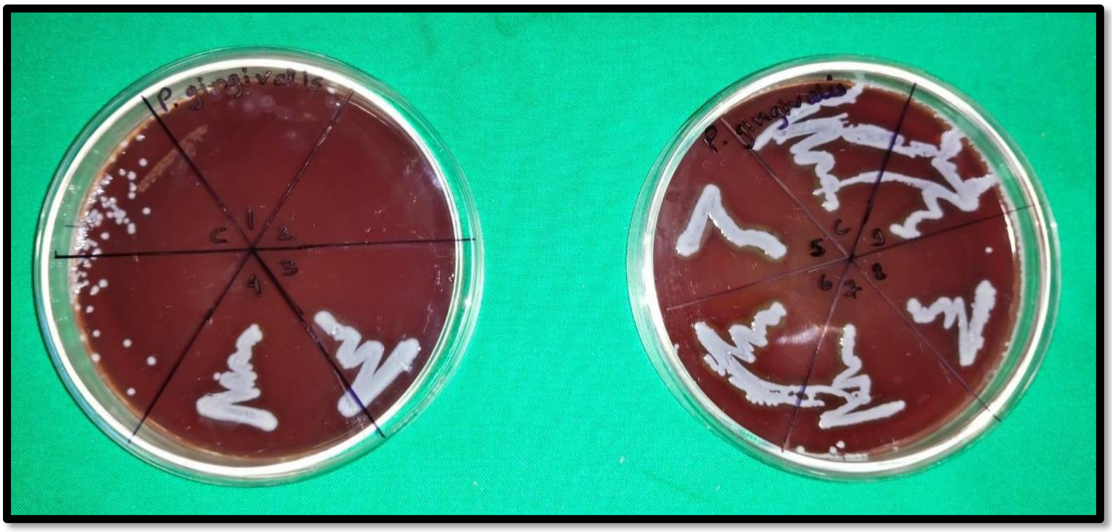


Figure 18: MBC of *Anethum graveolens* extract against *Fusobacterium nucleatum.*

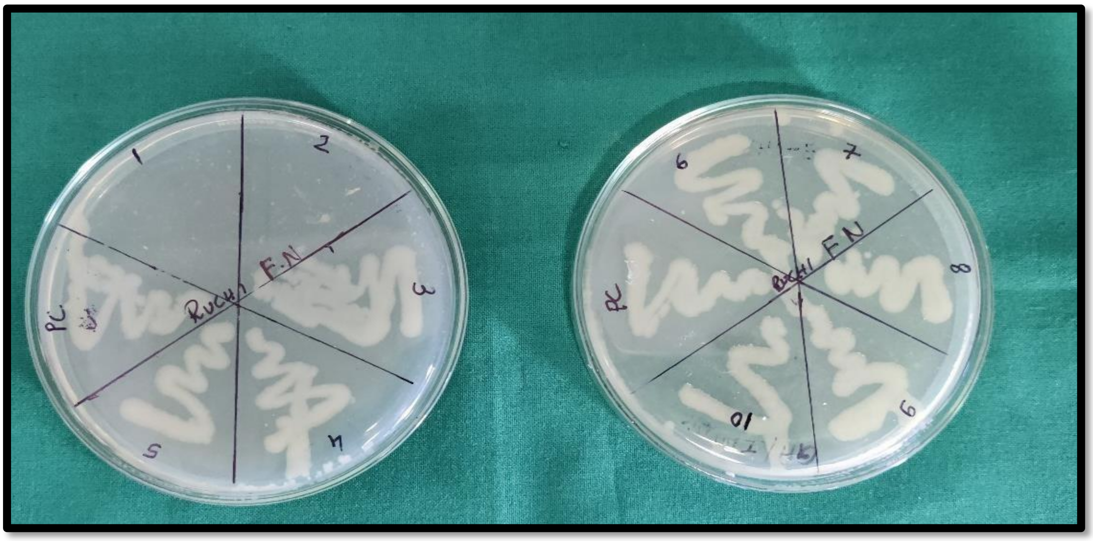


Figure 19: Magnetic stirrer

Figure 20: High speed propeller stirrer

Figure 21: *Anethum graveolens* gel

Figure 22: Agar well diffusion test for prepared *Anethum graveolens* gel and commercially available Chlorhexidine gel against *Aggregatibacter actinomycetemcomitans.*

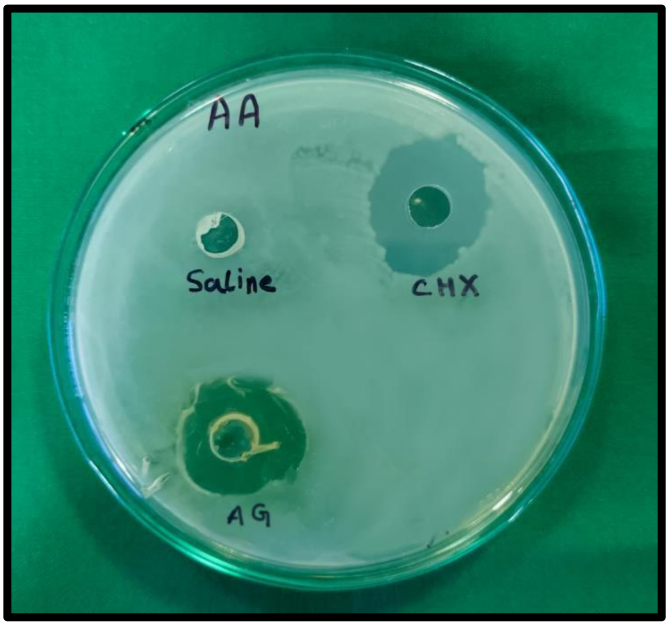


Figure 23: Agar well diffusion test for prepared *Anethum graveolens* gel and commercially available Chlorhexidine gel against *Porphyromonas gingivalis*

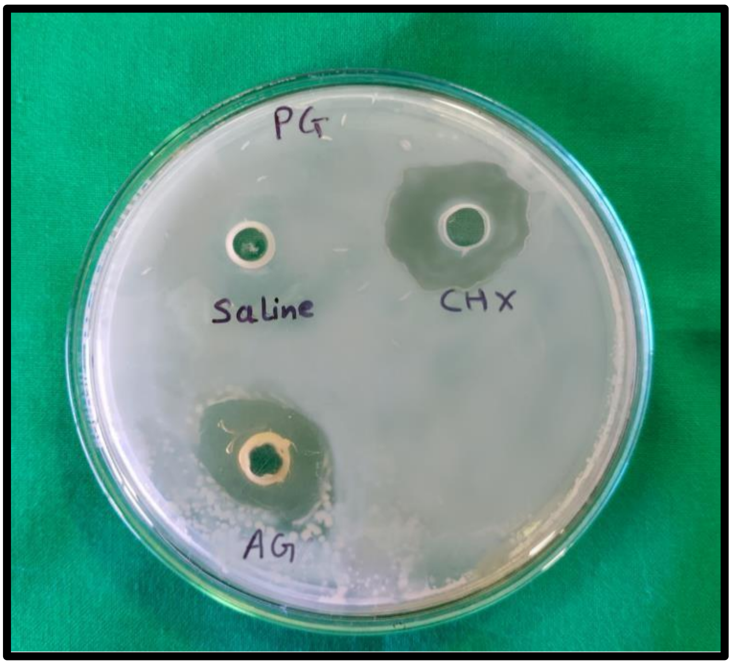
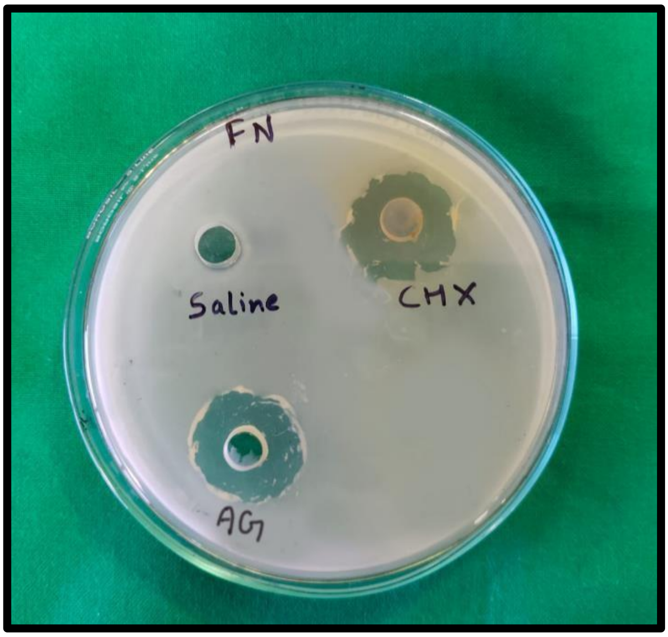


Figure 24: Agar well diffusion test for prepared *Anethum graveolens* gel and commercially available Chlorhexidine gel against *Fusobacterium nucleatum*



RESULTS AND OBSERVATIONS

MIC and MBC for *Anethum graveolens* extract was determined. The antimicrobial effects of 100 µl of 1% Chlorhexidine gel (positive control), 100 µl of *Anethum graveolens* gel (test) and 100 µl of Saline (negative control) was determined against standard ATCC strains namely- "*Porphyromonas gingivalis (P.g), Aggregatibacter actinomycetemcomitans (A.a)* and *Fusobacterium nucleatum (F.n)"* using agar well diffusion assay (zone of inhibition).

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

<table>
<tr><th>Extract Name</th><th colspan="2">A.a</th><th colspan="2">P.g</th><th colspan="2">F.n</th></tr>
<tr><td rowspan="3">Anethum graveolens</td><td>1.25</td><td rowspan="3">1.25</td><td>0.625</td><td rowspan="3">0.625</td><td>2.5</td><td rowspan="3">2.08</td></tr>
<tr><td>1.25</td><td>0.625</td><td>2.5</td></tr>
<tr><td>1.25</td><td>0.625</td><td>1.25</td></tr>
</table>

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

<u>Observations:</u> The Minimum inhibitory concentration of *Anethum graveolens* extract was 1.25 mg/ml against *A. actinomycetemcomitans*, 0.625 mg/ml against *P. gingivalis* and 2.08 mg/ml against *Fusobacterium nucelatum.*

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

<table>
<tr><th>Extract Name</th><th colspan="2">A.a</th><th colspan="2">P.g</th><th colspan="2">F.n</th></tr>
<tr><td rowspan="3">Anethum graveolens</td><td>1.25</td><td rowspan="3">1.25</td><td>2.5</td><td rowspan="3">2.5</td><td>2.5</td><td rowspan="3">2.5</td></tr>
<tr><td>1.25</td><td>2.5</td><td>2.5</td></tr>
<tr><td>1.25</td><td>2.5</td><td>2.5</td></tr>
</table>

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

Observations: The Minimum bactericidal concentration of *Anethum graveolens* extract was 1.25 mg/ml against *A. actinomycetemcomitans*, 2.5 mg/ml against *P. gingivalis* and 2.5 mg/ml against *Fusobacterium nucelatum*.

Table 3. 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

Observations: There was no growth noted w.r.t saline for all the three organisms. The zone of inhibition for Chlorhexidine gel was 15.6 mm for *A. actinomycetemcomitans,* 17 mm for *P. gingivalis*, and 15.3 mm for *F. nucleatum*. In comparison, for *A. graveolens* gel, it was 12.6 mm for *A. actinomycetemcomitans*, 13 mm for *P. gingivalis*, and 12 mm for *F. nucleatum.*

Table 4: Comparison of Three groups (Saline, 1% Chlorhexidine gel and *Anethum graveolen*s gel) against *A.a* using Kruskal Wallis ANOVA

Groups	Mean	SD	SE	Mean rank
Saline	0.00	0.00	0.00	2.00
1%Chlorhexidine gel	15.67	0.58	0.33	8.00
Anethum graveolens gel	12.67	1.53	0.88	5.00
H-value	7.5130			
p-value	0.0230*			
"Pair wise comparisons by Mann-Whitney U test"				
Saline vs1%Chlorhexidine gel	P=0.0809			
Saline vs Anethum graveolens	P=0.0809			
1%Chlorhexidine gel vs Anethum graveolens	P=0.0809			

*p<0.05

<u>Observations:</u> 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)."

Table 5: Comparison of Three groups (Saline, 1%Chlorhexidine gel and *Anethum graveolens gel*) against *P.g* using Kruskal Wallis ANOVA

Groups	Mean	SD	SE	Mean rank
Saline	0.00	0.00	0.00	2.00
1%Chlorhexidine gel	17.00	1.00	0.58	8.00
Anethum graveolens gel	13.00	1.00	0.58	5.00
H-value	7.4480			
p-value	0.0240*			
"Pair wise comparisons by Mann-Whitney U test"				
Saline vs1%Chlorhexidine gel	P=0.0636			
Saline vs Anethum graveolens	P=0.0636			
1%Chlorhexidine gel vs Anethum graveolens	P=0.0809			

*p<0.05

<u>Observations:</u> 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 6: Comparison of three groups (Saline, 1%Chlorhexidine gel and *Anethum* graveolens gel) against *F.n* using Kruskal Wallis ANOVA

Groups	Mean	SD	SE	Mean rank
Saline	0.00	0.00	0.00	2.00
1%Chlorhexidine gel	15.33	1.53	0.88	8.00
Anethum graveolens gel	12.00	1.00	0.58	5.00
H-value	7.4480			
p-value	0.0240*			
"Pair wise comparisons by Mann-Whitney U test"				
Saline vs 1%Chlorhexidine gel	P=0.0636			
Saline vs *Anethum graveolens*	P=0.0636			
1%Chlorhexidine gel vs *Anethum graveolens*	P=0.0809			

*p<0.05

Observations: 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 7: Comparison of three organisms (*A.a*, *P.g* and *F.n*) against saline (negative control) using Kruskal Wallis ANOVA

Organisms	Mean	SD	SE	Mean rank
A.a	0.00	0.00	0.00	5.00
P.g	0.00	0.00	0.00	5.00
F.n	0.00	0.00	0.00	5.00
H-value	0.0000			
p-value	1.0000			
"Pair wise comparisons by Mann-Whitney U test"				
A.a vs *P.g*	P=1.0000			
A.a vs *F.n*	P=1.0000			
P.g vs *F.n*	P=1.0000			

<u>Observations:</u> All the values for comparison of the three organisms (*A.a*, *P.g* and *F.n*) against saline was 0.

Table 8: Comparison of three organisms (*A.a, P.g* and *F.n*) against 1%Chlorhexidine gel (positive control) using Kruskal Wallis ANOVA

Organisms	Mean	SD	SE	Mean rank
A.a	15.67	0.58	0.33	4.17
P.g	17.00	1.00	0.58	7.17
F.n	15.33	1.53	0.88	3.67
H-value	3.0180			
p-value	0.2210			
"Pair wise comparisons by Mann-Whitney U test"				
A.a vs *P.g*	0.1904			
A.a vs *F.n*	0.8273			
P.g vs *F.n*	0.2752			

*p<0.05

<u>Observations:</u> The mean and standard deviation for *A.a* was 15.67 ±0.58, the mean and standard deviation for *P.g* was 17.00 ±1.00 and the mean and standard deviation for *F.n* was 15.33 ±1.53. The intergroup comparison of *A.a, P.g* and *F.n* against 1% Chlorhexidine gel showed no "statistically significant difference. (p=0.2210)"

Table 9: Comparison of three organisms (*A.a, P.g* and *F.n*) against *Anethum graveolens* gel (test) using Kruskal Wallis ANOVA

Organisms	Mean	SD	SE	Mean rank
A.a	12.67	1.53	0.88	5.33
P.g	13.00	1.00	0.58	6.00
F.n	12.00	1.00	0.58	3.67
H-value	1.2270			
p-value	0.5410			
"Pair wise comparisons by Mann-Whitney U test"				
A.a vs *P.g*	1.0000			
A.a vs *F.n*	0.6625			
P.g vs *F.n*	0.3827			

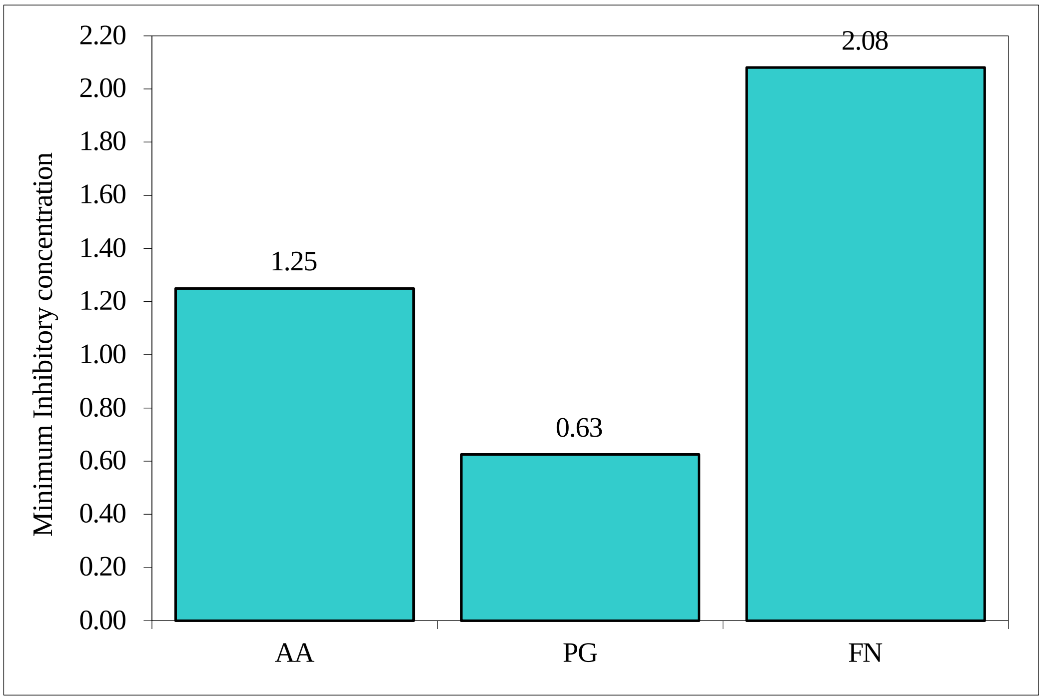
*p<0.05

<u>Observations:</u> The mean and standard deviation for *A.a* was 12.67 ±1.53, the mean and standard deviation for *P.g* was 13.00 ±1.00 and the mean and standard deviation for *F.n* was 12.00 ±1.00. The intergroup comparison of *A.a, P.g* and *F.n* against *Anethum graveolens* gel showed no "statistically significant difference. (p=0.5410)"

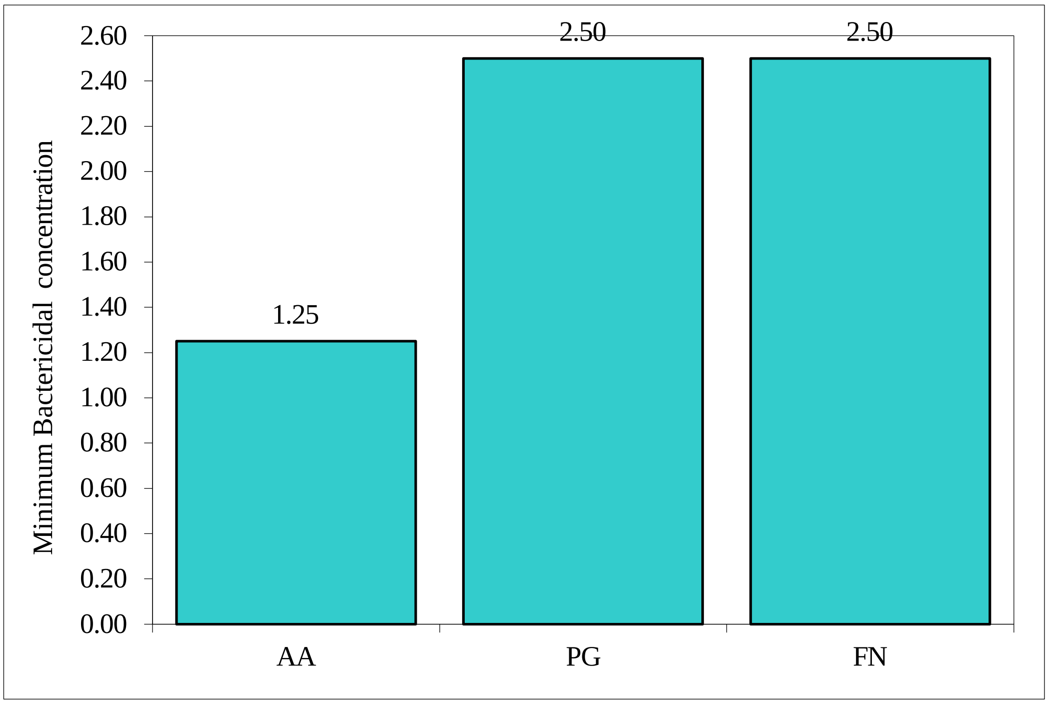
Table 10: Summary of Agar well diffusion (growth in mm) among three groups (Saline, 1% Chlorhexidine gel, *Anethum graveolens* gel) and three organisms (*A.a*, *P.g* and *F.n*)

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

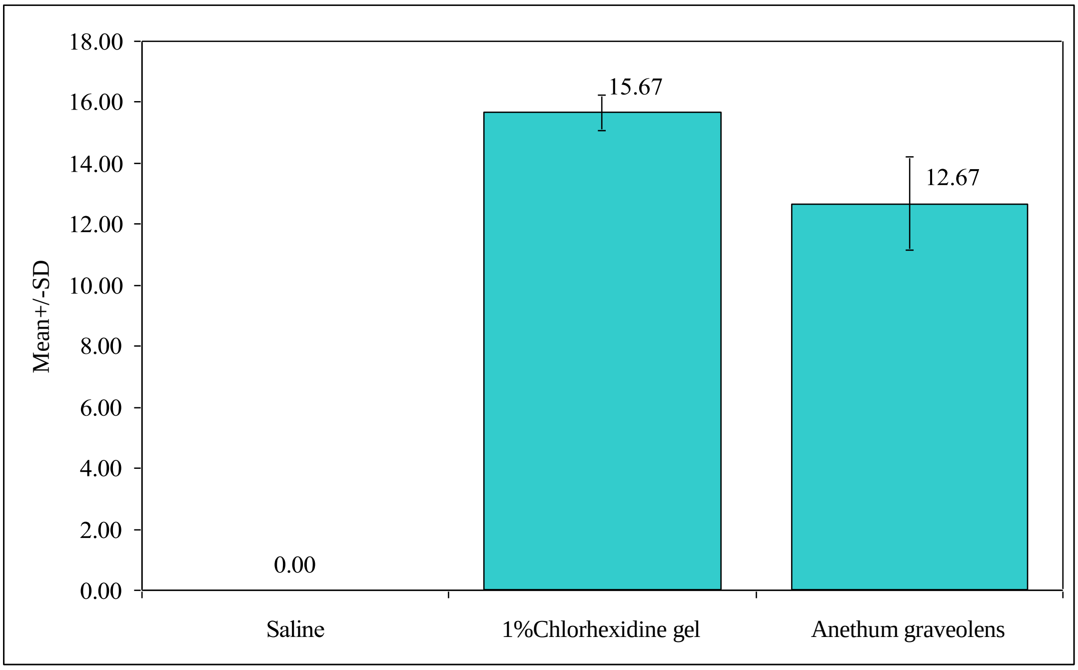
Graph 1: Comparison of Minimum Inhibitory concentration of *Anethum graveolens* extract against *A.a, P.g* and *F.n*



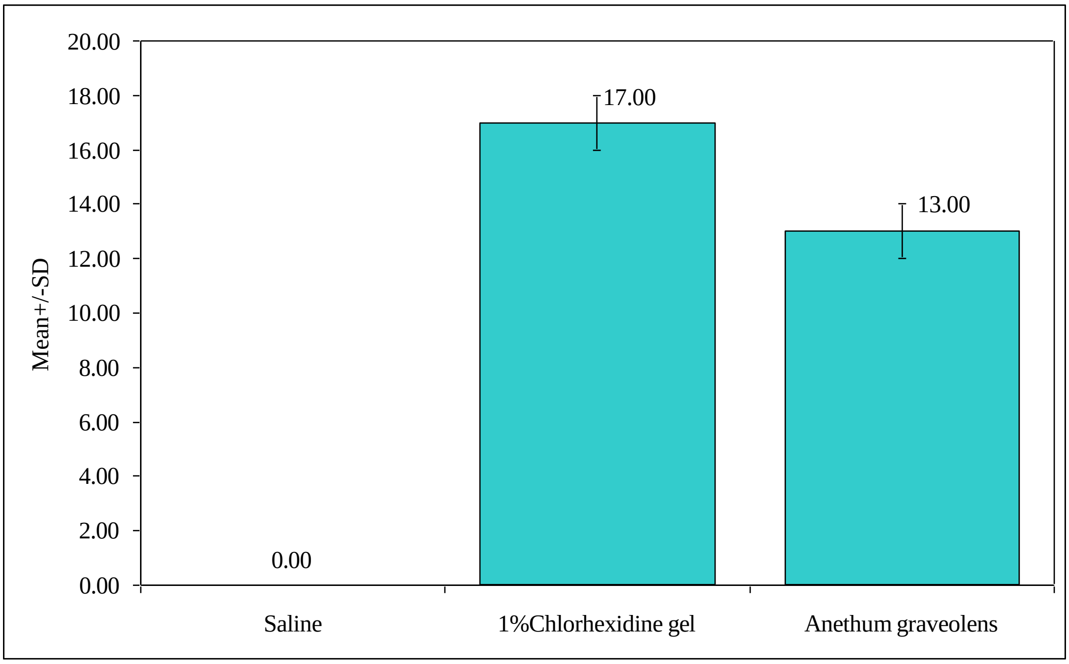
Graph 2: Comparison of Minimum Bactericidal concentration of *Anethum graveolens* extract against *A.a, P.g* and *F.n*



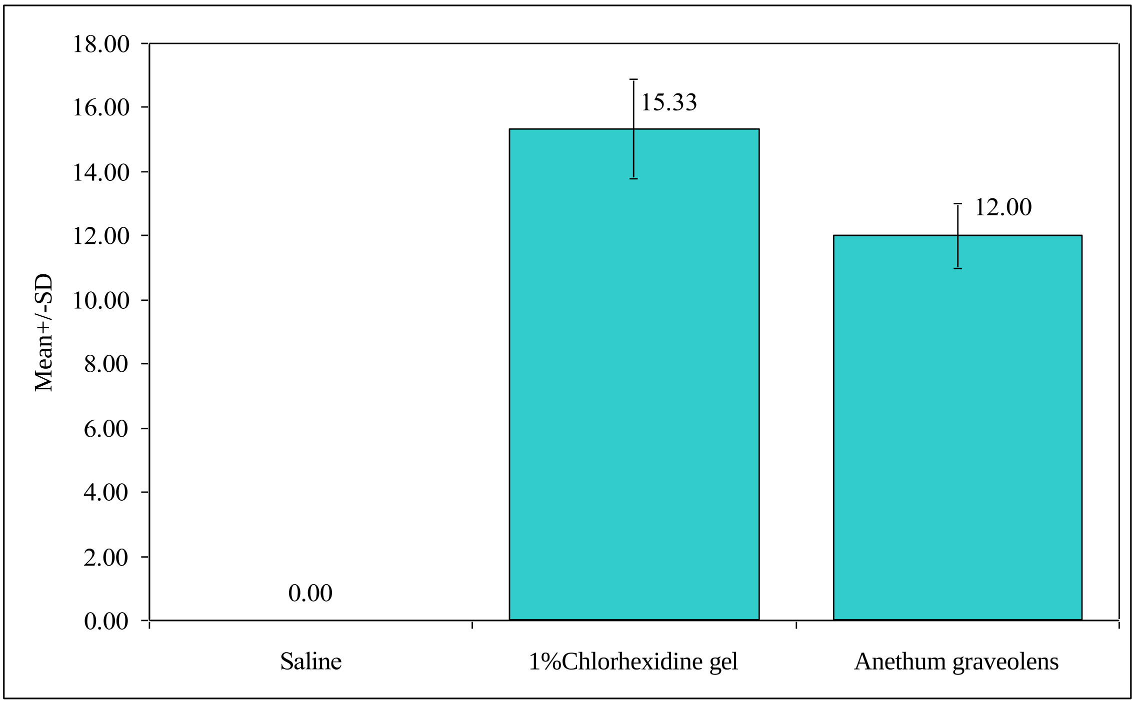
Graph 3: Comparison of three groups (Saline, 1%Chlorhexidine gel and *Anethum graveolens gel*) against *Aggregatibacter actinomycetemcomitans*.



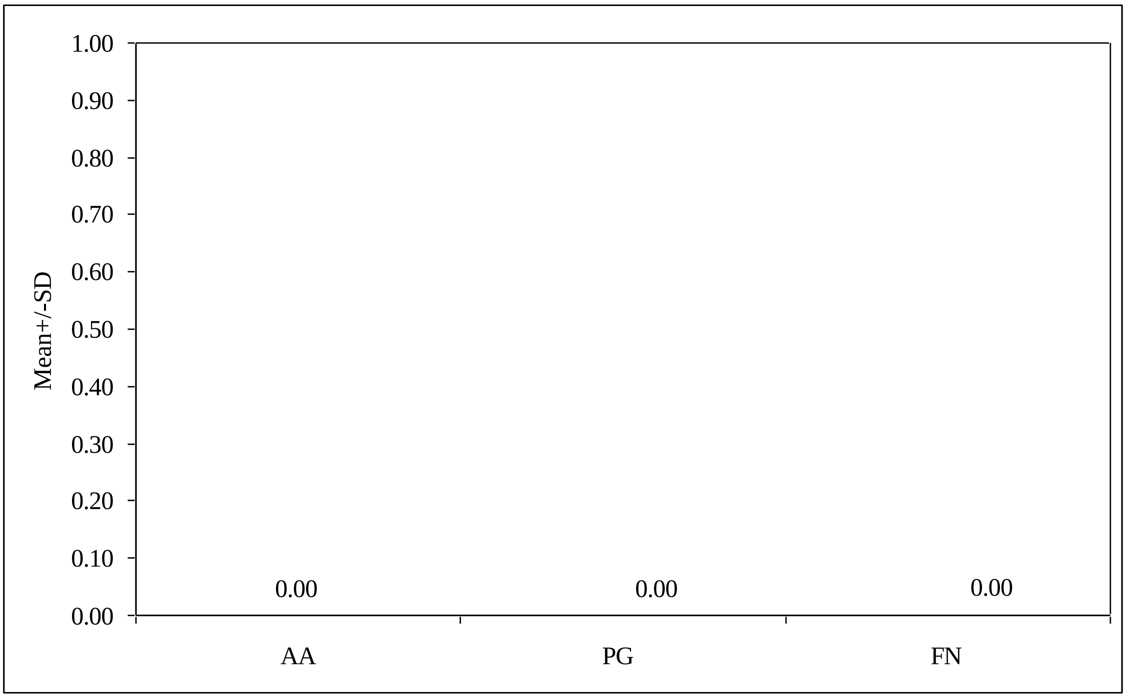
Graph 4: Comparison of three groups (Saline, 1%Chlorhexidine gel and *Anethum graveolens gel*) against *Porphyromonas gingivalis*



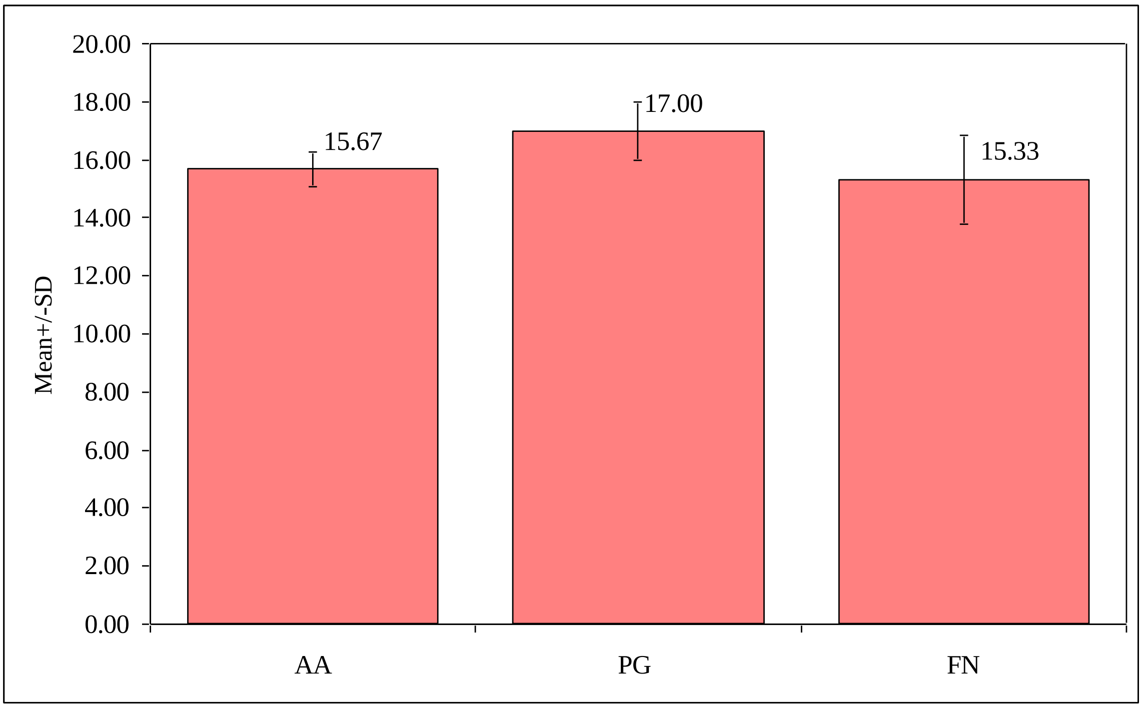
Graph 5: Comparison of Three groups (Saline, 1%Chlorhexidine gel and *Anethum graveolens gel*) against *Fusobacterium n*ucleatum.



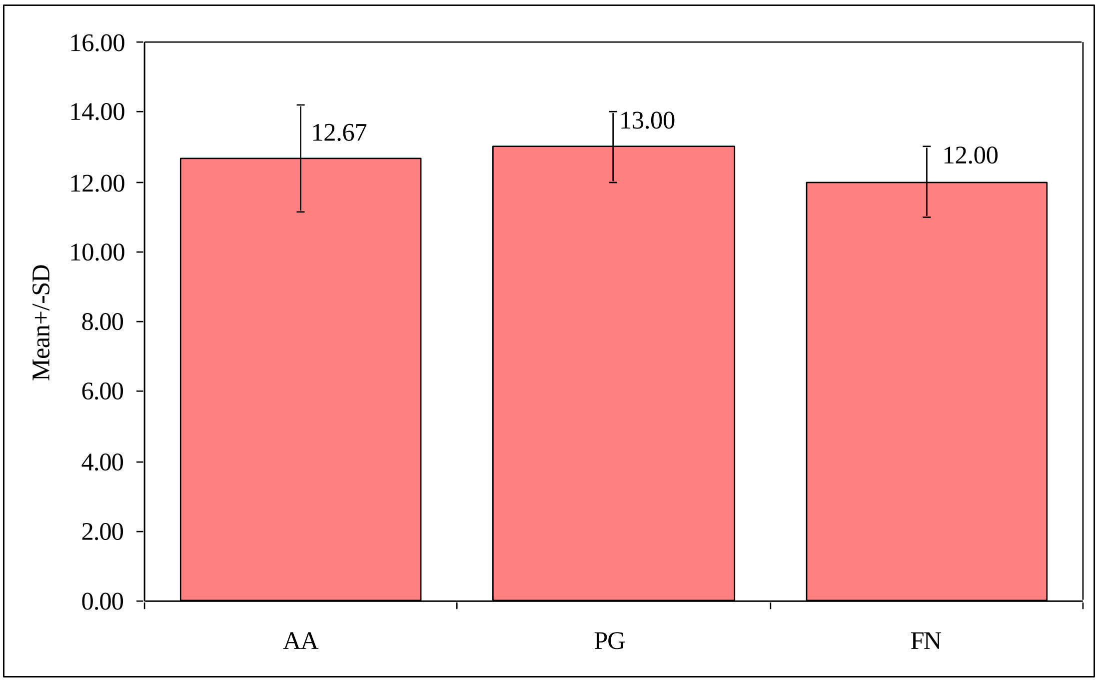
Graph 6: Comparison of Three organisms (*A.a, P.g* and *F.n*) against saline



Graph 7: Comparison of three organisms (*A.a*, *P.g* and *F.n*) against 1%Chlorhexidine gel



Graph 8: Comparison of three organisms (*A.a*, *P*.g and *F.n*) against *Anethum graveolens* gel



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.[40] Periodontitis correlates with elevated bacterial counts and a shift towards a higher presence of gram-negative species. Inflammation and periodontal tissue damage are attributed to the host's reaction to a microbial biofilm, predominantly comprising gram-negative pathogens.[41]

The primary bacteria linked to periodontal tissue degradation include "*P. gingivalis, A. actinomycetemcomitans, F. nucleatum, T. forsythia, P. intermedia, C. rectus, E. corrodens*, *Treponema*, and various Eubacterium species." Among them, *P. gingivalis* and *A. actinomycetemcomitans* are notable for their capacity to invade host tissues, strongly implicating them in severe periodontal breakdown. *F. nucleatum* serves as an intermediary colonizer, facilitating the attachment of commensal bacteria to both the tooth and epithelial surfaces, thus bridging the gap between these commensals and the true pathogens.[14]

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.[42]

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.[43]

The anatomy of periodontal pocket renders it an optimal site for the application of a local delivery system. Its irregular size and shape may vary, making it challenging to treat using conventional methods. Direct application to the pocket allows for the administration of drugs that cannot be taken orally due to systemic absorption limitations. An illustrative example is Chlorhexidine, an antiseptic drug with poor gastrointestinal absorption, which would not effectively reach the periodontal pocket if administered orally.[43]

Stabholz et al. observed a significant reduction in spirochetes and motile rods in the periodontal pocket after three days of exposure to sustained-release Chlorhexidine.[44] This effect is attributed to Chlorhexidine's binding to the negatively charged cell walls of microorganisms, altering their osmotic balance and resulting in cytoplasmic leakage or precipitation, ultimately leading to cell death or impaired function.

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. [16]

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. Mechanisms contributing to Chlorhexidine resistance in bacterial organisms include the presence of efflux pumps, as noted by Wassennar et al. in 2015, and mutations in the cell membrane structure.[45,46]

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. This compound has been utilized for generations as an alternative to synthetic drugs in the treatment of various ailments.

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. These compounds possess an aromatic nucleus with polar functional groups capable of forming hydrogen bonds with active sites on target enzymes.[24] These properties collectively make *A. graveolens* an effective agent in the treatment of periodontal diseases.

In the present study, we assessed the antimicrobial effectiveness of *A. graveolens* gel and compared it with the standard 1% Chlorhexidine gel. The Minimum Inhibitory Concentration (MIC) of the hydroethanolic extract of *A. graveolens* was found to be 1.25 mg/ml for *A. actinomycetemcomitans,* 0.625 mg/ml for *P. gingivalis* and 2.08 mg/ml for *F. nucleatum*. (Table 1) (Graph 1)

The Minimum Bactericidal Concentration (MBC) of the hydroethanolic extract of *A. graveolens* was 1.25 mg/ml for *A. actinomycetemcomitans*, 2.5 mg/ml for *P. gingivalis* and 2.5 mg/ml for *F. nucleatum*, as shown in (Table 2) (Graph 2). These findings underscore the significant antibacterial activity of the active constituents present in *A. graveolens*.

The antimicrobial effects of the prepared *A. graveolens* gel were evaluated using agar well diffusion assay. The zone of inhibition for Chlorhexidine gel was 15.6 mm for *A. actinomycetemcomitans,* 17 mm for *P. gingivalis,* and 15.3 mm for *F. nucleatum*. In comparison, for *A. graveolens* gel, it was 12.6 mm for *A. actinomycetemcomitans*, 13 mm for *P. gingivalis*, and 12 mm for *F. nucleatum* (Table 3). These results suggest that Chlorhexidine gel exhibited a wider zone of inhibition compared to *A. graveolens* gel. Although both Chlorhexidine gel and *A. graveolens* gel demonstrated comparable antibacterial activity, Chlorhexidine gel showed superior effectiveness.

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.

The intergroup comparison of saline, Chlorhexidine gel and *A. graveolens* gel was done using Kruskal Wallis ANOVA. The intergroup comparison of Saline, Chlorhexidine gel and *A. graveolens* gel for *A.a* showed statistically significant difference. ($p<0.05$) (Table 4) (Graph 3). The intergroup comparison of Saline, Chlorhexidine gel and Anethum graveolens gel for *P.g* showed statistically significant difference. ($p<0.05$) (Table 5) (Graph 4) The intergroup comparison of Saline, Chlorhexidine gel and *A. graveolens* gel for *F.n* also showed statistically significant difference. ($p<0.05$) (Table 6) (Graph 5)

The intergroup comparison of three organisms *A.a, P.g* and *F.n* was done using Kruskal Wallis ANOVA. There was no zone of inhibition noted with saline (control) group. (Table 7) (Graph 6) The intergroup comparison of three organisms *A.a, P.g* and *F.n* with Chlorhexidine gel showed no statistically significant difference.($p>0.05$) (Table 8) (Graph 7) The intergroup comparison of three organisms *A.a, P.g* and *F.n* with *A.graveolens* gel showed no statistically significant difference.($p>0.05$) (Table 9) (Graph 8)

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. [31]

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 s*pecies and Gram-negative bacteria such as E. *coli, Klebsiella pneumoniae*, and *P. aeruginosa"*.[32]

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.[32]

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.[34]

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.[37]

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. Thus, further investigation is essential to confirm and elucidate its clinical effectiveness against periodontal pathogens.

SUMMARY & CONCLUSION

The current study aimed to evaluate and compare the antimicrobial effectiveness of *Anethum graveolens* gel with Chlorhexidine gel against "*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum".*

The seeds of *Anethum graveolens* were procured and authenticated from "KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi." The *Anethum graveolens* gel utilized in the study was prepared and collected from "KAHER's KLE College of Pharmacy, Belagavi". Laboratory procedures were conducted at "KAHER's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi."

Hydroethanolic extract derived from *Anethum graveolens* seeds was prepared to determine the Minimum Inhibitory Concentration (MIC) using the Broth dilution method and the Minimum Bactericidal Concentration (MBC) using the streak method. *Anethum graveolens* gel was subsequently prepared from the extract's stock solution. For each of the bacteria *A.a, P.g* and *F.n,* three agar culture plates were prepared. On each plate, three uniform and aseptic wells were created: the first well contained saline (as negative control), the second well contained 1% Chlorhexidine gel (as positive control), and the third well contained *Anethum graveolens* gel (as test gel). An agar well diffusion assay was conducted to determine the zone of inhibition, aiding in assessing the susceptibility of *A.a, P.g* and *F.n* to both *Anethum graveolens gel* and Chlorhexidine gel (1%).

In light of the observations drawn from our study, the following conclusions can be made,

1. Minimum Inhibitory Concentration (MIC) of hydroethanolic extract of *Anethum graveolens was* 1.25 mg/ml for *Aggregatibacter actinomycetemcomitans*, 0.625mg/ml for *Porphyromonas gingivalis* and 2.08mg/ml for *Fusobacterium nucleatum*.

2. Minimum Bactericidal Concentration (MBC) of hydroethanolic extract of *Anethum graveolens was* 1.25 mg/ml for *Aggregatibacter actinomycetemcomitans*, 2.5 mg/ml for *Porphyromonas gingivalis* and 2.5 mg/ml for *Fusobacterium nucleatum.*

3. These results showed that the active constituents of *Anethum graveolens* had significant antimicrobial activity.

4. The zone of inhibition for Chlorhexidine gel (positive control group) was 15.6 mm for *Aggregatibacter actinomycetemcomitans*, 17mm for *Porphyromonas gingivalis* and 15.3mm for *Fusobacterium nucleatum*, whereas for *Anethum graveolens* gel (test gel) it was 12.6 mm for *Aggregatibacter actinomycetemcomitan*, 13mm for *Porphyromonas gingivalis* and 12 mm for *Fusobacterium nucleatum.*

The results of the current study suggest that the prepared *A. graveolens* gel demonstrated potent antimicrobial activity against the selected periodontal pathogens. But superior antimicrobial activity was noted with Chlorhexidine gel when compared to *Anethum graveolens* gel against *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

BIBLIOGRAPHY

1. Listgarten MA. Pathogenesis of periodontitis. Journal of clinical periodontology. 1986 May;13(5):418-25.

2. Papapanou PN, Sanz M Et al. Periodontitis: Consensus report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45 (Suppl 20): S162–S170.

3. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015 Jan;15(1):30-44.

4. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. Journal of dental research. 2012 Oct;91(10):914-20.

5. Mehrotra N, Singh S. Periodontitis. InStatPearls [Internet] 2022 May 8. StatPearls Publishing.

6. Rajeshwari HR, Dhamecha D, Jagwani S, Patil D, Hegde S, Potdar R, Metgud R, Jalalpure S, Roy S, Jadhav K, Tiwari NK. Formulation of thermoreversible gel of cranberry juice concentrate: Evaluation, biocompatibility studies and its antimicrobial activity against periodontal pathogens. Materials Science and Engineering: C. 2017 Jun 1;75:1506-14.

7. Haffajee AD, Socransky SS. Attachment level changes in destructive periodontal diseases. Journal of clinical periodontology. 1986 May;13(5) :461-72.

8. Frank RM. Bacterial penetration in the apical pocket wall of advanced human periodontitis. Journal of periodontal research. 1980 Dec;15(6):563-73.

9. Frank RM, Voegel JC. Bacterial bone resorption in advanced cases of human periodontitis. Journal of periodontal research. 1978 Jun;13(3):251-61.

10. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Nature Reviews Microbiology. 2012 Oct;10(10):717-25.

11. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. Journal of clinical pathology. 2008 May 1;61(5):577-87.

12. Christersson LA, Albini B, Zambon JJ, Wikesjö UM, Genco RJ. Tissue localization of Actinobacillus actinomycetemcomitans in human periodontitis: I. Light, immunofluorescence and electron microscopic studies. Journal of periodontology. 1987 Aug;58(8):529-39. 25)

13. Åberg CH, Kelk P, Johansson A. Aggregatibacter actinomycetemcomitans: virulence of its leukotoxin and association with aggressive periodontitis. Virulence. 2015 Apr 3;6(3):188-95.

14. Signat B, Roques C, Poulet P, Duffaut D. Role of Fusobacterium nucleatum in periodontal health and disease. Current issues in molecular biology. 2011 Jul;13(2):25-36.

15. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Current opinion in microbiology. 2015 Feb 1;23:141-7.

16. Szulc M, Zakrzewska A, Zborowski J. Local drug delivery in periodontitis treatment: A review of contemporary literature. Dental and medical problems. 2018 Jul 1;55(3):333-42.

17. Goodson JM, Cugini MA, Kent RL, Armitage GC, Cobb CM, Fine D, Fritz ME, Green E, Imoberdorf MJ, Killoy WJ, Mendieta C. Multicenter evaluation of

tetracycline fiber therapy: I. Experimental design, methods, and baseline data. Journal of periodontal research. 1991 Jul;26(4):361-70.

18. Brecx MC, Liechti T, Widmer J, Gehr P, Lang NP. Histological and clinical parameters of human gingiva following 3 weeks of chemical (chlorhexidine) or mechanical plaque control. Journal of clinical periodontology. 1989 Mar;16(3):150-5.

19. Goodson JM, Haffajee A, Socransky SS. Periodontal therapy by local delivery of tetracycline. Journal of Clinical Periodontology. 1979 Apr;6(2):83-92.

20. Greenstein G. Local drug delivery in the treatment of periodontal diseases: assessing the clinical significance of the results. Journal of periodontology. 2006 Apr;77(4):565-78.

21. Jones CG. Chlorhexidine: is it still the gold standard?. Periodontology 2000. 1997 Oct 1;15:55-62.

22. Sant a, dave d, patel p, zaver h. Omparative evaluation of anti-microbial efficacy of curcuma oral gel and chlorhexidine gel on the periodontal pathogens: an in-vitro study. Indo-European Journal of Dental Therapy and Research. 2017 Jan 1;5(1):86-389.

23. Kazemi M, Abdossi V. Chemical composition of the essential oils of Anethum graveolens L. Bangladesh Journal of Botany. 2015 Mar 26;44(1):159-61.

24. Jana S, Shekhawat GS. Anethum graveolens: An Indian traditional medicinal herb and spice. Pharmacogn Rev. 2010 Jul;4(8):179-84.

25. Hamza LF. Anethum graveolens: Physicochemical properties, medicinal uses, antimicrobial effects, antioxidant effect, anti-inflammatory and analgesic effects: A review. world. 2017;5(7).

26. Al-Snafi AE. The pharmacological importance of Anethum graveolens–A review. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(4):11-3.

27. Hasan F, Ikram R, Adel A, Abbas A, Ain Bukhari QU, Asadullah K. Treatment of periodontal diseases by the local drug delivery system using 1% chlorhexidine gel: A randomized clinical trial. Pak J Pharm Sci. 2021 Jan;34(1):41-45.

28. Popa M, Măruțescu L, Oprea E, Bleotu C, Kamerzan C, Chifiriuc MC, Grădișteanu Pircalabioru G. In vitro evaluation of the antimicrobial and immunomodulatory activity of culinary herb essential oils as potential perioceutics. Antibiotics. 2020 Jul 21;9(7):428.

29. Kumar R, Sharma G. Therapeutic evaluation of local drug delivery system containing 1.5% chlorhexidine used as an adjunct to scaling and root planing for the treatment of chronic periodontitis-A clinical and microbiologcal study. IP Int J Periodontol Implantol. 2019;4(3):103-6.

30. Sulthana A, Arun RT, Krishnaraj S, Sundaram R. Local drug delivery in the treatment of periodontal diseases. International Journal of Orofacial Biology. 2019 Jul 1;3(2):35.

31. Derakhshan S, Navidinia M, Ahmadi A. Antibacterial activity of Dill (Anethum graveolens) essential oil and antibiofilm activity of Cumin (Cuminum

cyminum) alcoholic extract. Infection Epidemiology and Microbiology. 2017 Dec 10;3(4):122-6.

32. Chahal KK, Kumar A, Bhardwaj U, Kaur R. Chemistry and biological activities of Anethum graveolens L.(dill) essential oil: A review. Journal of Pharmacognosy and Phytochemistry. 2017;6(2):295-306.

33. Arunachalam R, Rajeev V, Vedam V, Ganapathy S, Dhanavel J. Perioceutics in the management of Periodontal Disease. Journal of Young Pharmacists. 2017;9(1):08.

34. Eshwar S, Rekha K, Jain V, Manvi S, Kohli S, Bhatia S. Suppl-1, M8: Comparison of Dill Seed Oil Mouth Rinse and Chlorhexidine Mouth Rinse on Plaque Levels and Gingivitis-A Double Blind Randomized Clinical Trial. The open dentistry journal.2016;10:207.

35. Varga A, Aćimović M, Čabarkapa I, Filipčev B, Plavšić D, Nježić Z. Anethum graveolens seed oil: antibacterial activity against Escherichia coli. InProceedings of III International Congress Food Technology, Quality and Safety 2016. Institute of Food Technology, Novi Sad (Serbia).

36. Ruangamnart A, Buranaphalin S, Temsiririrkkul R, Chuakul W, Pratuangdejkul J. Chemical compositions and antibacterial activity of essential oil from dill fruits (Anethum graveolens L.) cultivated in Thailand. Mahidol Univ J Pharm Sci. 2015;42(3):135-43.

37. Badar NA, Arshad MU, Farooq UM. Characteristics of Anethum graveolens (Umbelliferae) seed oil: Extraction, composition and antimicrobial activity. International Journal of Agriculture and Biology. 2008;10(3):329-32.

38. Cosyn, Jan; Sabzevar, Mehran Moradi (2005). A Systematic Review on the Effects of Subgingival Chlorhexidine Gel Administration in the Treatment of Chronic Periodontitis. Journal of Periodontology, 76(11).

39. Daneshmand N, Jorgensen MG, Nowzari H, Morrison JL, Slots J. Initial effect of controlled release chlorhexidine on subgingival microorganisms. Journal of periodontal research. 2002 Oct;37(5):375-9.

40. Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006 Jun 15;6 Suppl 1(Suppl 1):S14.

41. Else Theilade; W. H. Wright; S. Börglum Jensen; Harald Löe (1966). Experimental gingivitis in man: II. A Longitudinal Clinical and Bacteriological Investigation

42. Dental Scaling and Root Planing for Periodontal Health: A Review of the Clinical Effectiveness, Cost-effectiveness, and Guidelines [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2016 Oct 17.

43. Steinberg D, Friedman M. Sustained-release delivery of antimicrobial drugs for the treatment of periodontal diseases: Fantasy or already reality? Periodontology 2000. 2020 Oct;84(1):176-87.

44. Stabholz A, Sela MN, Friedman M, Golomb G, Soskolne A. Clinical and microbiological effects of sustained release chlorhexidine in periodontal pockets. J Clin Periodontol. 1986 Sep;13(8):783-8.

45. Poppolo Deus F, Ouanounou A. Chlorhexidine in Dentistry: Pharmacology, Uses, and Adverse Effects. Int Dent J. 2022 Jun;72(3):269-277. Epub 2022 Mar 12.

46. Cieplik F, Jakubovics NS, Buchalla W, Maisch T, Hellwig E, Al-Ahmad A. Resistance Toward Chlorhexidine in Oral Bacteria - Is There Cause for Concern? Front Microbiol. 2019 Mar 22;10:587.

ANNEXURE – I – ETHICAL CLEARANCE LETTER

Research and Ethics Committee

KLE VK INSTITUTE OF DENTAL SCIENCES

A Constituent Unit of KLE Academy of Higher Education & Research

Accredited 'A' Grade by NAAC   Placed in Category 'A' by MHRD (GoI)

Nehru Nagar, Belagavi - 590 010, Karnataka State

☎: 0831-2470362   Web: http://www.kledental-bgm.edu.in

FAX: 0831-2470640   E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1573

CERTIFICATE

This is to Certify that the synopsis titled Comparative evaluation of the antimicrobial efficacy of anethum graveolens gel with chlorexidine gel against aggregatibacter actinomycetemcomitans, porphyromonas gingivalis and Fusobacterium nucleatum An in vitro study *Submitted by Dr.* ________ *P. G. Student / Staff, Guided by* ________ *from Department of* Dept of Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 23/3/24

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI.

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE – II – DRUG AUTHENTICATION CERTIFICATE

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

SHRI B.M.K. AYURVEDA MAHAVIDYALAYA

A constituent unit of KLE Academy of Higher Education & Research

Deemed-to-be-University

Central Research Facility

DRUG AUTHENTICATION REPORT

Outward No:-BMK/CRF/125/2022-23

Submitted By: K L E Ayurved Pharmacy

Submitted Date: 09//03/2022

Date of Issue: 12/03/2022

Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
					Common Name	Scientific Name	Family	Part Authenticated
Shatapushpa	*Anethum sowa* Roxb.	Apiaceae	Fruit	CRF/Auth/83/2022	Shatapushpa	*Anethum sowa* Roxb.	Apiaceae	Fruit

Signature:

Authentication Expert Name: Dr. Divya Khare

Date 12/03/2022

CRF SHAHAPUR, BELGAUM — Shri B. M. Kankanwadi Ayurved Mahavidyalaya K.L.E University

Signature of Coordinator

ASU Drug Testing Laboratory

ANNEXURE – III – BSRC REPORT

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA.
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; Web: www.klepkbsrc.org, Phone: 0831- 2444444, Extn. 4122
GSTIN/UIN: 29AABTK0881E1ZN

Report

TITLE OF RESEARCH: "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.

NAME OF THE STUDENT:

NAME OF THE GUIDE:

NAME OF THE CO-GUIDE:

Objective parameters:

1. Assessment of MIC of ethanolic extract of Anethum graveolens on *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*
2. Assessment of MBC of ethanolic extract of Anethum graveolens on *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*
3. To assess and compare the antibacterial efficacy of *Anethum graveolens* on *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

Laboratory Methods for assessing microbiome:

1. **Bacterial Culture:** *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum.*
2. **Method used:** Minimum inhibitory concentration, Minimum bactericidal concentration, Agar well diffusion.
3. **Extract concentration: Stock:** 200mg/10ml, **Working:** 5mg/ml

Dr. Prabhakar Kore Basic Science Research Center (BSRC) ★ KLE Academy of Higher Education and Research, Belagavi.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA.
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; Web: www.klepkbsrc.org, Phone: 0831- 2444444, Extn. 4122
GSTIN/UIN: 29AABTK0881E1ZN

Lab Investigations done in BSRC

Minimum Inhibitory concentration

Extract Name	AA		PG		FN	
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.

Minimum Bactericidal concentration

Extract Name	AA		PG		FN	
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.

Agar well diffusion

Groups	AA			PG			FN		
Saline	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
1%Chlorhexidine gel	16mm	16mm	15mm	17mm	18mm	16mm	15mm	17mm	14mm
Anethum graveolens	14mm	13mm	11mm	13mm	14mm	12mm	11mm	12mm	13mm

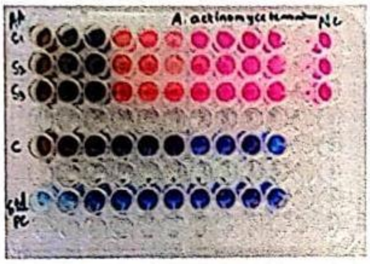
NZ: No Zone of Inhibition
mm: millimeter

Remarks

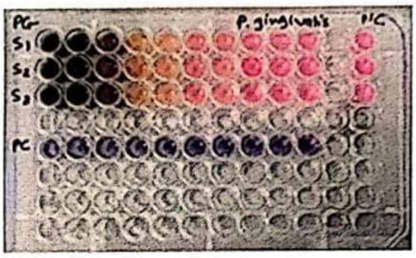
The results are satisfactory and relevant references have been followed.

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA.
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; Web: www.klepkbsrc.org, Phone: 0831- 2444444, Extn. 4122
GSTIN/UIN: 29AABTK0881E1ZN

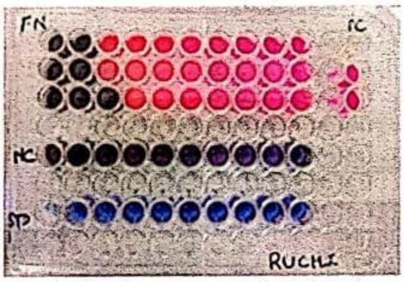
Minimum inhibitory concentration



Aggregatibacter Actinomycetemcomitans (Aa)



Porphyromonas gingivalis (Pg)

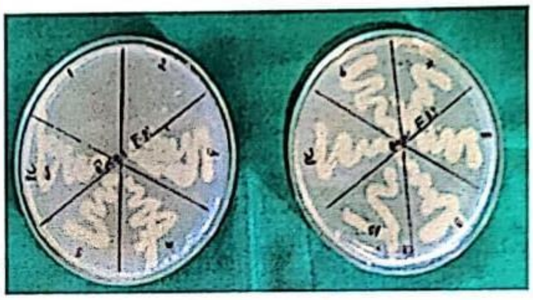


Fusobacterium nucleatum (Fn)

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA.
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; Web: www.klepkbsrc.org, Phone: 0831- 2444444, Extn. 4122
GSTIN/UIN: 29AABTK0881E1ZN

Minimum bactericidal concentration

Aggregatibacter Actinomycetemcomitans (Aa)

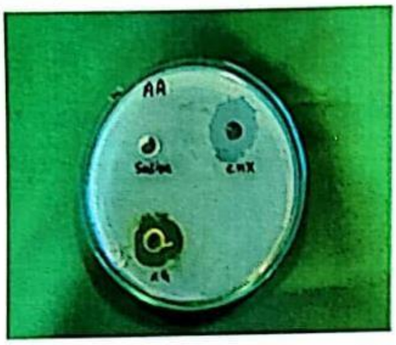


Fusobacterium nucleatum (Fn)

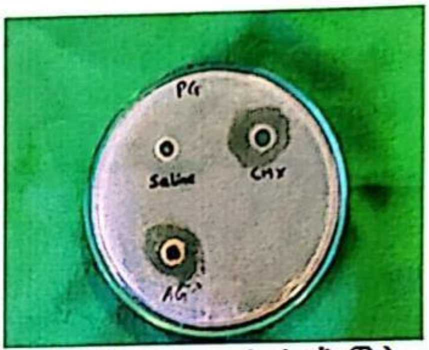
Porphyromonas gingivalis (Pg)

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA.
(Formerly known as KLE University) (Deemed-to-be-University u/s 3 of the UGC Act, 1956)
DR. PRABHAKAR KORE BASIC SCIENCE RESEARCH CENTER [BSRC], BELAGAVI, KARNATAKA.
III Floor, V. K. Institute of Dental Sciences Campus, Nehru Nagar, Belagavi - 590 010, Karnataka – INDIA
E-mail: research@kledeemeduniversity.edu.in; Web: www.klepkbsrc.org, Phone: 0831- 2444444, Extn. 4122
GSTIN/UIN: 29AABTK0881E1ZN

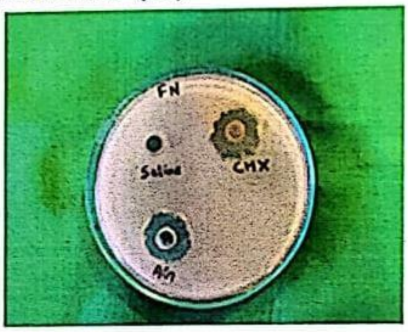
Agar well diffussion assay



Aggregatibacter Actinomycetemcomitans (Aa)



Porphyromonas gingivalis (Pg)



Fusobacterium nucleatum (Fn)

Dr. Prabhakar Kore Basic Science Research Center (BSRC) ★ KLE Academy of Higher Education and Research, Belagavi.

Dr. Suneel. Dodamani
11/01/2024

ANNEXURE – IV – PLAGIARISM CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) **Placed In Category 'A' by MHRD (GoI)**

☎: 0831-2470362 **Web: http://www.kledental-bgm.edu.in**
FAX: 0831-2470640 **E-mail:principal@kledental-bgm.edu.in**

Date : 2. 04. 2024 **Serial No. :** 163

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : POST GRADUATE
Batch & Year : 2021 - 2024
Department : PERIODONTICS

The soft copy of Research Work / Manuscript by . entitled "COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF ANETHUM GRAVEOLENS GEL WITH CHLORHEXIDINE GEL AGAINST AGGREGATIBACTER ACTINOMYCETEMCOMITANS, PORPHYROMONAS GINGIVALIS AND FUSOBACTERIUM NUCLEATUM....." under the guidance of ....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of ....3..........%, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

2/04/2024

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

ANNEXURE – V – BIOSTATISTICS CERTIFICATE

K L E VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES
(A Constituent unit of KLE Academy of Higher Education & Research
(Formerly known as KLE University) Deemed-to-be-University u/s 3 of the UGC Act, 1956)

J.N.M.C. Campus, Nehru Nagar, Belagavi-590 010, Karnataka, India
Accredited 'A+' grade by NAAC (3rd Cycle) Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640

Web: http://www.kledental-bgm.edu.in
E-mail : principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that Biostatics aspect of the Dissertation/Research work of **Post Graduate Student,** under the guidance of M.D.S, **Professor, Department of Periodontics,** entitled **"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."** has been done under my guidance and completed satisfactorily.

Place: Belagavi

Date : 29|01|24

Name & Signature of Biostatistician

Dr. S. B. JAVALI Ph.D.
Sr. Associate Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programme
BELAGAVI-590010.

ANNEXURE – VI- WAIVER FORM

Department of Periodontics

KAHER's KLE VK Institute of Dental Sciences, Nehru Nagar, Belagavi.

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.

Waiver form

Standard bacterial strains from BSRC will be used in this study. It is not feasible to obtain informed consent from the donors of these strains. However, I assure that confidentiality of the participant information will be ensured and no identifying information related to the study participants will be disclosed in any report/publication arising from the study.

POST GRADUATE STUDENT
DEPARTMENT OF PERIODONTICS
KAHER's KLE VK INSTITUTE OF
DENTAL SCIENCES
BELAGAVI-590010

PROFESSOR
DEPARTMENT OF PERIODONTICS
KAHER's KLE VK INSTITUTE OF
DENTAL SCIENCES,
BELAGAVI-590010