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**“TO ASSESS AND COMPARE THE  
ANTIMICROBIAL ACTIVITY OF PSORALEA  
CORYLIFOLIA GEL AND CHLORHEXIDINE GEL  
AGAINST PORPHYROMONAS GINGIVALIS AND  
AGGREGATIBACTER ACTINOMYCETEMCOMITANS  
– AN IN-VITRO STUDY ”**

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**By**

**Dr. MINAL MANOLKAR  
REG NO.IK0219003**

**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)**

**Under the Guidance of**

**Dr. Vinayak Kumbhojkar M.D.S**

**DEPARTMENT OF PERIODONTICS  
KAHER'S KLE VISHWANATH KATTI  
INSTITUTE OF DENTAL SCIENCES, KAHER,  
NEHRU NAGAR, BELAGAVI -10, KARNATAKA.**

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*Place: Belagavi*

***Dr. MINAL MANOLKAR***

## LIST OF ABBREVIATIONS

CHX	Chlorhexidine
MIC	Mean inhibitory concentration
MBC	Mean bactericidal concentration
Aa	<i>Aggregatibacter actinomycetemcomitans</i>
Pg	<i>Porphyromonas gingivalis</i>

## **ABSTRACT**

### **INTRODUCTION:**

Periodontitis is a complex infectious disease with several etiologic and contributory factors. The disease process has a mainly bacterial origin where primary colonizers in turn cause an environment which is favorable for development of secondary colonizers like *Porphyromonas gingivalis* (PG) and *Aggregatibacter actinomycetemcomitans*. These organisms are an intrinsic part of the dental biofilm and are known to be adherent to it. They also have the capacity to invade tissues locally. These characteristics of the bacteria play an important role in the pathogenesis of the disease. Scaling and root planing (SRP) is the first step and an essential phase of periodontal treatment, but it alone cannot eliminate tissue invading pathogens which suggest the need for adjuvant antimicrobial therapy. Chlorhexidine is considered the gold standard in antimicrobial agents used for treatment of periodontitis but it has side effects like bitter taste, staining of teeth and tongue, increased supragingival calculus formation and parotid swelling. This has led to an increase in the demand for herbal medicine as they show fewer side effects and they make low cost drugs.

The purpose of the study was to assess and compare the antimicrobial activity of *Psoralea corylifolia* gel and chlorhexidine gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

### **AIM:**

To assess and compare the antimicrobial activity of *Psoralea corylifolia* gel and chlorhexidine gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

## **MATERIALS AND METHODS:**

This is an experimental in-vitro microbial study. The hydroalcoholic seed extract of *Psoralea corylifolia* (*Bakuchi*) was prepared through maceration. The extract was then filtered using Whatman No.1 filter paper and using the New Brunswick scientific Excella E24 Incubator Shaker Series, the filtrate was further evaporated at room temperature.

MIC and MBC of the *Psoralea Corylifolia* extract against standard bacterial strains of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* revived from the repository of the research centre was determined using broth dilution method and streaking on blood agar plates. The gel was then prepared accordingly using Carbopol 940. The antibacterial activity of the prepared *Psoralea gel* was tested and compared to Chlorhexidine gel using the agar well diffusion assay.

## **RESULTS:**

The MIC of *Psoralea corylifolia* extract against *Aggregatibacter actinomycetemcomitans* was observed at 8.33 mg while for *Porphyromonas gingivalis* it was 4.16 mg. The MBC of the extract for *Aggregatibacter actinomycetemcomitans* was 10mg and was not determined for *Porphyromonas gingivalis*. MIC of Chlorhexidine against *Aggregatibacter actinomycetemcomitans* was found to be 0.041 mg while for *Porphyromonas gingivalis* it was not detected. The MBC of chlorhexidine for *Aggregatibacter actinomycetemcomitans* was 0.047 mg while *Porphyromonas gingivalis* showed complete inhibition.

## **CONCLUSION:**

In light of the observations drawn from our study we conclude that *Psoralea corylifolia* seed extract shows bacteriostatic activity against *Aggregatibacter actinomycetaemcomitans* and *Porphyromonas gingivalis*. The gel prepared from *Psoralea corylifolia* seed extract did not show bacteriocidal activity against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. On comparison with Chlorhexidine, *Psoralea corylifolia* seed extract gel showed no diffusion activity and hence was only bacteriostatic.

**KEYWORDS:** Dental plaque, *Psoralea corylifolia*, Herbal extract, Bakuchi, Periodontal disease.

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## INTRODUCTION

Periodontitis is a complex polymicrobial infectious disease with several etiologic and contributory factors. The disease is known to begin early in childhood or adolescence but more often occurs in early years of adulthood and can develop in the later years of life as well. <sup>[1]</sup>

The disease process has a mainly bacterial origin which compounded with susceptible human host conditions like compromised wound healing processes; decreased immune function causes the shift of bacterial flora from commensal to pathogenic in the dental biofilm. Streptococcus species colonize the dental biofilm first causing oxygen depletion in the local environment which is favorable for development secondary colonizers like *Porphyromonas gingivalis* (P.g) and *Aggregatibacter actinomycetemcomitans* (A.a). <sup>[2,3]</sup>

Presently, destructive periodontal disease can present in two forms -chronic periodontitis and aggressive periodontitis<sup>[4]</sup> where *A. actinomycetemcomitans* has been firmly associated with a localized form of aggressive (juvenile) periodontitis and *P. gingivalis*, *A. actinomycetemcomitans*, *Tannerella forsythia* (formerly, *Bacteroides forsythus*), *Treponema denticola*, and *Eikenella corrodens* have been implicated in the occurrence of a chronic form of periodontal disease that occurs mainly in adults (chronic periodontitis).<sup>[5]</sup> These organisms are an intrinsic part of the dental biofilm and are known to be adherent to it. They also have the capability to invade tissues locally. These characteristics of the bacteria determine the role they play in the pathogenesis of the periodontal disease<sup>[6]</sup>

Scaling and root planing (SRP) is the first step and is a crucial part of periodontal treatment, but it singularly cannot eliminate the tissue invading pathogens which suggest the need antimicrobial therapy using antimicrobial agents as adjuncts to SRP.<sup>[6]</sup> Chlorhexidine is described as having hydrophilic and hydrophobic groups and is considered the gold standard in antimicrobial agents used in treatment of periodontitis. The antiseptic molecule attaches itself to bacterial cell membranes and at low concentration causes increased permeability of the cell membrane of the bacteria causing leakage of intracellular components (bacteriostatic effect). While at high concentration, it causes precipitation of bacterial cytoplasm leading to cell death. (bactericidal effect).<sup>[7]</sup> It is commercially available in various forms like gel, varnishes, toothpaste and mouthwashes. Apart from its advantages it also has certain disadvantages like bitter taste, staining of teeth and tongue, increased calculus formations supragingivally and parotid swellings.<sup>[6,7]</sup>

Indigenous herbs have been used for treatment of various diseases in several traditional systems of medicine. These herbs have become increasingly popular over the past few decades attributing to their vast chemical diversity. Since their discovery, there has been a steady increase in the demand for medicine derived from herbal plants as they show fewer side effects and they make efficient low cost drugs. The relative safety, easy availability, and affordability, has made these drugs pre-eminent in pioneering drug research, resulting in the discovery of newer molecules.<sup>[8]</sup>

*Psoralea corylifolia*, popularly known as Bakuchi, is a versatile Indian herb. Ayurvedic and Chinese medicine have used *Psoralea corylifolia*, for its wide range of healing properties such as antioxidant, antimicrobial, and anti-inflammatory

properties since the ancient times. *Psoralea* seed extract derived compounds, especially its flavonoids Corylin and Bavachin, have been found to stimulate osteoblast proliferation, suggesting the possibility of promoting bone formation.<sup>[8]</sup> It has also been seen to be effective against *S. mutans*, which is a major causative organism for dental caries.<sup>[9]</sup>

A suitable vehicle impregnated with drugs is appropriately designed to be used as a local drug delivery agent (LDD). It is used in conjunction with non-surgical periodontal therapy in the form of gel, chips, fibres, ointments or polymers.

Antimicrobials that can be locally administered into the mucosa include metronidazole, chlorhexidine, minocycline, doxycycline and tetracycline, which, when inserted in periodontal pockets, can inhibit or eliminate periodonto-pathogenic microorganisms. LDDs are available in the form of a chip, microsphere, gel or patch. Amongst these, gels are dilute, cross-linked semisolid systems in which the active drug molecules are evenly distributed and do not flow at steady state.<sup>[9]</sup>

Hence, the aim of the present study was to assess and compare the antimicrobial activity of the *Psoralea corylifolia* gel and chlorhexidine gel on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

## **AIM OF THE STUDY**

To assess and compare the antibacterial activity of the *Psoralea corylifolia* gel and chlorhexidine gel on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

### **OBJECTIVES OF THE STUDY:**

1. To assess antimicrobial activity of *Psoralea corylifolia* gel on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*
2. To assess the antibacterial activity of chlorhexidine gel on *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*.
3. To compare the antibacterial activity of *Psoralea corylifolia* gel against chlorhexidine gel on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

## REVIEW OF LITERATURE

1. **Sung-Im Kim et al 2015**, attempted to compare the antibacterial effect of natural herbal extracts and mouth rinsing solutions against *Streptococcus mutans*. This study measured the minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Psoralea corylifolia* extracted with distilled water and ethanol, respectively against *S. mutans*. The results further were compared to mouth rinsing solutions for their antibiotic effect. MIC of ethanolic extract of *P. corylifolia* was at 20µg/ml and MBC was 20µg/ml. It was concluded that when compared to mouth rinsing solution, *Psoralea corylifolia* had growth inhibitory effect and the minimum bactericidal effect at lowest concentration. No cytotoxicity was found on human fibroblast cells when tested with this extract<sup>[9]</sup>
2. **Khushboo P. S. et al 2010** highlighted the literature available on *P. corylifolia* with regards to its various pharmacologic activities, clinical effects and uses. It was found that the plant is versatile, that is the roots, stems, leaves, and seeds show anti-inflammatory, antibacterial and antioxidant properties. It contains essential oils, coumarins, alkaloids, flavonoids, and terpenoids which are the active constituents. Amongst the flavonoids, Corylin and Bavachin mostly have a stimulating effect on osteoblast proliferation and could thus promote formation of bone. There has been research since the ancient times into the healing properties of the plant against various skin diseases, including leprosy, psoriasis, and leukoderma. The plant is known to harbour different kinds of bioactive agents with diverse chemical structures and varied pharmacologic

activities, suggesting that *P. corylifolia* could function as a "lead" for the evolution of new agents for the treatment of various diseases.<sup>[8]</sup>

3. **Bina Gidwani et al 2010** performed an animal study on nine albino rats to check the anti-inflammatory activity of *Psoralea corylifolia*. These rats were distributed to three groups, Group 1 – normal saline (control), Group 2- *Psoralea corylifolia* (test), Group 3 – Diclofenac sodium(standard). Before the drug was administered the thickness of the hind paw of the rats was measured using vernier callipers followed by administration of drugs orally. To induce inflammation in the hind paws of the rats, subcutaneous injection of 0.1 ml of 1% w/v carrageenan in water was injected followed by evaluation of the paw thickness at different time intervals. The drug activity was measured as percent inhibition of oedema. Percentage inhibition for the *P. corylifolia* extract was found to be 44% and for standard it was 55% which led to conclusion that the extract possess some anti-inflammatory activity.<sup>[12]</sup>
4. **SeungLee al (2012)** investigated the anti-inflammatory activity of *Psoralea corylifolia* seeds on Interleukin 6 induced STAT 3 activation which is considered an effectual mediator in the immune-inflammatory pathways of various inflammation induced diseases. Seven flavonoids derived from methanol extracts of *Psoralea corylifolia*, their mean inhibitory concentrations and mean cytotoxic concentration effect induced by IL-6 on STAT3 promoter activity was determined. Among them, compounds like Corylifol A and Corylin exhibited the most formidable inhibition of IL-6 induced STAT3 promoter activity. Additionally, these compounds also inhibited IL-6 induced STAT3 phosphorylation. By inhibiting these key processes, the authors

concluded that *P. corylifolia* derived flavonoids can be useful agents in treating inflammatory diseases.<sup>[13]</sup>

5. **Archana Moon et al, 2012** did a study to evaluate antimicrobial activity of the leaf and callus extracts of *Psoralea corylifolia* against periodontitis causing microbes that were resistant to multiple drugs such as Amoxycillin, Penicillin, Cephalexin, Streptomycin etc. Plant collection and processing, inoculum preparation, and activity testing of methanolic extracts of *Psoralea* was done. Clinical isolates of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* showed resistance to the above antibiotics and were treated with leaf MeOH extract of *Psoralea corylifolia* and corresponding callus on agar plates. MeOH extracts seemed effective at concentration of 30 mg/ml to 100mg/ml and the methanolic callus extract at all concentrations for these two periodontal pathogens.<sup>[14]</sup>
6. **Naznin A. Khatune et al 2004**, did an in-vitro study to check antibacterial activities of bioactive compounds isolated from *Psoralea* seeds against certain gram positive and gram-negative bacteria. Tested compounds were Psoralidin , Bakuchicin , mixture (1:1) of Psoralen and Angelicin by disk diffusion method. Psoralidin showed notable inhibition of gram negative organisms *S. sonnei* and *S.flexneri* while Psoralen and Angelicin exhibited the highest inhibitory effect against Gram positive organism *Staphylococcus aureus*.<sup>[15]</sup>

## **MATERIALS AND METHODS:**

### **Methodology of extract preparation:**

The powder was extracted by maceration in 70% ethanol. Approximately 200g of the powder were soaked in 1400 ml of ethanol and 600ml of water (2.3:1) for 72 h at room temperature (Fig 1). The extract filtration was done through Whatman No.1 filter paper (Fig 2). Evaporation of the filtrate was done using the New Brunswick scientific Excella E24 Incubator Shaker Series. The dried extract was sterilized overnight by UV-irradiation. Stock solution of extract was prepared by dissolving 200mg of dried crude extract in 10 ml dimethyl sulfoxide saline (DMSO) at pH 7.0 prepared with concentration of 20 mg/ml and kept at 4°C in the dark to prevent oxidation before being used.



Fig 1: Maceration of extract



Fig 2: Filtration



Fig 3: New Brunswick scientific Excella E24 Incubator shaker series



Fig 4: Labotech Bacteriological Incubator

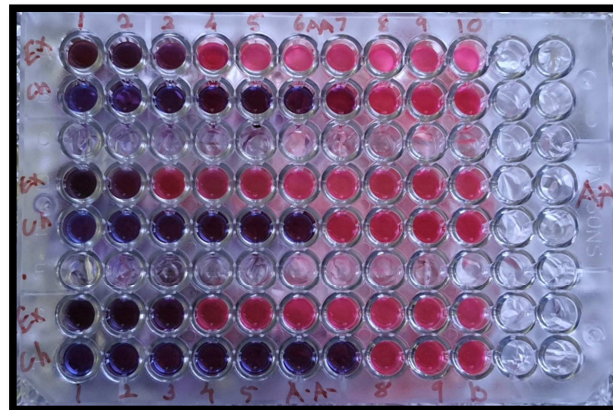


Fig 5: MIC of extract and Chlorhexidine against *Aggregatibacter actinomycetemcomitans*

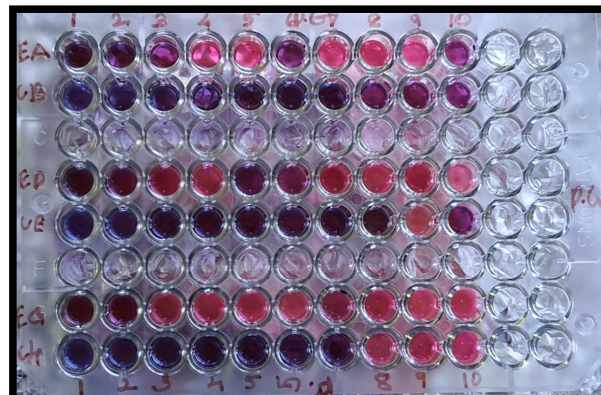


Fig 6: MIC of extract and Chlorhexidine against *Porphyromonas gingivalis*

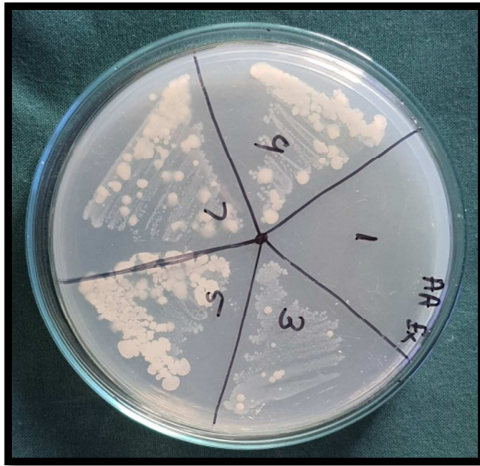


Fig 7: MIC of extract against *Aggregatibacter actinomycetemcomitans*

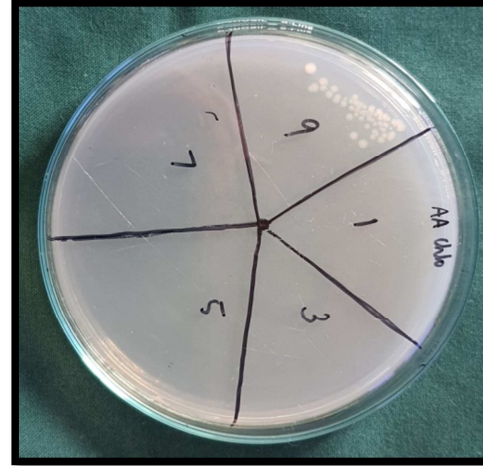


Fig 8: MIC of CHX against *Aggregatibacter actinomycetemcomitans*

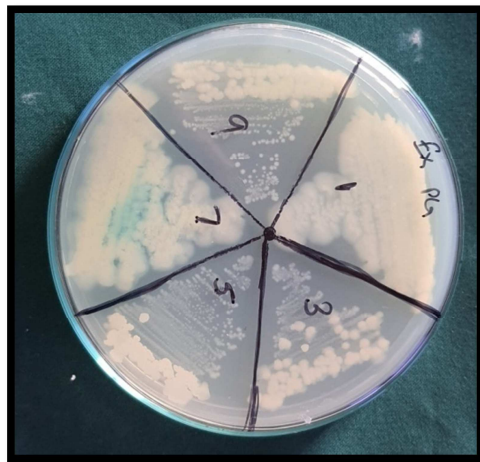


Fig 9: MIC of extract against *Porphyromonas gingivalis*

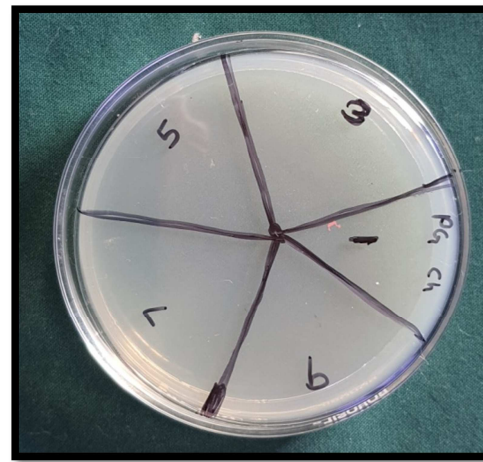


Fig 10: MIC of CHX against *Porphyromonas gingivalis*

**Inoculum preparation:**

Preparation of inoculum was carried out in BHI broth. The standard colonies of the same morphological type of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were collected from an agar culture plate. The colonies were picked with a sterile loop, and were transferred into a tube containing 5 mL of BHI broth. This stock culture was incubated at 37°C for 8–14hrs until it attained the turbidity of the 0.5 McFarland standard. The turbidity of actively thriving bacterial culture was calibrated with broth to match the turbidity of 0.5 McFarland guidelines.

**Broth dilution method [Resazurin] for determining Minimum Inhibitory Concentration**

Broth dilution was done in a sterilized 96 well plate. This was carried out with triplicates. Total of 100 µl of broth was added to all the 10 wells in triplicates. Further in the first well 100 µl of extract was added and serially diluted to required concentrations up to tenth well. Similarly, same procedure was carried out in other two rows of the well plates. The 96 well plates were kept for incubation in McIntosh and Fildes' anaerobic jar followed by addition of resazurin reagent after 48 hours and was observed after 4 hours for possible colour change. The colour change from blue/violet to slight pink/pink/magenta was recorded as MIC of emulsion. The results were recorded by taking good quality photographs.

**Note: Separate 96 well plates used for each bacteria and extract.**

<b>SL No.</b>	<b>Ingredients</b>	<b>Concentration (mg)</b>	<b>Uses</b>
1	Bakuchi	20	Active ingredient
2	Carbopol	60	Gel forming agent
3	Tween 80	20	Dispersing agent
4	Propylene glycol	60	Humectant and dispersing agent
5	Sodium methyl paraben	1	Bactericidal agent
6	Sodium propyl paraben	0.2	Bactericidal agent
7	Sodium benzoate	10	Bacteriostatic agent
8	Trietholamine	10	pH adjusting agent to pH7
9	Distilled water qs	100	Solvent

**Gel preparation:**

60 ml of distilled water was used to soak the Carbopol 940 at room temperature, which was then stirred continuously using a magnetic stirrer for 24 hours. The Bakuchi extract was uniformly dispersed by triturating it in a mortar & pestle along with Tween 80 & propylene glycol. A preservative was then added along with 30 ml of distilled water & stirred with a magnetic stirrer for 30 mins. The Bakuchi extract along with Carbopol 940 was further added to the preservative solution. The required quantity was achieved by adding distilled water to the total volume. To form a uniformly distributed gel, Triethanolamine was added drop wise using a high speed propeller stirrer for 10 mins. The gel thus obtained was maintained & stored in an air tight container.

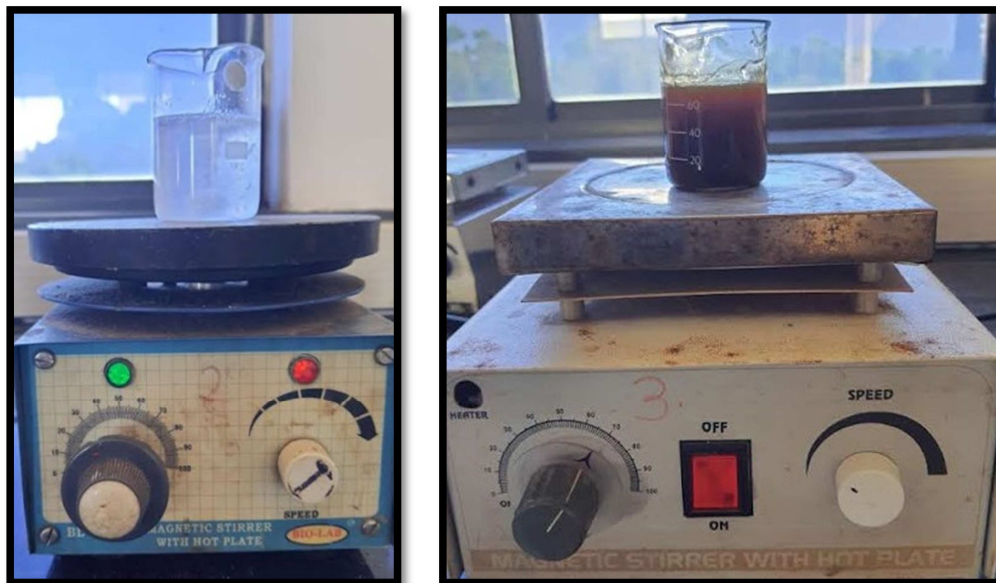


Fig 11: Magnetic stirrer



Fig 12: High speed propeller stirrer

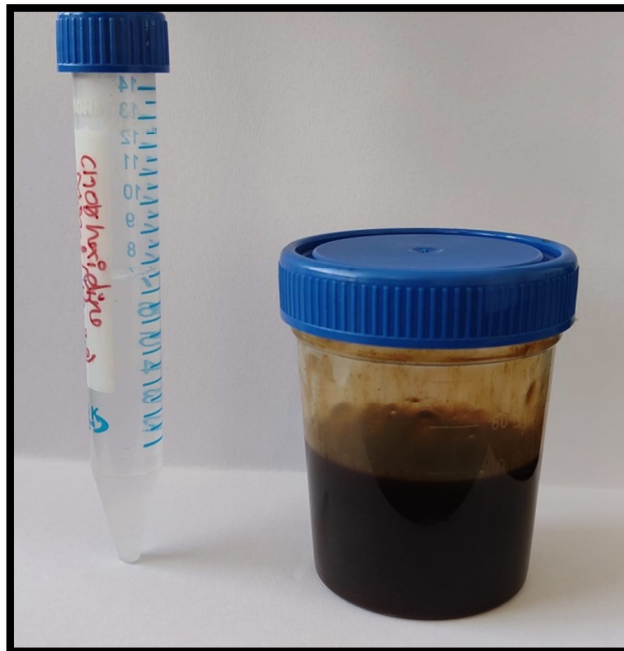


Fig 13: Prepared *Psoralea corylifolia* gel

#### **Agar well diffusion assay**

The agar well diffusion assay was performed on bacteriological agar plates. The Brain Heart Infusion agar was prepared by adding 5.2 g BHI agar to 100 ml of distilled water and was sterilized in steam sterilizer. It was then kept to cool at room temperature for 10-15min following which the agar plates were poured and allowed to solidify. The bacterial broth cultures of *A.a* and *P.g*, were taken [0.5 McFarland's] and spread all over on prepared BHI agar plates [100  $\mu$ l] with sterile cotton spreader. Following this, uniform aseptic wells were made using a cork borer. To these wells, sample reagents [100  $\mu$ l chlorhexidine and 100  $\mu$ l extract] were added and placed in 37°C, CO<sub>2</sub> incubator [jar of desiccator]. They were observed for diffusion through 24-72 hours of incubation. Growth pattern was observed on the plates and results were noted against Chlorhexidine as standard.

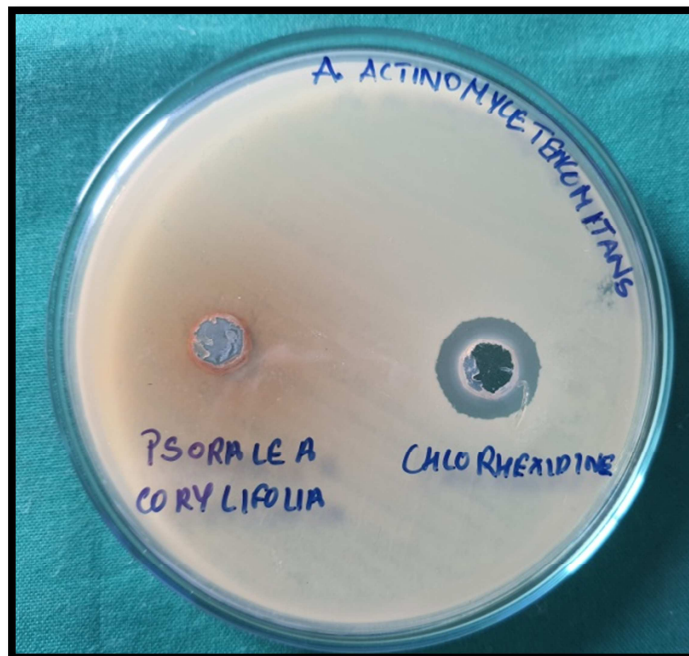
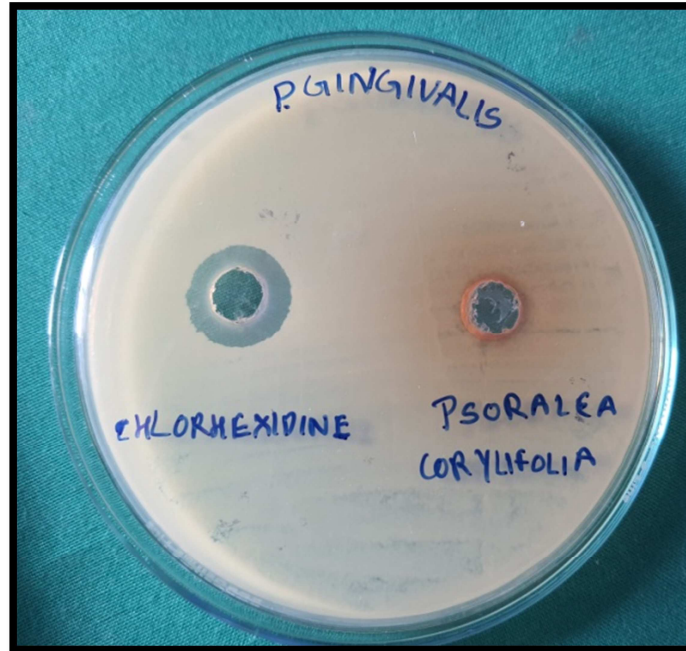


Fig 14: Agar well diffusion for *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

## **RESULTS AND OBSERVATIONS**

### **Antibacterial susceptibility tests**

The antibacterial effects of *Psoralea corylifolia* extract were evaluated using “Broth dilution assay” (Resazurin) for its “minimum inhibitory concentration (MIC)” (**Table 1**) and Agar plate assay for minimum bactericidal concentration (MBC) (**Table 2**). The effects were observed against standard strains of anaerobic bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

The control group agent selected was Chlorhexidine extract/gel, regarded as a gold standard antimicrobial agent. The test group for the MIC and MBC test was *Psoralea corylifolia* (Bakuchi) extract. The well diffusion assay was performed using 100 µl of Chlorhexidine gel (control) and 100 µl of *Psoralea corylifolia* (test).

**Stock (*Psoralea corylifolia*): 200mg/10ml Working - 20mg/ml**

**Chlorhexidine: Working - 4mg/ml**

Table. 1. Minimum inhibitory concentration of *Psoralea corylifolia* extract

Sr.No.	Samples	Minimum inhibitory concentration (MIC) in milligram (mg)			
		<i>A.comitans</i>	Average	<i>P.gingivalis</i>	Average
1	<i>Psoralea corylifolia</i> (extract)	5	<b>8.33</b>	2.5	<b>4.16</b>
		10		5	
		10		5	
4	Chlorhexidine (control)	0.063	<b>0.041</b>	ND	
		0.031			
		0.031			

**Table. 2. Minimum bactericidal concentration of *Psoralea corylifolia* emulsion**

		<i>Aggregatibacter actinomycetemcomitans</i>		<i>Porphyromonas gingivalis</i>	
1	<i>Psoralea corylifolia</i>	ND	10	ND	
		15		ND	ND
		15		ND	
2	Chlorhexidine	0.063	0.047	Showed complete inhibition	
		0.016			
		0.063			

The antibacterial effects of the prepared *Psoralea corylifolia* gel was evaluated against the same organisms through the agar well diffusion assay. The results of the agar well diffusion assay are listed in Table 3.

**Table 3. Agar well diffusion assay of *Psoralea corylifolia* gel against *A.comitans* and *P.gingivalis***

<b>Sl. No</b>	<b><i>Aggregatibacter actinomycetemcomitans</i></b>	<b>Average</b>	<b><i>Porphyromonas gingivalis</i></b>	<b>Average</b>
Chlorhexidine	18mm	<b>18 mm</b>	18 mm	<b>19 mm</b>
	18mm		19mm	
	19mm		20mm	
<i>Psoralea corylifolia</i>	<b>NZI</b>	<b>NZI</b>	<b>NZI</b>	<b>NZI</b>

mm : millimeter , NZI :No zone of Inhibition

**Table 4: Mean and Standard deviation**

	<i>Aggregatibacter actinomycetemcomitans</i>		<i>Porphyromonas gingivalis</i>	
<i>Psoralea corylifolia</i>	MIC	MBC	MIC	MBC
Mean ±SD	8.33±2.35	10±7.07	4.16±1.17	ND
<i>Chlorhexidine</i>				
Mean±SD	0.04±0.01	0.04±0.02	ND	ND

## **DISCUSSION:**

Dental plaque is a heterogenous grid matrix primarily composed of bacteria in matrix of salivary glycoproteins and extra-cellular polysaccharides. It occurs as a grayish yellow substance present on intra oral hard surfaces of oral cavity. The plaque can be present supra-gingivally or sub-gingivally.

Its association with diseases of periodontium is site specific. The plaque present on the marginal gingiva helps in initiation of gingivitis, while the plaque present supra-gingivally aids in calculus formation and the tissue associated subgingival plaque is important in tissue destruction. Most commonly found bacteria to which periodontal tissue breakdown is attributed to are *P. gingivalis*, *T. forsythia*, *P. intermedia*, *C. rectus*, *E. corrodens*, *F. nucleatum*, *P. micros*, *A. actinomycetemcomitans*, *Treponema* and *Eubacterium* species. Among these *P. gingivalis* and *A. actinomycetemcomitans* are known to invade host tissues and whose association has been strongly incriminated in a destructive (aggressive) form of periodontitis <sup>[10]</sup>

Developed in the 1940s by Imperial Chemical Industries, England, Chlorhexidine was first promoted in 1954 as an antiseptic for skin wounds. It was used initially for disinfection of the oral cavity prior to surgery in dentistry and its capability to inhibit plaque was first investigated by Schroeder in 1969. The results of his study were later validated in 1970 by a decisive study carried out by Schiott and Loe. Chlorhexidine is an antibacterial agent that binds strongly to cell membrane of bacteria.

At low concentration it alters the cell membrane permeability that results in leakage of intracellular components. At high concentration, chlorhexidine causes cell death through precipitation of bacterial cytoplasm leading to a bactericidal effect. It is because of these properties that Chlorhexidine is considered the gold standard antimicrobial agent used in treatment of periodontitis. However, its usage causes side effects such as altered taste sensation, increased precipitation of calculus, staining of teeth and rarely parotid swelling. Hence, the direction of periodontal research is towards developing plant based effective antimicrobial agents that cause fewer side effects and are economically priced.<sup>[7]</sup>

*Psoralea corylifolia* Linn (Bakuchi), a traditional and popular Chinese medicinal herb has officially been listed in Chinese Pharmacopoeia. It is a small, upright, herb which grows through the year, reaching up to 60–120 cm in height throughout tropical and sub-tropical areas (semi-arid regions) of Central and East India. All the parts of the plant, that is the root, stem, leaves have displayed medicinal value. Seeds of the plant appear brownish black, oval, flattened, kidney shaped and ex-albuminous with straw-colored test, having a pervasive-bitter taste with aromatic smell.<sup>[8]</sup>

Phytochemical evaluation of the seeds has shown the presence of an essential oil (0.05%), a non-volatile terpenoid oil, a dark brown resin (8.6%), and some remnants of alkaloidal substances.<sup>[2,6,8]</sup> The phytochemistry of seeds of *Psoralea* showed presence of flavonoids, such as corylifolean, corylifolin, psoralidin, isopsoralidin, bavachin, isobavachin, bavachinin, bavachalcone, corylifolinin, bakuchicin, isobavachalcone, 7-O-methyl bavachin, bavachromanol, corylin, corylidin, corylinal, 4-O-methyl bavachalcone, isoneobavachalcone,

neobavaisoflavone, bavachromene, and neobavachalcone, bakuchalcone. *P. corylifolia* can be used to treat osteoporosis and bone fractures because it promotes bone calcification. There is evidence that its flavonoids, particularly Corylin and Bavachin, stimulate osteoblastic proliferation, which would help to build bone. <sup>[8]</sup> When non-polar crude fractions of *Psoralea corylifolia* seed extract was administered orally to mice with rachitic diseases and monitored biologically, it was found that the phosphorus levels were significantly raised. Additionally, histomorphometrically a significant increase in bone calcification was revealed. <sup>[16]</sup> This shows that there is an effect of *Psoralea corylifolia* on bone calcification and bone formation.

In periodontitis where there is a bacterial mediated breakdown of the alveolar bone this perhaps could be beneficial however the primary requisite of any herbal preparation used for local delivery would be its ability to induce bacterial killing. We therefore decided to explore this property of *Psoralea corylifolia*.

Sung-Im Kim et al 2015, evaluated the efficacy of *Psoralea corylifolia* seed extract on *Streptococcus mutans*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was observed for the same and the results showed significant action at 20 µl/ml concentration <sup>[9]</sup> Similar results were observed in an in-vitro study done by Harumi Katsura et al, 2001, where the *Psoralea* extract showed antimicrobial activity against *Streptococcus mutans* and *P. gingivalis*, with MIC being observed at 20 µl/ml. <sup>[9]</sup>

In the present study, antibacterial efficacy of a traditional Chinese herb *Psoralea corylifolia* was evaluated where hydroalcoholic extraction of the seeds of the plant was carried out. The MIC of *Psoralea corylifolia* extract (determined using broth dilution assay) against *Aggregatibacter actinomycetemcomitans* was observed at

8.33 mg while for *Porphyromonas gingivalis* it was 4.16 mg (**Table 1**). The MBC of the extract (determined using agar plate assay) for *Aggregatibacter actinomycetemcomitans* was 10mg and was not determined for *Porphyromonas gingivalis* (**Table 2**). MIC of Chlorhexidine (determined using broth dilution assay) against *Aggregatibacter actinomycetemcomitans* was found to be 0.041 mg while for *Porphyromonas gingivalis* it was not detected (**Table 1**). The MBC of chlorhexidine (determined using agar plate assay) for *Aggregatibacter actinomycetemcomitans* was 0.047 mg while *Porphyromonas gingivalis* showed complete inhibition (**Table 2**). These results show that the active constituents of *Psoralea coryfolia* show significant anti-bacterial activity. The antimicrobial effects of the prepared *Psoralea* gel were assessed using agar well diffusion assay and it determined that 100 µl of prepared *Psoralea* gel did not show any zone of inhibition on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in comparison to the control group (Chlorhexidine) where, the zone of inhibition was 18mm for A.a and 19 mm for P.g(**Table 3**). Mean and standard deviation for MIC of A.a using *Psoralea corylifolia* was  $8.33 \pm 2.35$  and MBC was  $10 \pm 7.07$  and MIC for P.g was  $4.16 \pm 1.17$ . (Table 4) Mean and standard deviation for MIC of A.a using Chlorhexidine was  $0.04 \pm 0.01$  and for MBC was  $0.04 \pm 0.02$ . (**Table 4**) This implies that the prepared *Psoralea coryfolia* gel showed bacteriostatic effects against the chosen periodontal organisms but it did not have a significant bactericidal effect. This can be attributed to the vast number of biochemical ingredients that are present in the extract, the specific one however need to be further identified and explored. Furthermore the beneficial effects on bone remodeling that can be harnessed for a favorable periodontal post non-surgical therapy outcome in patients also can be evaluated in future research.

## SUMMARY AND CONCLUSION

Periodontal disease is a complex disease mainly caused by the presence of plaque sub-gingivally. This plaque is known to harbor a high proportion of Gram negative periodontopathic micro-organisms such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

These bacteria cause widespread inflammation and destruction of the periodontium when they remain unchecked. The aim of non-surgical periodontal therapy is to eradicate these bacteria from the subgingival areas of the periodontal pocket to allow restoration of periodontium to healthy state. Often, antimicrobial agents like Chlorhexidine are used as adjuncts to periodontal therapy to improve treatment outcomes but they are known to cause unpleasant side effects.

Hence, the focus of the present investigation was to assess and contrast the antibacterial activity of a gel prepared from *Psoralea*, a versatile plant that has shown significant antibacterial and bone formation promotive activity and Chlorhexidine gel on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Hydroalcoholic plant seed extract was obtained through maceration and filtration and a working solution of 40mg/ml was used for assessment of antibacterial activity using Broth dilution assay (Resazurin) for its “minimum inhibitory concentration” (MIC) and agar plate assay for minimum bactericidal concentration (MBC). The effects were observed against standard strains of anaerobic bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* revived from the repository of the research centre.

Once the MIC and MBC values were determined, a carbopol based gel was prepared whose antibacterial effects were evaluated against the same organisms through the agar well diffusion assay.

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

1. *Psoralea corylifolia* seed extract shows bacteriostatic activity against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.
2. Gel prepared from *Psoralea corylifolia* seed extract did not show bactericidal activity against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.
3. On comparison with Chlorhexidine, *Psoralea corylifolia* seed extract gel showed no diffusion activity and hence was only bacteriostatic.

It can be said that the antibacterial activity showed by *Psoralea corylifolia* maybe due to the action of secondary metabolites. Further investigations at biomolecular levels and clinical applications of the same are required to elucidate and prove the efficacy of *Psoralea corylifolia* against periodontal pathogens.

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## 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

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 E-mail: [principal@kledental-bgm.edu.in](mailto:principal@kledental-bgm.edu.in)



Sl. No. : 1509

**CERTIFICATE**

*This is to Certify that the synopsis titled*

*To assess and compare the antimicrobial activity of psoralea corylifolia and chlorhexidine gel against porphyromonas gingivalis and Aggregatibacter*

*Actinomyces actinomycetans - An in vivo study. Submitted by*

*Dr. Minal Manolkar. P. G. Student /*

*Staff, Guided by Dr. Vinayak Kumbhghkar from Department of*

*Periodontics - has been critically evaluated by*

*committee members and granted ethical clearance to conduct the above*

*mentioned study*

**Date :** 28/11/21

**Member Secretary**

Research and Ethical Committee  
 KLEVK Institute of Dental Sciences  
 Belagavi

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

**Chairman**

Research and Ethical Committee  
 KLEVK Institute of Dental Sciences  
 Belagavi

CHAIRMAN  
 Research and Ethical Committee  
 KLEVK Institute of Dental Sciences  
 Belagavi

## ANNEXURE – II – DRUG AUTHENTICATION CERTIFICATE



**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**



Submitted By: KLE Ayurveda Pharmacy  
 Submitted Date: 27/11/2021

Date of Issue: 29/11/2021

SN	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Ayurvedic Name	Scientific Name	Family	Part Authenticated
1	Bakuchi	<i>Psoralea corylifolia</i> Linn.	Fabaceae	Seed	CRF/Auth 33/2021	Bakuchi	<i>Psoralea corylifolia</i> Linn.	Fabaceae	Seed

Signature:

Authentication Expert Name: Dr. Divya Khare

Date: 29/11/2021



Signature of Coordinator  
 ASU Drug Testing Laboratory

## ANNEXURE – III – MIC and MBC RESULTS

**Dr. Prabhakar Kore Basic Science Research Center, KLE Academy of  
Higher Education and Research**

**Report**

**Title of Research:** “To assess and compare the antimicrobial activity of *Psoralea corylifolia* and Chlorhexidine against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*”

**Student Name:** Dr. Minal Manolkar

**Guide:** Dr. Vinayak Kumbhojkar

**Objective Parameters:**

1. Assessment of MIC and MBC of *Psoralea corylifolia* seed extract

**Laboratory Methods for assessing microbiome:**

1. **Bacterial Culture:** *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*
2. **Method used:** Minimum inhibitory concentration, Minimum bactericidal concentration
3. **Extract concentration:** Stock 200mg/10ml Working : 20mg/ml

**Lab Investigations done in BSRC**

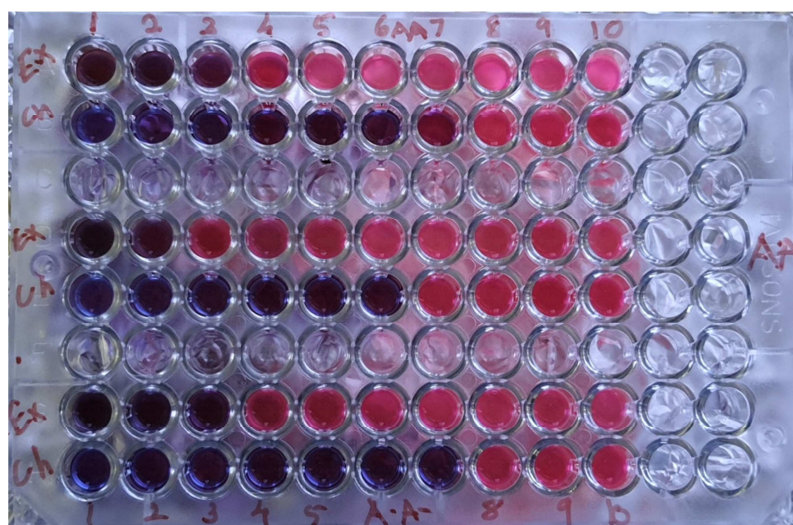
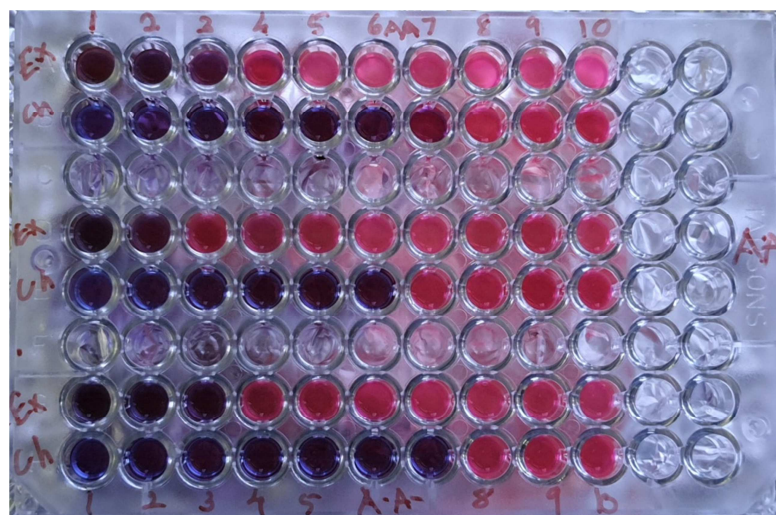
Sl.No.	<i>Aggregatibacter actinomycetemcomitans</i>				<i>Porphyromonas gingivalis</i>		
	MIC		MBC		MIC	MBC	
<b>Psoralea . corylifolia(extra ct)</b>	5	8.33	ND	10	2.5	4.16	Showed complete inhibition
	10		15		5		
	10		15		5		
<b>Chorhexidine</b>	0.031	0.041	0.063	0.047	Showed Complete inhibition		
	0.063		0.016				
	0.031		0.063				

All values are expressed in mg/ml against tested organism

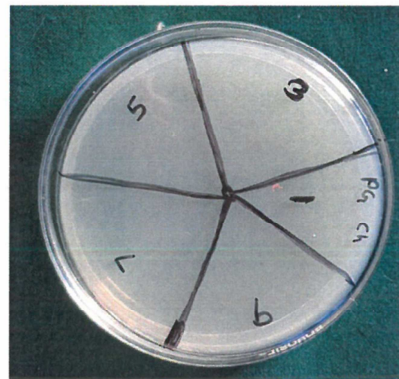
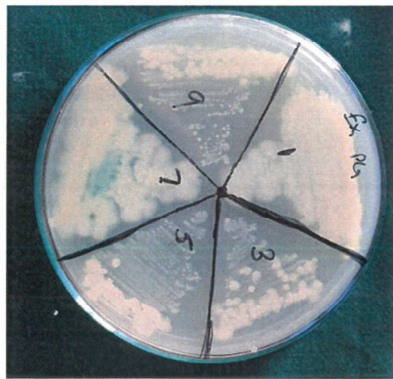
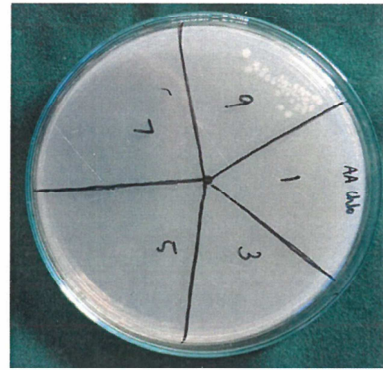
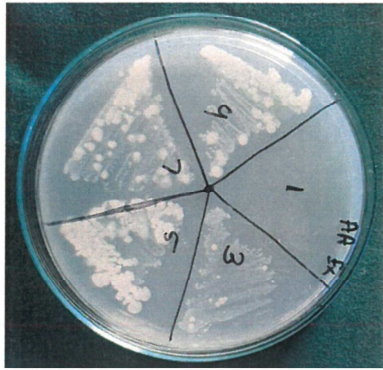
**Remarks**

The results are satisfactory and relevant references have been followed

**Minimum Inhibitory Concentration :**



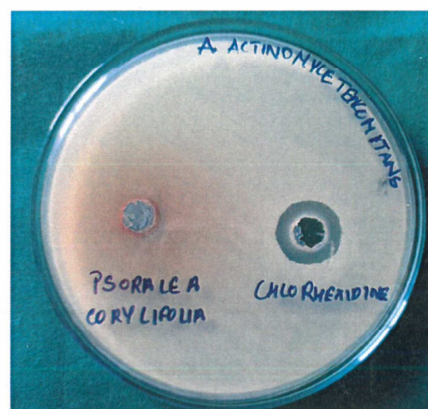
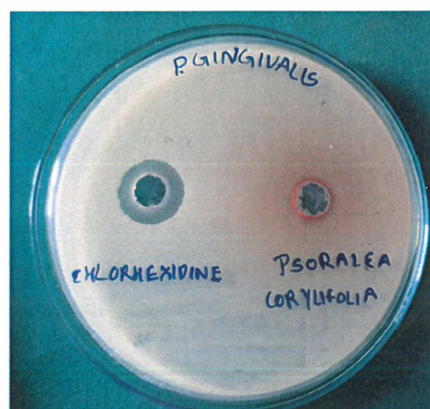
Minimum Bactericidal Concentration



Agar well Diffusion Assay:

Sl. No	<i>Aggregatibacter actinomycetemcomitans</i>	Average	<i>Porphyromonas gingivalis</i>	Average
Chlorhexidine	18mm 18mm 19mm	18 mm	18 mm 19 mm 20 mm	19 mm
<i>Psoralea corylifolia</i>	NZI	NZI	NZI	NZI

mm : millimeter NZI :No zone of Inhibition



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