"TO EVALUATE AND COMPARE THE ANTIBACTERIAL EFFICACY OF ALOE VERA AND NEEM GEL WITH DOXYCYCLINE GEL AGAINST PORPHYROMONAS GINGIVALIS AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS - AN IN-VITRO STUDY"

By

REG NO. IK0220003

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 -10, KARNATAKA.

2020 – 2023

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH,
BELAGAVI, KARNATAKA

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LIST OF ABBREVIATIONS

Aa	*Aggregatibacter actinomycetamcomitans*
BHI	Brain Heart Infusion
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
conc	Concentration
DMSO	Dimethyl Sulfoxide
DO	Doxycycline
F.n	Fusobacterium nucleatum
GCF	Gingival crevicular fluid
GI	Gingival Index
Hrs	Hours
LDD	Local Drug Delivery
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
Pg	*Porphyromonas gingivalis*
PI	Plaque Index
P. i	*Prevotella intermedia*
PPD	Pocket Probing Depth
RAL	Relative Attachment Level
SD	Standard Deviation
SRP	Scaling and root planing
µg	Microgram
µL	Microlitre
%	Percentage

ABSTRACT

INTRODUCTION

Periodontitis is a complex infectious disease with several etiologic and contributory factors. The disease process has a mainly bacterial origin were primary colonizers in turn cause an environment which is favorable for development secondary colonizers like *Porphyomonas 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 pocket formation providing an ideal niche for the growth of gram negative anaerobes. Often the non-surgical phase i.e., Scaling and root planing (SRP) alone cannot eliminate tissue invading pathogens which suggest the need for adjuvant antimicrobial therapy to enhance the results at the localized sites. The concept of controlled drug delivery was proposed in the late 1970s for their potential benefit to reach the base of the periodontal pocket and prolonged substantivity allowing the antimicrobial effect to occur. Doxycycline is considered the gold standard for local drug delivery used for the treatment of periodontitis but it has side effects like growth retardation and enamel hypoplasia and also has harmful effects on bone and tooth development. This has led to increasing 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 evaluate and compare the antibacterial efficacy of herbal plant extract gel; Aloe Vera and Neem with Doxycycline Gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

AIM

To assess and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

MATERIALS AND METHODS

This is an experimental in-vitro microbiological study. The ethanolic extract of Aloe Vera and Neem 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 ethanolic extract of Aloe vera and Neem against standard bacterial strains of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* revived from the repository of the research center 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 Aloe vera and Neem *gel* was tested and compared to the prepared 10% Doxycycline gel using the agar well diffusion assay. The data were entered in Excel and analyzed statistically using the SPSS software version. Descriptive analysis was done for zone of inhibition. The intergroup comparison between the test and the control group was determined using an unpaired t-test while the intragroup comparison between the two test groups was determined using a one-way ANOVA test. All statistical tests were performed at a significance level of 5% ($p<0.05$).

RESULTS

The MIC of ethanolic extract of Aloe vera and Neem against *Porphyromonas gingivalis* was observed at 1.25 mg and 0.21 mg respectively while *for Aggregatibacter actinomycetemcomitans* was observed at 1.25 mg and 2.5 mg respectively. The MBC of the extract for *Porphyromonas gingivalis* was 2.5 mg and 0.312 mg respectively and for *Aggregatibacter actinomycetemcomitans* 2.5 mg and 5 mg respectively. The zone of inhibition for positive control group i.e., doxycycline gel was 33.7 mm for *Porphyromonas gingivalis* and 33.3 mm for *Aggregatibacter actinomycetemcomitan* whereas for Aloe vera gel it was 7.6 mm for *Porphyromonas gingivalis* and 3.3 mm for *Aggregatibacter actinomycetemcomitan,* and for Neem gel it was 24.3 mm for *Porphyromonas gingivalis* and 23.3 mm for *Aggregatibacter actinomycetemcomitan.*

CONCLUSION

In light of the observations drawn from our study we conclude that ethanolic extract of Aloe vera and Neem shows bacteriostatic activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetaemcomitans.* The gel prepared from Aloe vera and Neem leaf extract did show antibacterial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetaemcomitans.* In comparison with Doxycycline gel, Aloe vera and Neem gel showed reduced diffusion activity and hence, showed reduced sensitivity for *Porphyromonas gingivalis* and *Aggregatibacter actinomycetaemcomitans.* But Neem gel showed greater bacterial sensitivity when compared to Aloe vera gel.

KEYWORDS: Aloe vera, Dental plaque, Doxycycline, Herbal extract, Neem, Periodontal disease.

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INTRODUCTION

Periodontitis is the most common and serious widespread health problem because of its high prevalence rate of 25% to 55% worldwide, leading to tooth loss, and deteriorating the standard of life. [1] It is a complex disease that results due to dysbiosis between the commensal microbiome and the immune and inflammatory systems of the tissues. Moreover, genetic, environmental, and behavioral factors also play an important part in the initiation and progression of the disease.

Periodontal disease prevalence rate ranged from 24.4% in adults between the age of 30 to 34 years to 70.1% in adults aged 65 years and older. [2] Within hours of birth, the sterile oral cavity gets colonized by facultative and aerobic bacteria, [3] while on the 2nd day more anaerobic bacteria can be detected in the infant's edentulous mouth. [4,5] An adult human oral cavity comprises of about 700 commonly occurring species which can be present in any individual at any given time. [6.7] Scanty population of pathogenic bacteria is present along with commensals in the oral environment. [8]

Bacterial colonization in the oral environment is considered the primary etiology of periodontal disease while secondary etiology can be dental plaque, calculus, anatomical features like developmental grooves, short trunk, cervical enamel projections, overhanging restorations, stress, smoking, etc. [9] All of these factors gradually cause a shift of the Gram-positive population which is associated with health to a predominant Gram-negative bacterial population that is correlated with gingival and periodontal disease marking the progression of gingivitis to periodontitis [10]

Organisms strongly associated with periodontitis are *P. gingivalis, A. actinomycetemcomitans, Tannerella forsythia* (formerly, *Bacteroides forsythias*), *Treponema denticola*, and *Eikenella corrodens* causing local and systemic inflammation that would lead to the destruction of the periodontal ligament, resorption of the alveolar bone and the migration of the junctional epithelium along the tooth surface. [(11)]

During the advanced stage of the disease, the inflammation extends deeper into the tissues leading to the destruction of periodontal fibers and the gingival epithelium migrates apically forming periodontal pockets. [(12)] In periodontal disease, bacteria invade the apical most and lateral areas of the periodontal pocket wall in the intercellular spaces of the epithelium [(13,14)] The presence of bacteria such as P.g [(15)], A.a [(16,17,18)], and P.i [(15)] was reported by Hillmann and colleagues in periodontal pockets. These bacteria produce various virulent factors that cause the degradation of periodontal tissues. Because of the pathogenic potential of P.g and A.a, their importance in disease progression is utmost. More rapid degeneration in the pocket epithelium is seen in association with A.a including micro clefts and necrotic areas.

Porphyromonas gingivalis is among the major periodontal pathogens and also the most virulent micro-organisms in the pathogenesis of the periodontal disease. [(19)] It is a non-motile, asaccharolytic, gram-negative obligate anaerobic rod that forms black-pigmented colonies on blood agar plates. It is found more in deeper pockets than in shallow periodontal pockets and is present in 87.75% of sub-gingival plaques samples [(20)] of chronic periodontitis patients. P. gingivalis depends on the fermentation of amino acids for its survival. The count of P. gingivalis in diseased sites increases drastically as the disease progresses and is found in lower or non-detectable counts in sites with health. [(21)]

The second most prevalent and commonly isolated bacteria in an infected periodontal pocket is ***Aggregatibacter actinomycetemcomitans.*** This virulent periodontopathogen was 1st isolated in 1975 by Killian and Schiott in plaque samples of localized juvenile periodontitis patients, currently known as localized aggressive periodontitis. [(22)] It is a gram-negative, non-sporing, non-motile, facultative anaerobic coccobacillus. A.a has the potential to grow both supra and subgingivally as it is a microaerophilic organism. [(23)] The presence of A.a was demonstrated in every diseased tissue that was examined, with the colonies being present in both the gingival tissues as well as inside the phagocytic cells within the tissues. [(24)] The increase in A.a CFU was correlated with its presence in the periodontal tissue and periodontal pockets. [(24)]

Pockets provide an ideal niche for the growth of Gram-negative, facultative anaerobes. If not treated at the right time, the disease can progress into an even more destructive phase leading to an alveolar bone boss accompanied by tooth loss eventually. Therefore, the elimination of local factors and sub-gingival microflora are considered utmost in treating periodontal disease.

Periodontal therapy includes both mechanical and chemical therapeutic agents which help in the reduction or elimination of microbial biofilm. Conventional plaque control (scaling and root planing [SRP] and ultrasonic debridement) is considered the 1st and the crucial part of periodontal treatment have reduced effectiveness singularly because it lacks accessibility to micro-organisms in the sub-gingival environment. [(25)] Adjunctive chemotherapies enhance the results at sites not responsive to conventional mechanical therapy. [(25)]

The application of systemic antibiotics for treating periodontitis is restricted, because of the need for a higher dosage to achieve the desired concentration in GCF,

the onset of bacterial resistance, and the side effects of the drug. [26] It is also a known fact that systemic administration of antibiotics could lead to hypersensitivity allergic reactions, urticarial rash, vaginal candidiasis, liver toxicity, gastrointestinal disorder, etc. [76] Even mouthwashes are not able to reach the deeper tissues. Hence to overcome these drawbacks, the development of drug systems in the form of direct sub-gingival administration has been employed for the past 30 years. [27,28]

Goodson et al 1st in 1979 proposed the concept of controlled drug delivery for the treatment of periodontal diseases. [29] The potential effect of using this form of system is that it was able to reach the base of the periodontal pocket and it sustained for an adequate amount of time for its antimicrobial effect to occur. [30] It can be cautiously used in patients who are medically compromised and in whom periodontal surgery is contraindicated. [31] Controlled drug delivery has various transport systems such as Gels, Strips, Fibers, Films, Injectable systems, Nanoparticle systems, Microspores, and many more. Antimicrobials that can be locally administered into the mucosa include metronidazole, chlorhexidine, minocycline, doxycycline, and tetracycline, which, when inserted in periodontal pockets, can suppress or annihilate periodontopathogenic microorganisms.

The 1st locally applied controlled-release gel was of tetracycline base and was incorporated into the microtubular excipient halloysite, coated with chitosan to delay drug release. [30] Tetracycline is a broad-spectrum bacteriostatic antibiotic that works by inhibiting the protein synthesis in the bacterial cell wall. The availability of gel in a syringe form made its delivery into a subgingival environment even easier.

10% doxycycline hyclate for local drug delivery is commercialized as Atridox (DenMat, Lompoc, USA) which is a pale yellow to yellow viscous liquid and is available as a two-syringe (Syringe A - 450 mg of Atrigel and Syringe B - 42.5 mg

of active doxycycline) system, releases sub-gingival doxycycline. The viscous liquid solidifies in the subgingival environment upon contact with the crevicular fluid and subsequently allows for controlled release of the drug for a period of 7 days. It has the ability to achieve GCF conc. of 1500 ug/ml 2 hours, 1000 ug/ml at 18 hours and 140ug/ml at seven days after application, which is well above the MIC for periodontal pathogens [66] In an in-vitro study, the susceptibility of A.a to doxycycline was found to be 32-fold higher when compared to metronidazole. [37] But it tends to produce side effects like growth retardation and enamel hypoplasia and also has harmful effects on bone and tooth development. [38]

In this contemporary era, people are more inclined towards organic products, and also researchers are coming up with herbal alternatives because of their various medicinal and beneficial properties including antibacterial, antioxidant, immune-regulatory, and anti-inflammatory potentials proving as an effective antidote for various common ailments. Also, it is more preferred because they are cost-effective, relatively safe, and have decreased development of resistance, toxicity, and lesser side effects including hypersensitivity reactions, staining of teeth, etc. when compared to the conventional antimicrobial agents.

Aloe barbadensis miller commonly known as Aloe vera is a perennial, xerophytic, pea-green-colored succulent plant that belongs to the *Liliaceae* family.

The word has Arabic origin where “Alloeh” means “shining bitter substance” while Latin word “vera” means “true”. [39] In India, it is found in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra, and Tamil Nadu. [39] For thousands of years, Aloe vera has been used as a medicine for various ailments like bruises, skin infections, hair loss, burns, sinusitis, gastrointestinal (GI) pain, and hemorrhoids. [40]

The triangular fleshy leaves comprise three layers: 1. The **inner gelatinous layer** contains 99% of water and amino acids, lipids, glucomannans, sterols, and vitamins, 2. The **middle latex layer** is the yellow bitter sap containing glycosides, and anthraquinones, and 3. The **outer thick rind** synthesizes proteins and carbohydrates and is also known to have a protective function. [39] Aloe vera comprises 75 potentially known active constituents namely vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, various amino acids, etc. [39] Vitamins like A, C, and E are antioxidants and neutralize free radicals, bradykinase, an enzyme when applied topically helps in reducing inflammation, Mannose-6-phosphate, Acemannan (glucomannans) and recently found glycoprotein called Alprogen has anti-allergic and anti-inflammatory properties. [39] Aloin and emodin both acts as antibacterials, antivirals, and analgesics. [39]

Because of the above-mentioned properties, Aloe vera has also been widely used in the field of dentistry. [41,42,43,44,45,46] It is being used in the field of Periodontology to treat gingivitis [49] and periodontitis. Aloe vera reduces inflammation [47,48], swelling, and bleeding associated with periodontal disease and also promotes wound healing [48] due to the presence of the growth substance mannose-6-phosphate.

Azadirachta indica also known as Neem is an evergreen tree with various medicinal values and belongs to the Meliaceae family. It is derived from a Persian word “Azad” meaning free, “Dirakat” meaning tree and “indica” which means India, hence, it meant “free tree of India” Neem is available in most tropical and subtropical parts of India, Pakistan, and Bangladesh and is of great medicinal value and is distributed widely in the world. [53] Neem is being used in Ayurveda, Unani and Homeopathic medicine and has also made its way into modern medicine. [54] Every

part of neem i.e., its leaves, twigs, and bark are traditionally used for the treatment of inflammation, infections, fever, and skin and dental problems. [54]

The leaves possess anti-bacterial [63], anti-viral, anti-fungal, anti-inflammatory, antioxidant, immunomodulatory [55], and anti-nociceptive properties. It is also said to display anti-cariogenic, anti-mutagenic, antimalarial, anti-hyperglycemic, antifertility, and hepato-protective properties. [53,54] Neem twigs have been used in old times as an alternative to tooth brushing, to relieve toothache, and to treat oral malodor. [53,54] Neem bark and seeds also have anti-malarial, anti-fungal, anti-bacterial, anti-oxidant, and anti-fertility effects. [53,54,56]

The main active phytochemical of neem is Azadirachtin; others are gedunin, nimbidin, nimbolides, salanin, nimbin, valassin, and meliacin all of which possess anti-microbial activity. [53] All these properties make neem an effective agent for its use in periodontal therapy.

Hence, considering the benefits of herbal drugs this in-vitro study was carried out to assess and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

AIMS AND OBJECTIVES

AIM OF THE STUDY

To evaluate and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

OBJECTIVES OF THE STUDY:

1. To determine the Minimum Inhibitory Concentration (MIC) of ethanolic extract of Aloe vera and Neem on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.
2. To determine the Minimum Bactericidal Concentration (MBC) of ethanolic extract of Aloe vera and Neem on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*
3. To assess and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Porphyromonas gingivalis.*
4. To assess and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Aggregatibacter actinomycetemcomitans*.

REVIEW OF LITERATURE

Aloe vera (*Aloe barbadensis miller*), is a popular traditional Indian medicinal plant that has been used since the early 1700s and many wars had been fought to obtain control over its growing area in North Africa [(40)]. It is an evergreen short stemmed cactus-like plant that grows up to 60 – 100 cm in height in tropical climates and low rainfalls. The margin of the leaf is serrated and has white thorns. Aloe Vera has a colorless mucilaginous gel secreted by parenchymatous cells that contains majorly water and about 1-2% active compounds. Anthroquinones and their derivatives like Barbaloin-IO-aloe emodin-9 anthrone, Isobarbaloin and chromones in the leaves exert a strong purgative effect and are potent anti-microbial agents.

Neem (*Azadirachta indica*), an Indian native tree considered as "divine tree" and a village pharmacy. Parts of neem trees such as the leaf, kernel oil, bark, flower, fruit, twig, gum, and seed pulp are used for medicinal purposes. The most active phytochemical constituent of neem is azadirachtin and various other constituents possess antibacterial, antifungal, antiviral, antipyretic, anti-inflammatory, antimalarial, antitumor, and diuretic effects [(54)].

All these properties make both Aloe and Neem effective agents in the treatment of periodontal diseases.

1. **Jadhav AN et al 2021;** The authors conducted a systematic review and meta-analysis to evaluate the effect of Aloe vera in various forms such as gel, mouthwash, and dentifrice on GI and PI in comparison to various allopathic products such as chlorhexidine, metformin, chlorine dioxide, fluoridated toothpaste, and alendronate. A comprehensive electronic search for Randomized controlled trials was conducted on PubMed/MEDLINE, GOOGLE SCHOLAR, and HAND SEARCH of reference list of archived articles published till January 2020. The authors concluded from the results obtained from the meta-analyses that the beneficial effects of *Aloe vera* improved the

periodontal parameters and hence may be considered as a safe alternative drug delivery agent for the management of periodontal diseases in future [66].

2. **Anushree Choudhary et al 2019;** In this study, the authors conducted a clinical and microbiological study that evaluated the effect of Aloin, (*Aloe vera* extract) as a LDD as an adjunct to SRP. The aim was to compare the clinical and microbiological effect of Aloin on the levels of P.g and A.a. 30 CGP cases with 90 sites were selected; age ranged from 25-55 years with chronic periodontitis, with probing depth of >5 mm and radiographic evidence of bone loss were included in the study. These sites were then divided into three groups with 30 sites each. Group A was treated with aloin alone as LDD. Group B was treated with SRP and Aloin, whereas Group C received only SRP. Plaque samples were collected at baseline, 7^{th} and 30^{th} day for quantitative and qualitative analysis of P. gingivalis and A.A comitans. Aloin gel was applied again on the 7^{th} and 15^{th} days. Clinical parameters, including PI, GI, PPD and RAL were recorded at baseline, $7^{th,}$ and 30^{th} day. A custom-made stent of acrylic and UNC-15 periodontal probe was used to standardize the measurement of PPD and RAL. For microbiological analysis, samples were processed within 24 hours for isolation of strict anaerobes and were placed on non-selective blood agar plates. They were incubated in vacuum desiccator at 37º C under anaerobic conditions for 7 days. Identification was based on cell morphology, gram stain reaction, biochemical and enzymatic tests. Highly significant difference between the groups B and other two was seen at 15^{th} and 30^{th} day and no difference between group A and C was seen in terms of PI and GI. The mean PPD and RAL on comparing from baseline to 30th day in Group A;(32.45%), Group B;(49.73%), Group C;(31.63%), and Group A;(1.80%), Group B;(16.48%), Group C; (0.48%) respectively, indicating a highly statistically significant reduction in pocket depth and significant gain in RAL in Group B. On comparing from baseline to 7th day, there was statistically significant reduction found in the colonies of P.g and A.a in Group B when compared to the other groups, and on comparing from 7th to

30th day, there is no significant reduction was found among the groups respectively. This recolonization of micro-organisms at 30th day was observed due to the lack of bioavailability of the drug. The authors advised that the gel should be used at 7 days' intervals after the treatment. The authors concluded that when compared to SRP alone, greater benefits in reduction of PPD, gain in RAL and reduction in P.g and A.a when Aloin as LDD was used as an adjunct to SRP in the treatment of chronic generalized periodontitis [(51)].

3. **Lwin HY et al 2019;** The authors conducted a randomized control clinical trial to assess the efficacy of locally delivered Azadirachta indica (neem) extract gel as an adjunct to nonsurgical periodontal therapy in the management of chronic periodontitis. 44 sites from systemically healthy patients age ranging from 35-59 years old, having PPD of 3-5mm without furcation involvement were selected for the study. Dried neem leaves were used to prepare 25% of Neem gel for the study. Experiment was divided into test and control groups equally. Thorough history, clinical examination, and SRP was done for all the selected individuals. The test group received locally delivered neem extract gel and then periodontal pack was placed. On 3rd week follow-up, oral hygiene status was checked and on the 6th week clinical parameters i.e, PI, GI, PPD, and CAL were recorded again. Statistically significant reduction in PI and GI was seen in both the test and the control groups but no significant changes between the groups on the 6th week were recorded. Both reduction in PPD and gain in CAL was highly statistically significant in the test group as compared to the control group. No patient reported any discomfort or any adverse reaction was reported in any of the subjects in the study. Within the limitations of the study, the authors concluded that improved clinical results can be seen when neem extract gel is used as an adjunct to SRP [(67)].

4. **Abdelmagyd HA et al 2019;** Aim of their paper was to analyze the literature published in the research related to herbal medicine as an adjunct for scaling and root planning in past ten years. An internet search using search engines - Google, Research gate and PubMed

was carried out. It was noted that the two most populated countries i.e., India and China have been using herbal medicines for over 2000 years. The natural phytochemicals present in herbal drugs have demonstrated to be effective substitutes to artificial antimicrobial agents. The herbs frequently in recent clinical trials for treatment of periodontitis are Babul (*Acacia catechu*), Aloe vera (*Aloe barbadensis Mille*r), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*), Lemongrass (*Cymbopogon citratus*), Tulsi (*Ocimum sanctum*), *Coriandrum sativum,* and Meswak (*Salvadora persica*). The authors concluded that herbal medicine is proving to be potential effective competitor to modern medicine as an adjunct to SRP. However, more evidence number of clinical trials are required to further establish herbal medication as a reliable treatment modality for periodontal therapies [(68)].

5. **Jain S et al 2016;** The main aim of the authors in this study was to determine the anti-microbial and inhibitory activities of various concentration of aloe vera gel against oral pathogenic bacteria. Subgingival calculus samples and periapical and periodontal abscess contents were collected from 20 patients and transferred to thioglycolate broth. This samples were then incubated in blood agar at 37ºC for 48 hours in anaerobic jar. Bacterial colonies were identified using gram staining method and biochemical fermentation tests. Each isolated colony of identified species was cultured separately in Muller-Jilton broth at 37ºC for 24 hours. Aloe vera gel was obtained from the solid mucilaginous layer of the mature fresh leaves and stored in a sterile container. MIC was measured using the microdilution method and antibacterial property by the disc diffusion method. The samples collected from patients confirmed the presence of strains of *A.a*, *Clostridium bacilli*, *S. mutans,* and *Steph. Aureus. AVG showed the antibacterial property at higher concentrations (100% and 50%) while* at lower concentrations (25%, 12.5%) there was no effect on the bacteria. At 100% AVG concentration average zone of inhibition measured was 6.9mm, Ciprofloxacin (30mcg) showed 7.4mm, and Ofloxacin (5mcg) showed 4.6 mm in *A.a respectively.* MIC of AVG against *A.a* was found to be at 25%. The authors concluded with the obtained results that Aloe vera gel was effective against both gram-

positive and gram-negative bacteria proving it to be an effective alternative bacterial agent to prevent and treat some oral infectious diseases at higher concentration [(49)].

6. **Vennila K et al 2016;** Evaluated effectiveness of herbal local drug delivery systems as an alternative for systemic therapy in managing the chronic periodontal disease. In this study, 10% neem oil chip was used as a local drug delivery system in 20 otherwise healthy patients with the bilateral periodontal probing depth of 5-6 mm. After scaling and root planning (SRP), 10% non-absorbable neem chip was placed in the pocket in one side of the arch. Other side received SRP only. Clinical parameters such as PI, GI, Sulcus bleeding index, and PPD were recorded on the baseline, 7^{th} day, and 21^{st} day. Plaque samples were obtained for a microbiological study on the baseline, 7^{th} day and 21^{st} day. P.g strains were seen using quantitative and qualitative PCR assay. The mean PI and GI reduced significantly from baseline to 7^{th} day and 21^{st} day respectively. At test site, highly significant reduction in the mean PPD at 21^{st} day from the baseline was observed. Qualitative PCR assay showed the presence of P.g strains in the Group I (control site) and near complete absence of P.g in Group II (test site) at the 7^{th} day. The authors concluded that sustained release systems prevent the recolonization of pathogens for long period, a significant reduction in the clinical parameters compared with conventional therapy and a significant reduction in qualitative and quantitative counts of P.g in response to neem local drug delivery [(57)].

7. **Mehta WP et al 2015;** The author conducted a clinico-microbilogical study that compared and evaluated the efficacy of Neem and Tetracycline as an LDD when used as an adjunct to SRP. The study included a total of 15 systemically healthy patients in the age group of 25-55 years with atleast 3 non-adjacent sites with pocket ranging from 4 to 7 mm and giving no previous history of allergy to tetracycline. For the study, 25% of neem was incorporated into collagen fiber delivery system. Baseline parameters for PI, GI and PPD and plaque samples for microbiological analysis for A.a, P.g, P.i, and F.n were obtained, After SRP was performed, all the patients were divided into three groups were Group A- SRP, Group

B- SRP with tetracycline fibers, and Group C- SRP along with neem fibers. The clinical and microbiological parameters were further evaluated at 1 month and 3 month intervals. Significant reduction in PI, GI and PPD was seen at 1-month interval from baseline was observed in all the three groups. But at 3-month interval, slight increase in PI and GI was seen in respect to all the groups as compared to 1 month which was not statistically significant. At 1-month interval from baseline, statistically significant reduction in A.a, P.g, P.i, and F.n was found in all 3 groups but at 3-month interval the count increase slightly when compared to 1 month. The bacterial count was higher in the control group as compared to the test group. Intergroup comparison of microbiological analysis, two test group showed higher reduction as compared to control group. Slightly better results were seen in the neem gel group. The authors concluded that LDD of tetracycline and neem enhanced the periodontal status clinically and reduced pathogenic bacteria counts [(59)].

8. **Kudalkar et al 2014;** This in-vitro study was designed to evaluate the anti-inflammatory effect of Neem and Aloe vera as compared to subantimicrobial dose doxycycline on MMP-2 and MMP-9. A total of 30 patients with PPD/CAL of 5 mm or more and having bleeding on probing. Gingival tissue samples were collected from these patients, washed in saline and then transferred into small sterile plastic vials containing transport medium. Extraction of MMP- 2,9 was done by mixing the tissues with Tris buffer, homogenized with Triton X-100 2.5% and centrifuged at 6000 rpm for 30 min at 4°C. The resultant supernatant was separated and used for analysis. The Neem solution was prepared by dissolving 15 mg of Neem extract in 10 ml of distilled water (1500 μg/ml). The Aloe vera solution was prepared by dissolving 20 mg of Aloe vera in 10 ml of distilled water (2000 μg/ml). The doxycycline solution was prepared by dissolving 3 mg of doxycycline in 10 ml of distilled water (300 μg/ml). For comparison 50 μl of gingival tissue extract was preincubated with freshly prepared solution of 50 μl each of *Neem*, Aloe vera, doxycycline solution separately, for 60 min at room temperature in separate vials. The mean values showed an 82.1%, 53.5%, and 20.9% reduction in the MMP-2 activity with the addition of

doxycycline, Neem and Aloe vera respectively. The mean values showed an 82.7%, 52.5%, and 20.4% reduction in the MMP-9 activity with the addition of doxycycline, Neem and Aloe vera respectively. On the basis of the data derived from the current study, the authors concluded that both Neem and Aloe vera had inhibitory effects on MMP-2 and MMP-9[(46)].

9. **Ahamed S et al 2013;** In this study, the authors made an attempt to know the efficacy of controlled local drug delivery of doxycycline as an adjunct for treating chronic periodontitis. A total of 12 patients aged ranging from 25 to 55 years were selected from the outpatient department for the study. 30 sites each with 6-7 mm pockets were divided into test group which received SRP and Atridox gel placement and control group received only SRP. Plaque samples were collected before recording any clinical parameters and were placed in sterile pellets. The clinical parameters included PI, BOP, GI recorded at baseline, 30^{th}, 90^{th} and 180^{th} days while PPD, and CAL at baseline, 90^{th} and at 180^{th} day. After thorough scaling and root planning, the test group received Atridox gel which was formulated by mixing two syringes as instructed by the manufacturers. Post-op instructions were given to all the patients included in the study. Intragroup comparison for both BI and PI showed a statistically significant reduction in both the test and the control groups while intergroup comparison was found to have no statistical significance. Intragroup comparison for BOP showed a reduction in the percentage of bleeding scores at test sites for both groups while intergroup comparison showed highly statistically significant results at 180^{th} days from baseline for the experimental group. Both groups showed a statistically significant reduction in the percentage of bleeding scores at 180^{th} day from baseline. But the mean reduction in probing pocket depth was found to be highly statistically significant in the experimental group. the mean gain in attachment in experimental group was found to be highly significant when compared to control group. In microbiological analysis, reduction in both cocci and non-motile bacilli was observed form baseline to 90^{th} day in both the experimental and control group but the difference among the groups were not significant. Reduction in motile bacilli and spirochete was seen to be statistically

significant in the experimental group. The results of the study demonstrated that the sites in experimental group showed better results as compared to the control group. Adjunctive use of 10% doxycycline hyclate gel clearly demonstrated superior results in clinical parameters in the management of periodontal diseases [33].

10. **Fani M et al 2012;** In the present in-vitro study, authors investigated the inhibitory activities of Aloe vera gel on 20 isolates of cariogenic (*Streptococcus mutans*), periodontopathic (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*) and an opportunistic periodontopathogen (*Bacteroides fragilis*) isolated from patients with dental caries and periodontal diseases. S. mutans were isolated from carious teeth and then was subcultured to obtain pure cultures of the organisms. P.g, A.a and B.f was isolated from either localised aggressive periodontitis or aggressive periodontitis patients having the deepest pockets (PPD $\geq$ 6 mm) by using sterile paper points and then subcultures to obtain pure cultures of individual bacteria. Aloe vera gel was prepared by cutting the leaves transversely after washing it properly with fresh water and removing their epidermides. 100 grams of mucilaginious gel was mixed in 1L od 2% DMSO and stored at 4ºC. For antimicrobial susceptibility testing both qualitative using disk diffusion method and quantitative using Broth microdilution method. Undiluted Aloe vera gel gel produced significant growth inhibition. It was noted that the greater the concentration, the larger the zone of inhibition. At 100% Aloe vera gel conc, zone of inhibition of S. mutans was widest 54 mm, A.a was 38mm, B.f was 40 mm and P.g was 32 mm. At 50% Aloe vera gel conc, zone of inhibition of S. mutans was widest again 30 mm, A.a was 21 mm, B.f was 22 mm and P.gingivalis was 17 mm and at 25% Aloe vera gel conc, zone of inhibition of S. mutans was 17 mm, A.a was 12 mm, B.f was 12 mm and P.g was 9 mm. The mean MIC values for Aloe vera gel measured against clinical isolates of S. mutans, A.a, B.f and P.g were 12.5, 25, 50, and 25 μg/ml, respectively. The authors based on their data concluded that, Aloe vera gel at optimum concs in toothpastes and mouthwashes can be used for prevention of dental caries and periodontal diseases [50].

11. **Deo V et al 2011;** The aim of this clinical study was to evaluate the efficacy of doxycycline hyclate 10% as an adjunct to SRP for the treatment of chronic periodontitis. 60 healthy individuals were selected aged 30 – 45 having one persistent pocket of 5-7mm around a molar/premolar. Patients were then divided into two groups; one group received SRP followed by local delivery of doxycycline hyclate 10% and the other group received SRP followed by local delivery of gel containing placebo. Clinical measurements were recorded at 6 weeks following the initial visits (baseline) and again at 6 months that included PI, and periodontal bleeding index (PBI). PPD, CAL, and gingival recession. After measuring the baseline values, a thorough SRP was done under LA for both groups. The test group received 10% doxycycline hyclate in a bio-absorbable vehicle, which was supplied as an Atrigel delivery system. A 23-gauge blunt cannula was attached to the delivery syringe and gel was expressed into the periodontal pocket. The control sites were treated by SRP followed by local delivery of a gel containing glycerine as a placebo. Patients were then recalled at 6 months to record the measurements. The mean PPD reduction and mean CAL gain in the test and the control group were statistically significant but significantly greater in the test group. The mean increase in REC at the 6-month examination increased significantly in both groups but no statistically significant difference was found. The authors concluded that the use of doxycycline hyclate 10% as an adjunct to SRP provides more favorable and statistically significant reductions in PPD and gains in CAL compared to SRP [(32)].

12. **Larsen T 1991;** The aim of this study was to investigate the occurrence of Doxycycline resistant bacteria in subgingival plaque and oral cavity after local delivery of doxycycline. Five patients with periodontitis were selected for the study. Baseline microbiological samples were collected from both the test and the control site. Thorough SRP was done for all the patients after sample collection and then 10 μg/ml locally delivered doxycycline was inserted in the periodontal pockets once a week for 3 weeks. Subgingival microbiological samples were collected after the removal of supragingival plaque at the end of treatment

i.e., 3rd, 13th, 26th, and 52nd week. Samples were incubated at 35°C in an anaerobic chamber containing 70% N_2, 20% H, and 10% C02 for 7 days. Doxycycline-resistant bacteria at baseline was 0.7% at the test site and 8.9% at the control site which increased to 21.7% at test site after doxycycline application. It gradually decreased to 1.7% at 13th week while the control site showed no increase in Doxycycline-resistant bacteria. The author concluded that, local application of doxycycline in periodontal pocket caused a transient increase in resistance in oral microflora which returned to normal by the end of 3rd month [(69)].

MATERIALS AND METHODS

ARMAMENTARIUM:

For extract preparation

- Aloe vera leaves
- Neem leaves
- 90% Ethanol
- Grinder
- Sieve
- Weighing Scale
- Beaker
- Conical Flask
- Mortar & Pestle
- Whatman No. 1 filter paper

For MIC & MBC

- Ethanolic extract of Aloe vera and Neem
- ATCC strains of *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans.*
- Weighing Scale
- DMSO
- Eppendorf Tubes
- Vortex mixture
- Micropipettes (10 µL, 100 µL, 1000 µL)
- BHI broth and agar
- Anaerobic Jar
- Incubator
- Blood agar plates
- Platinum Inoculum loops
- Electric loop sterilizer

For gel preparation

- Ethanolic extract of Aloe vera and Neem
- Doxycycline (10%)
- Carbopol 940
- Tween 80
- Sodium Methyl paraben
- Sodium Propyl paraben
- Distilled water
- Glycerine
- Digital weighing scale
- Glass funnel
- Glass measuring cylinder
- Pipette
- Propylene glycol
- Sodium methyl paraben
- Sodium propyl paraben
- Sodium benzoate
- Triethanolamine

For antimicrobial activity of Aloe vera, Neem, and Doxycycline gel

- MHA
- 90mm diameter petri plates
- Sterile cork borer
- Aloe vera gel
- Neem gel
- Doxycycline gel (10%)

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.

This study was conducted in the Department of Periodontics, KAHER's KLE V K Institute of Dental Sciences, Belagavi utilizing the outpatient department facilities.

The laboratory procedure was undertaken at KAHER's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi.

The leaves of Aloe vera and Neem were collected and authenticated from KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi.

The preparation of ethanolic leaf extract of Aloe vera and Neem was prepared at KAHER's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi.
The Aloe vera gel, Neem gel and Doxycycline gel (10%) were prepared and collected from KAHER's KLE College of Pharmacy, Belagavi.
The experiment was divided into two groups:

Group 1: Control group (no gel added), Doxycycline gel (10%), Aloe vera gel and Neem gel against *P. gingivalis*.

Group 2: Control group (no gel added), Doxycycline gel (10%), Aloe vera gel, and Neem gel against *A. actinomycetemcomitans*.

METHODOLOGY

1. Extract preparation:

The fresh leaves of Aloe vera and neem were collected and authenticated from KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi, respectively and stored in airtight containers. After washing the leaves with distilled water, the leaves were dried at 70ºC for 2 hours and then powdered. Approximately 200g of Neem powder was soaked in 1600 ml of 90% ethanol and 50g of Aloe vera powder in 200ml of 90% ethanol for 72 h at room temperature. The extracts were filtered using Whatman No.1 filter paper (Fig 3). Evaporation of the filtrate was done using the "New Brunswick scientific Excella E24 Incubator Shaker Series" until it was concentrated. The extract was sterilized overnight by UV irradiation and was stored at 4ºC. The stock solution of extract was prepared by dissolving 200mg of crude extract in 10 ml 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: Fresh Aloe vera leaves

Fig 2: Fresh Neem leaves

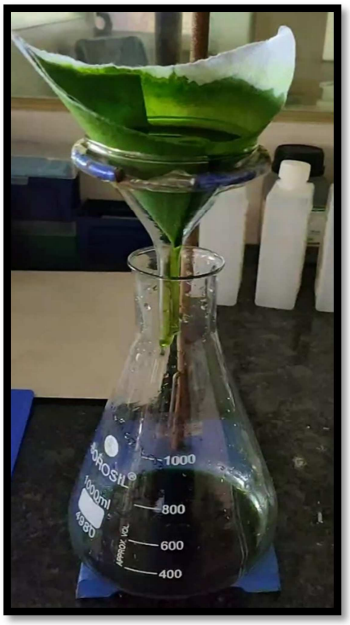

Fig 3: Filtration

Fig 4: New Brunswick scientific Excella E24 Incubator shaker series

Fig 5: Labotech Bacteriological Incubator

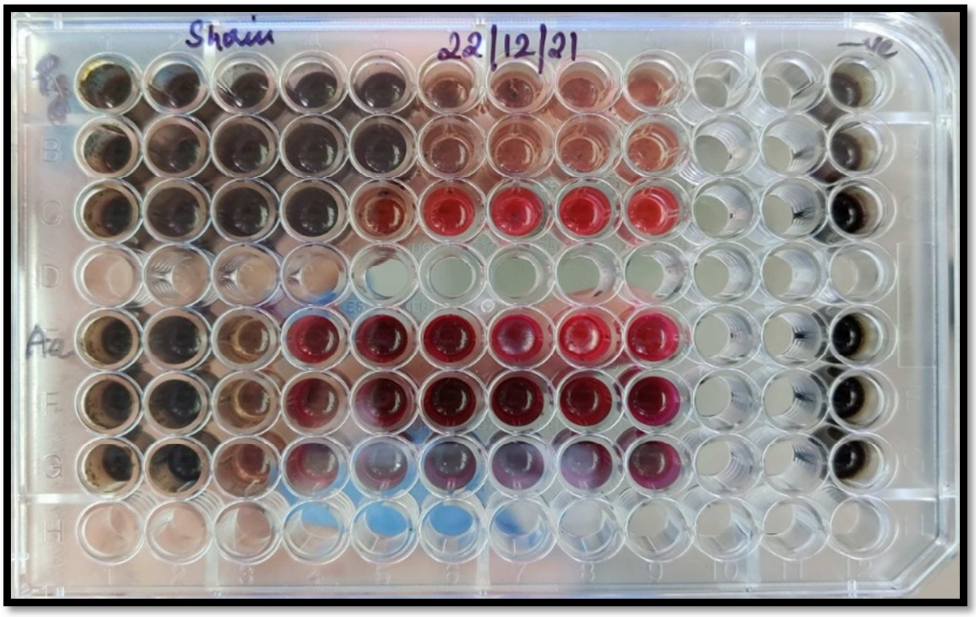

Fig 6: MIC of Neem extract against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*

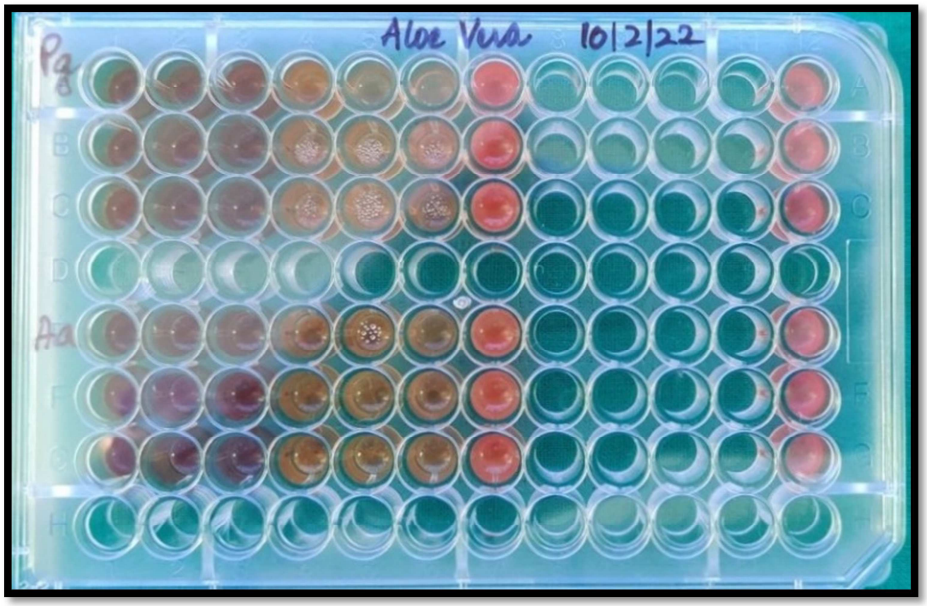

Fig 7: MIC of Aloe vera extracts against *Aggregatibacter actinomycetemcomitans*

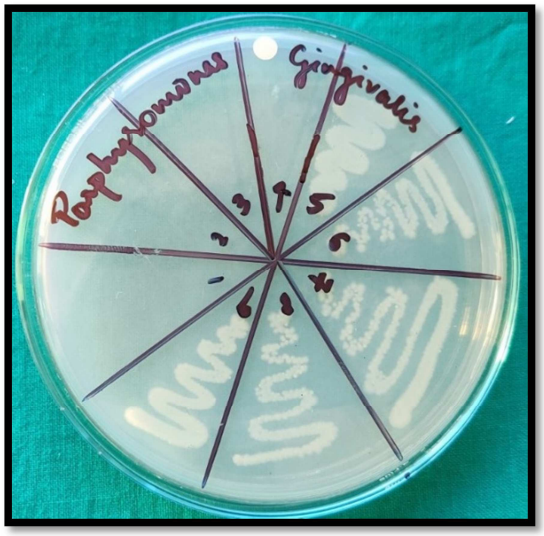

Fig 8: MBC of Neem extract against *Porphyromonas gingivalis*

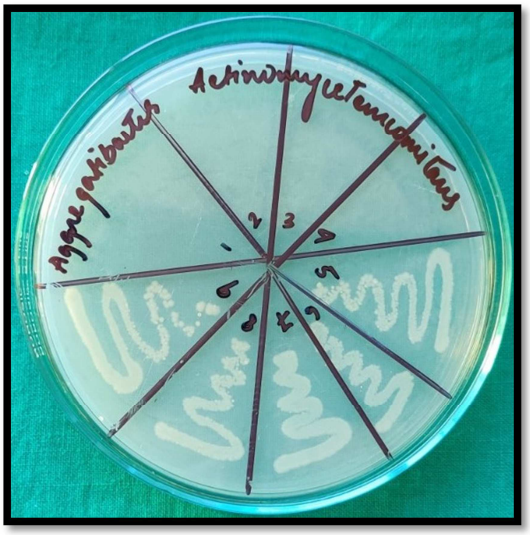

Fig 9: MBC of Neem extract against *Aggregatibacter actinomycetemcomitans*

Fig 10: MBC of Aloe vera extract against *Porphyromonas gingivalis*

Fig 11: MBC of Aloe vera extract against *Aggregatibacter actinomycetemcomitans*

Fig 12: Magnetic stirrer

Fig 13: High speed propeller stirrer

Fig 14: Prepared Doxycycline, *Aloe vera* and Neem

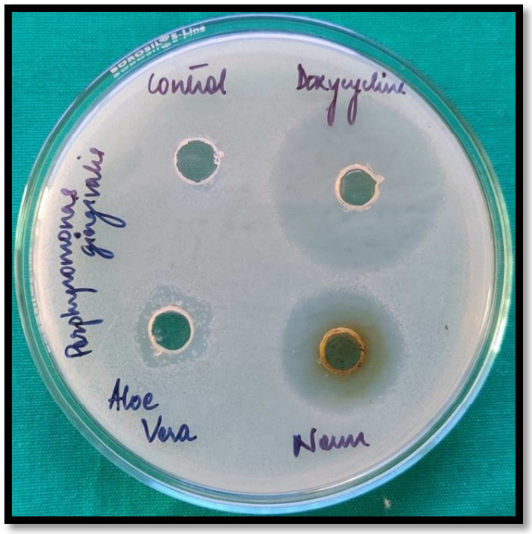

Fig 15: Agar well diffusion for *Porphyromonas gingivalis*

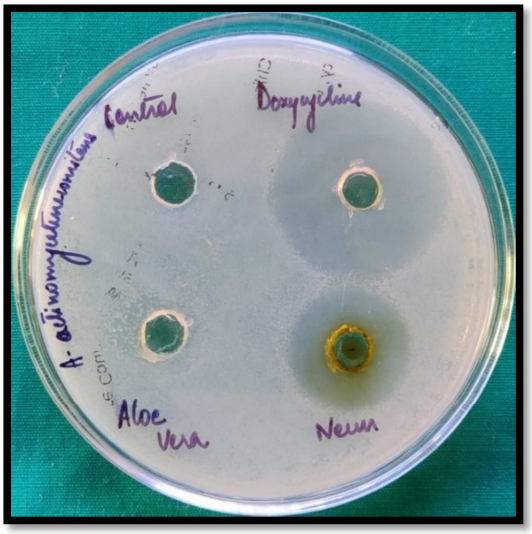

Fig 16: Agar well diffusion for *Aggregatibacter actinomycetemcomitans*

2. **Inoculum preparation:**

BHI broth and ATCC strain of *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans* were used for the preparation of inoculum. A sterile loop was used to pick the colonies, and was 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 complement the turbidity of 0.5 McFarland guidelines.

3. **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. A total of 100 µl of broth was added to all 10 wells in triplicates. In the first well 100 µl of the extract was added and serially diluted to the required concentrations up to the ninth well for Neem and the seventh well for Aloe vera. A similar procedure was performed in the other two rows of the well plates. The 96 well plates were kept for incubation in McIntosh and Fildes' anaerobic jar (Fig 5) followed by the addition of resazurin reagent after 48 hours and was observed after 4 hrs 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 quality photographs (Fig 6 & Fig 7).

Note: Separate 96 well plates were used for each extract.

4. Minimum Bactericidal Concentration (MBC)

MBC was checked using the MIC values of both Aloe vera and neem extracts with the help of BHI agar plates in triplicates. With the help of inoculating loop, streakes were made on the BHI agar plates. The plates were sealed with the paraffin film and were incubated in the bacteriological incubator for 12 hrs. At the end, minimum concentration at which the bacteria did not show any growth was considered as MBC value. (Figure 8 - 11)

Both Aloe vera and Neem gel were prepared by considering the MIC and MBC results obtained from the above procedures.

5. Gel preparation:

SL No.	Ingredients	Concentration (mg)	Uses
1.	Aloe vera	20	Active ingredient
2.	Neem	20	Active ingredient
3.	10 % Doxycycline	20	Active ingredient
4.	Carbopol	60	Gel forming agent
6.	Tween 80	20	Dispersing agent
7.	Propylene glycol	60	Humectant and dispersing agent
8.	Sodium methyl paraben	1	Bactericidal agent
9.	Sodium propyl paraben	0.2	Bactericidal agent
10.	Sodium benzoate	10	Bacteriostatic agent
11.	Triethanolamine	10	pH adjusting agent to pH7
12.	Distilled water qs	100	Solvent

60 mg of Carbopol 940 was soaked in distilled water at room temperature, which was then stirred continuously using a magnetic stirrer for 24 hours. The 10% Doxycycline, Neem, and Aloe vera extract were uniformly dispersed by triturating it in a mortar & pestle along with Tween 80 & propylene glycol. 30 ml of distilled water was then added along with the preservative & stirred with a magnetic stirrer for 30 mins. The Doxycycline, Neem, and Aloe vera 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 dropwise using a high-speed propeller stirrer for 10 mins. The gel thus procured was stored in an airtight container. (Figure 14)

6. Agar well diffusion assay

The "agar well diffusion assay" was performed on bacteriological agar plates. The BHI agar plate was prepared by adding 5.2 g BHI agar to 100 ml of distilled water and was sterilized in a steam sterilizer. It was then kept at room temperature for 10-15min to cool 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 µL] with a sterile cotton spreader. Following this, uniform aseptic wells were made using a cork borer. To these wells, sample reagents [100 µL doxycycline, 100 µL neem extract, and 100 µL aloe vera extract] were added and placed in 37℃, CO_2 incubator [jar of desiccator]. They were observed for diffusion through 24-72 hours of incubation. A growth pattern was observed on the plates and results were noted against Doxycycline as standard. (Figure 15 and 16).

RESULTS AND OBSERVATIONS

MIC and MBC for Aloe vera and Neem extract was determined. The antibacterial effects of 100 µl of Doxycycline gel (control) and 100 µl of Aloe vera and Neem extract (test) was determined against standard ATCC strains namely "*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans"* using well diffusion assay (zone of inhibition).

Minimum Inhibitory Concentration (MIC)

The antibacterial effects of Aloe vera and Neem extract were evaluated using Broth dilution assay (Resazurin) for its "minimum inhibitory concentration (MIC)"(**Table 1**)

Table. 1. Minimum inhibitory concentration (MIC) of Aloe vera and Neem extract in milligram (mg).

Sr.No.	Samples	*Porphyromonas gingivalis*		*Aggregatibacter actinomycetemcomitans*	
		MIC	**Average**	**MIC**	**Average**
1	Aloe vera **(extract)**	1.25	1.25	1.25	1.25
		1.25		1.25	
		1.25		1.25	
2	Neem **(extract)**	0.156	0.21	2.5	2.5
		0.156		2.5	
		0.312		2.5	

Minimum Bactericidal Concentration (MBC)

Agar plate assay was used to determine "minimum bactericidal concentration (MBC)" of Aloe vera and Neem extract (**Table 2**).

Table. 2. Minimum bactericidal concentration of Aloe vera and Neem extract in milligram (mg).

Sr.No	Samples	*Porphyromonas gingivalis*		*Aggregatibacter actinomycetemcomitans*	
		MBC	**Average**	**MBC**	**Average**
1.	Aloe vera **(extract)**	2.5	2.5	2.5	2.5
		2.5		2.5	
		2.5		2.5	
2.	Neem **(extract)**	0.312	0.312	5	5
		0.312		5	
		0.312		5	

The antibacterial effects of the prepared Aloe vera, Neem and Doxycycline 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 Aloe vera, Neem and Doxycycline gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

Groups	***Porphyromonas gingivalis***			***Aggregatibacter actinomycetemcomitans***		
Doxycycline	34 mm	32mm	35mm	34 mm	34mm	32mm
Aloe vera	13 mm	10mm	0	10 mm	0	0
Neem	26 mm	25mm	22mm	24 mm	22mm	24mm

mm: millimeter, NZI: No zone of Inhibition

Descriptive analysis of zone of inhibition is described in **Table 4**.

Table 4. Mean and SD for Zone of Inhibition.

Groups	*Porphyromonas gingivalis*		*Aggregatibacter actinomycetemcomitans*	
	Mean	**SD**	**Mean**	**SD**
Doxycycline	33.67	1.53	33.33	1.15
Aloe vera	11.50	2.12	10.00	-
Neem	24.33	2.08	23.33	1.15

The intergroup comparison between the test and the control group was determined using an unpaired t-test while the intragroup comparison between the two test groups was determined using a one-way ANOVA test. The results of these inter and intra-group comparisons are listed in **Table 5**.

Table 5. Inter and Intragroup comparison of Doxycycline, Aloe vera, and Neem gel against P. gingivalis and Aa

	Porphyromonas gingivalis		***Aggregatibacter actinomycetemcomitans***	
	t-test	**p-value**	**t-test**	**p-value**
Doxycycline gel – Aloe gel	12.7	0.0008	-	-
Doxycycline gel – Neem gel	6.3	0.0033	10.6	0.0004
Neem gel - Aloe vera gel	6.7	0.0068	-	- -

The inter and intragroup comparison between the test groups and the control group were found to be statistically significant for P.g at p-value<0.05 while for A.a only the intergroup comparison between the doxycycline gel and neem gel was statistically significant at p-value<0.05.

DISCUSSION

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

Its association with diseases of the periodontium is site specific. The plaque present on the marginal gingiva causes gingivitis, aids in calculus formation, and the tissue-associated subgingival plaque is important in tissue destruction. The most commonly found bacteria to which periodontal tissue breakdown is attributed 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 [10]

Conventional therapy i.e., SRP often have reduced effectiveness in the subgingival environment. Hence, adjunctive chemotherapies are provided for better clinical and microbiological outcomes. These chemotherapies can be in the form of local or systemic antibiotics. Locally delivered agents are preferred more because they actively achieve desirable concentration in the subgingival environment and reduce bacterial resistance and systemic side effects of the drug.

Doxycycline is a broad-spectrum bacteriostatic agent that works by inhibiting bacterial protein synthesis. It is applied subgingivally to act against various gram-positive and gram-negative aerobic and anaerobic bacteria, spirochetes, and

mycoplasma. It maintains substantivity in the subgingival environment by solidifying from flowable gel on contact with saliva, water or aqueous fluids. Boren et al [67,68], Garrett et al [69] and Johnson et al [70] reported that doxycycline showed superior results in terms of CAL gain, PPD reduction, reduced bleeding on probing and an overall reduction of periodontal pathogens at 180 days.

However, the use of doxycycline has been contraindicated in pregnant patients because of its teratogenicity and also in infancy and early childhood up to the age of 8 years. Tetracycline has been known to cross the placental barrier and is found in fetal tissues causing enamel hypoplasia of the developing teeth and growth impairment of fetal long bones. In a study by Zambon JJ (1983), 82% of 19 clinical isolates of A.a were found resistant to tetracycline and carried the tetB resistance determinant.

To overcome these drawbacks, various alternative sources are being searched for curing oral diseases. One among these is traditional herbal medicines. Aloe Vera and Neem contain natural phytochemicals that have been considered useful alternatives to synthetic drugsand have been used therapeutically for a long time to treat many diseases.

Aloe vera (*Aloe barbadensis miller*), a popular traditional Indian medicinal plant that has been used since the early 1700s and many wars had been fought to obtain control over its growing area in North Africa [40]. Free Anthroquinones and their derivatives like Barbaloin-IO-aloe emodin-9 anthrone, Isobarbaloin and chromones in Aloe Vera leaves exert a strong purgative effect and are potent anti-microbial agents.

Neem (*Azadirachta indica*), an Indian native tree considered as "divine tree" and a village pharmacy. Parts of neem trees such as the leaf, kernel oil, bark, flower, fruit, twig, gum, and seed pulp are used for medicinal purposes. Neem possesses antibacterial, antifungal, antiviral, antipyretic, anti-inflammatory, antimalarial, antitumor, and diuretic effects [(54)].

All these properties make both Aloe and Neem effective agents in the treatment of periodontal diseases. Bhat et al [(40)] concluded that the subgingival application of Aloe vera gel in periodontal pockets after mechanical debridement resulted in encouraging findings in terms of clinical parameters.

In the present study, the antibacterial efficacy of traditional Indian Herbs namely Aloe vera and Neem was evaluated where ethanolic extraction of the leaves of the plant was carried out and compared with the standard 10% doxycycline gel. The MIC of Aloe vera and Neem extracts (determined using broth dilution assay) against P.g was observed at 1.25 mg and 0.21 mg respectively while for A.a it was 1.25 mg and 2.5 mg respectively (**Table 1**).

The MBC of the Aloe vera and Neem extracts (determined using agar plate assay) for P.g was 2.5 mg and 0.312 mg respectively while for A.a it was 2.5 mg and 5 mg respectively (**Table 2**). These results show that the active constituents of both Aloe vera and Neem show significant anti-bacterial activity.

The antimicrobial effects of the prepared Aloe vera and Neem gel were assessed using agar well diffusion assay. 100 µl of prepared Aloe vera gel showed a zone of inhibition of 7.6 mm for P.g and 3.3 mm for A.a. For Neem gel zone of inhibition was 24.3 mm for P.g and 23.3 mm for A.a. For positive control group (10%

doxycycline gel) the zone of inhibition was 33.7 mm for P.g and 33.3 mm for A.a (**Table 3**). This implies that Doxycycline gel had the widest zone of inhibition when compared to Neem gel and Aloe vera gel.

The mean and standard deviation of the zone of inhibition for P.g with Doxycycline gel was 33.67±1.53, Aloe vera gel was 11.50±2.12 and Neem gel was 24.33±2.08. Zone of inhibition for A.a with Doxycycline gel was 33.33±1.15, Aloe vera gel was 10±0.0 and Neem gel was 23.33±1.15 (**Table 4**).

The intergroup comparison between the Doxycycline gel, Aloe vera gel, and Neem gel was determined using an unpaired t-test while the intragroup comparison (Doxycycline gel – Aloe gel, Doxycycline gel – Neem gel, Neem gel - Aloe vera gel) was determined using one-way ANOVA test. The results of these inter and intra-group comparisons are listed in **Table 5**. For P.g, the comparison between Doxycycline gel – Aloe gel, Doxycycline gel – Neem gel, and Neem gel - Aloe vera gel was found to be statistically significant ($p<0.05$). For A.a. the comparison between Doxycycline gel – Neem gel was found to be statistically significant ($p<0.05$). The above findings suggest that all three prepared gels showed antibacterial activity but Doxycycline gel was found to be more efficacious than the Neem and Aloe vera gel against both the pathogens. Among the test groups, Neem gel was found to have better antibacterial activity than the Aloe vera gel.

Comparable outcomes were recorded by Jain S et al [(49)] in an in-vitro study where Aloe vera gel was compared with Ciprofloxacin and ofloxacin. Aloe vera showed anti-bacterial property at 100% and 50% concentration against A.a while at lower concentration, no effect was seen against the bacteria. At 100% concentration, zone of inhibition for A.a was 6.9 mm, Ciprofloxacin (30mcg) showed 7.4mm, and

Ofloxacin (5mcg) showed 4.6 mm in *A.a respectively. MIC of Aloe vera gel against A.a was 25%.*

Contradictory results were reported by Fani M et al [50] in an in-vitro study where the zone of inhibition for undiluted aloe vera gel 100% was 38 mm for A.a and 32mm for P.g, at 50% concentration, zone of inhibition for A.a was 21mm and P.g was 17 mm while at 25% concentration, zone of inhibition for A.a was 12 mm and P.g was 9 mm. The mean MIC values for A.a and P.g were found to be 25 μg/ml.

Aloin gel can significantly reduce CFU for both P.g and A.a in the group that received both SRP and the gel [51]. Aloe vera can also be used to control inflammation and bacterial contamination around implants [52].

Various studies stated that Neem and Aloe vera extracts in the form of gel, chips or mouthwashes can be used as an adjunct to SRP with significant improvement in clinical parameters and reduction of P.g and A.a count [46,57,58,59,67].

In the present study a statistically significant difference among the three groups was observed($p<0.05$). Neem gel had a wider zone of inhibition than the Aloe vera gel against both P.g and A.a while the positive control group doxycycline gel had the widest zone of inhibition of all the groups.

The findings of the present study imply that, the prepared Neem gel showed potent anti-bacterial effects against the chosen periodontal pathogens when compared to Aloe vera gel but Doxycycline gel had superior antibacterial activity against P.g and A.a when compared to Neem and Aloe vera gel.

SUMMARY & CONCLUSION

The purpose of the present study was "to evaluate and compare the antibacterial efficacy of Aloe vera gel and Neem gel with Doxycycline gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans"*. The Aloe vera gel, Neem gel, and Doxycycline gel (10%) were prepared and collected from "KAHER's KLE College of Pharmacy, Belagavi". The laboratory procedure was performed at "KAHER's Dr. Prabhakar Kore Basic Science Research Center (BSRC), Belagavi".

Ethanolic extracts of Aloe vera and Neem were prepared from the fresh leaves for the determination of "Minimum Inhibitory Concentration (MIC)" using the Broth dilution method and "Minimum Bactericidal Concentration (MBC)" using the streak method. Aloe vera gel, Neem gel, and Doxycycline gel (10%) were prepared from the stock solution of extracts. Two bacterial agar culture plates each of "*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans"* was prepared and 4 uniform aseptic wells were made; 1st well was kept empty (negative control), 2nd well with doxycycline (positive control group), 3rd and 4th well with Aloe vera gel and Neem gel (test groups) and Agar well diffusion assay was performed for the determination of the "zone of inhibition". This would help us measure the susceptibility of "*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans"* against the prepared Aloe vera gel, Neem gel, and Doxycycline gel (10%).

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

1. "Minimum Inhibitory Concentration (MIC)" of ethanolic extract of Aloe vera and Neem on *Porphyromonas gingivalis* was 1.25 mg, and 0.21 mg and on *Aggregatibacter actinomycetemcomitans* was 1.25 mg, and 2.5 mg respectively.

2. "Minimum Bactericidal Concentration (MBC)" of ethanolic extract of Aloe vera and Neem on *Porphyromonas gingivalis* was 2.5 mg and 0.312 mg and for *Aggregatibacter actinomycetemcomitan* was 2.5 mg and 5 mg respectively.

3. These results show that the active constituents of both Aloe vera and Neem had significant anti-bacterial activity.

4. The zone of inhibition for positive control group i.e., doxycycline gel was 33.7 mm for *Porphyromonas gingivalis* and 33.3 mm for *Aggregatibacter actinomycetemcomitan* whereas for Aloe vera gel it was 7.6 mm for *Porphyromonas gingivalis* and 3.3 mm for *Aggregatibacter actinomycetemcomitan*, and for Neem gel it was 24.3 mm for *Porphyromonas gingivalis* and 23.3 mm for *Aggregatibacter actinomycetemcomitan.*

5. This implies that the prepared Neem gel showed potent anti-bacterial effects against the chosen periodontal pathogens when compared to Aloe vera gel but both had reduced effect when compared to the Doxycycline gel. Hence, Doxycycline gel had superior antibacterial activity against P.g and A.a when compared to Neem and Aloe vera gel.

Under the limitations of the study it can be concluded that further investigations at biomolecular levels to identify the active phytochemical constituents responsible for the antibacterial effect and clinical applications of Aloe vera and Neem gel are required to elucidate and prove the clinical efficacy against periodontal pathogens.

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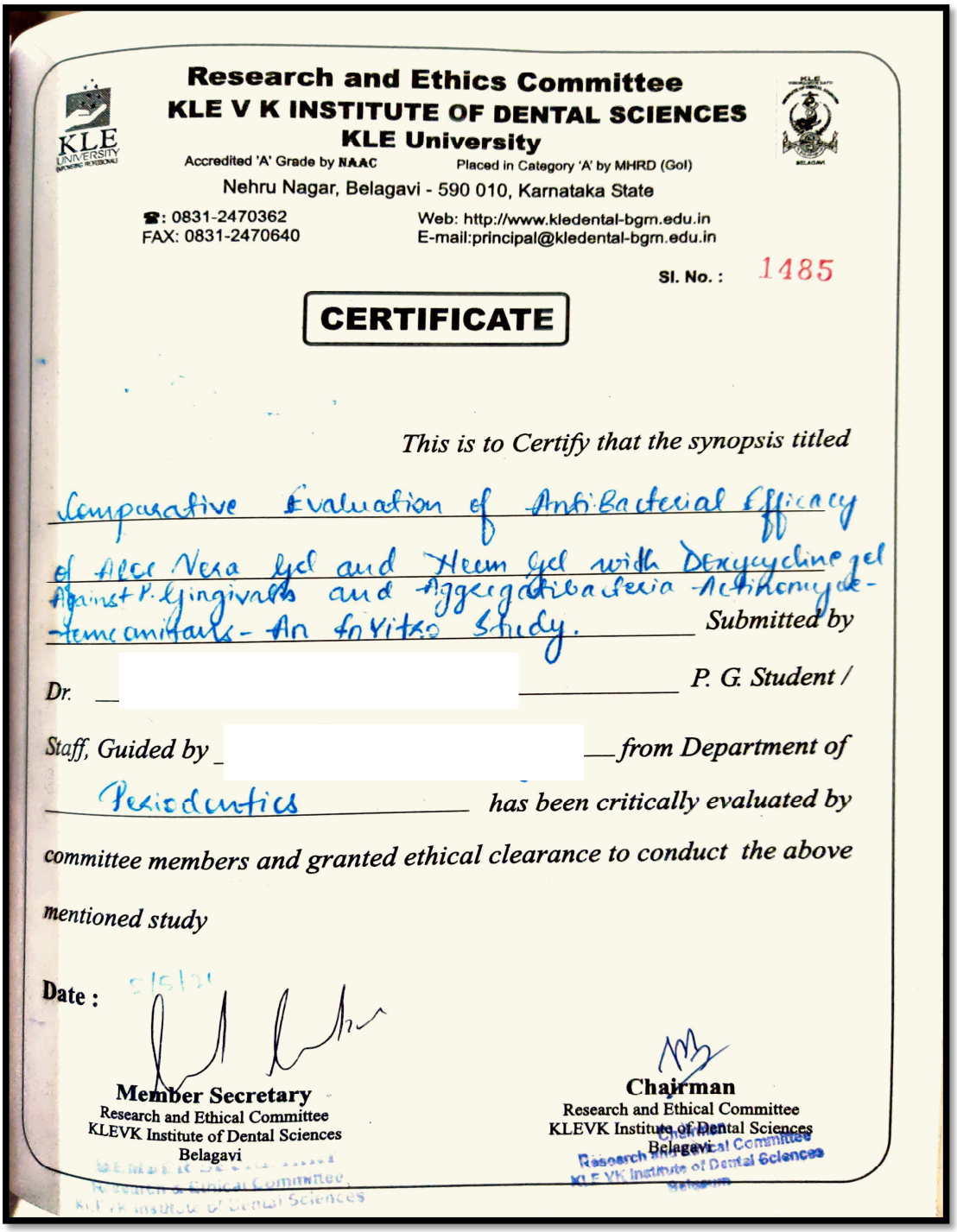
ANNEXURE – I – ETHICAL CLEARANCE LETTER



Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University

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

Nehru Nagar, Belagavi - 590 010, Karnataka State

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Sl. No. : 1485

CERTIFICATE

This is to Certify that the synopsis titled

Comparative Evaluation of Anti-Bacterial Efficacy of Aloe Vera Gel and Neem Gel with Doxycycline gel Against P. gingivalis and Aggregatibacteria Actinomycetemcomitans - An in Vitro Study.

Submitted by

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

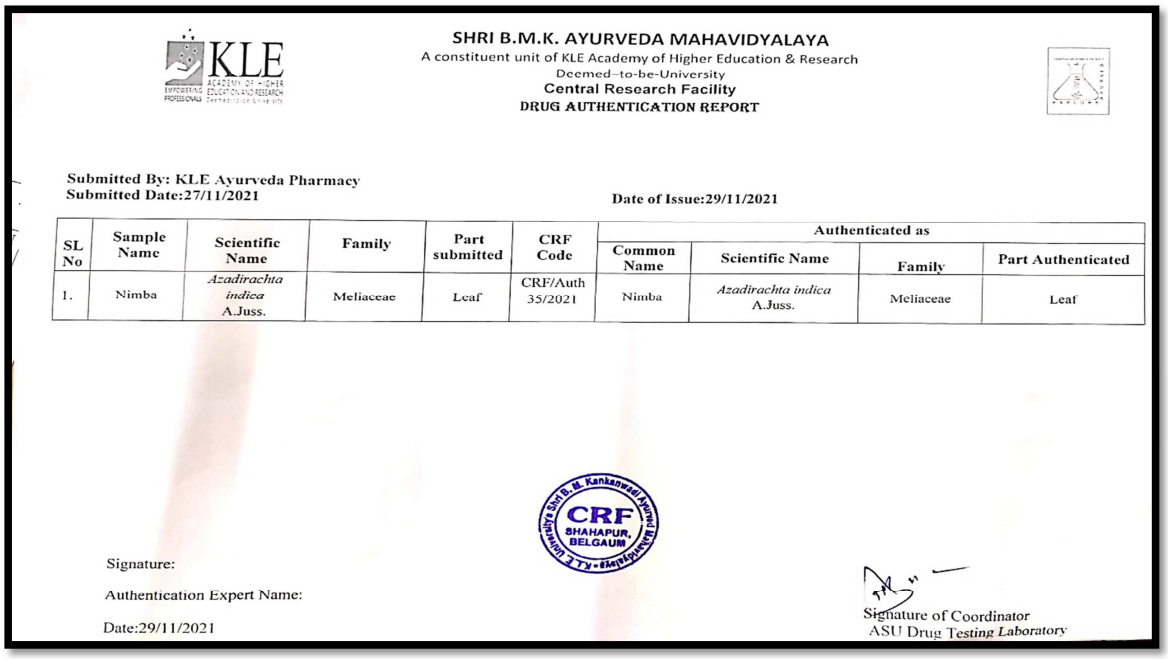
Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

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

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE – II – DRUG AUTHENICATION 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

SL No	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Common Name	Scientific Name	Family	Part Authenticated
1.	Nimba	*Azadirachta indica* A.Juss.	Meliaceae	Leaf	CRF/Auth 35/2021	Nimba	*Azadirachta indica* A.Juss.	Meliaceae	Leaf

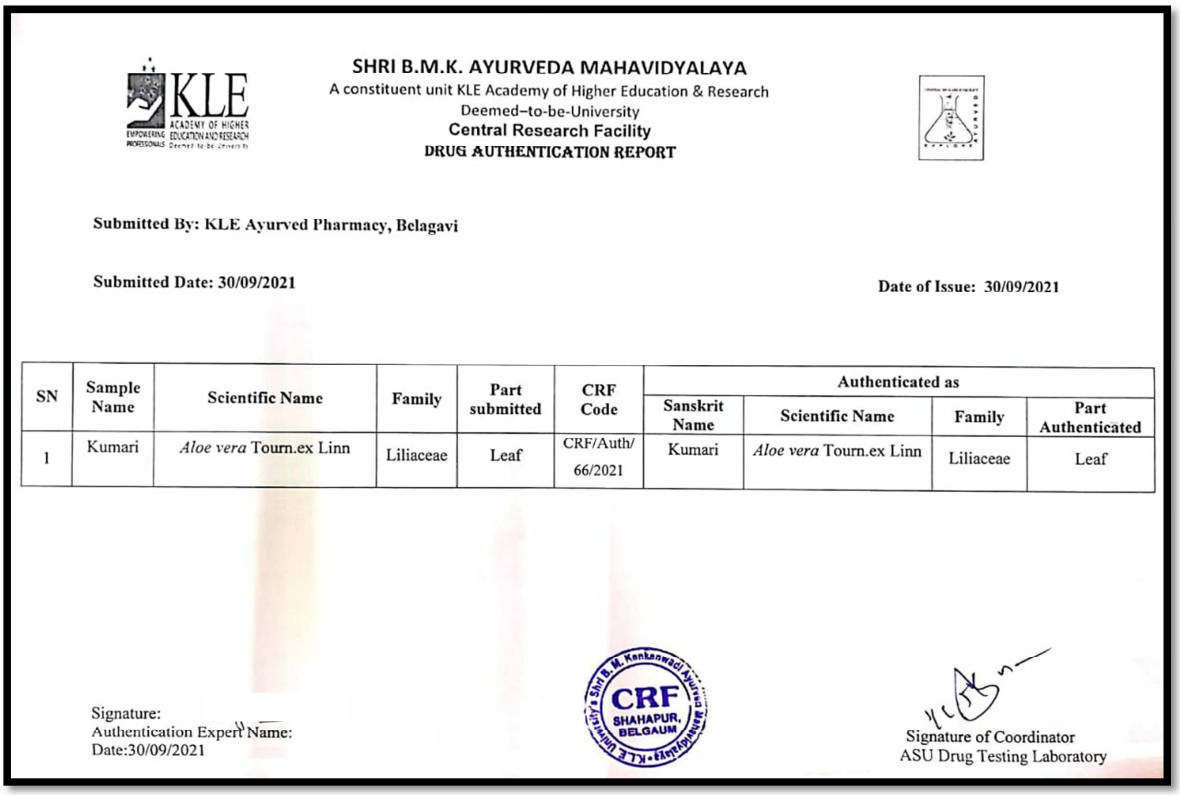
CRF SHAHAPUR, BELGAUM

Signature:

Authentication Expert Name:

Date:29/11/2021

Signature of Coordinator
ASU Drug Testing Laboratory



SHRI B.M.K. AYURVEDA MAHAVIDYALAYA
A constituent unit KLE Academy of Higher Education & Research
Deemed–to-be-University
Central Research Facility
DRUG AUTHENTICATION REPORT

Submitted By: KLE Ayurved Pharmacy, Belagavi

Submitted Date: 30/09/2021

Date of Issue: 30/09/2021

SN	Sample Name	Scientific Name	Family	Part submitted	CRF Code	Authenticated as			
						Sanskrit Name	Scientific Name	Family	Part Authenticated
1	Kumari	*Aloe vera* Tourn.ex Linn	Liliaceae	Leaf	CRF/Auth/ 66/2021	Kumari	*Aloe vera* Tourn.ex Linn	Liliaceae	Leaf

CRF SHAHAPUR, BELGAUM

Signature:
Authentication Expert Name:
Date:30/09/2021

Signature of Coordinator
ASU Drug Testing Laboratory

ANNEXURE – III – MIC and MBC RESULTS

KAHER's Dr. Prabhakar Kore Basic Science Research Center [BSRC]
KLE Academy of Higher Education and Research [KAHER]

Report

TITLE OF THE STUDY: "To evaluate and compare the antibacterial efficacy of *Aloe vera* and Neem gel with doxycycline gel against *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans* - an in-vitro study."

NAME OF THE STUDENT: Dr.

NAME OF THE GUIDE: Dr.

NAME OF THE CO-GUIDE: Dr.

Objective Parameters:

1. Assessment of MIC and MBC of *Aloe vera* & Neem leaf 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:** 5mg/ml

Lab Investigations done in BSRC

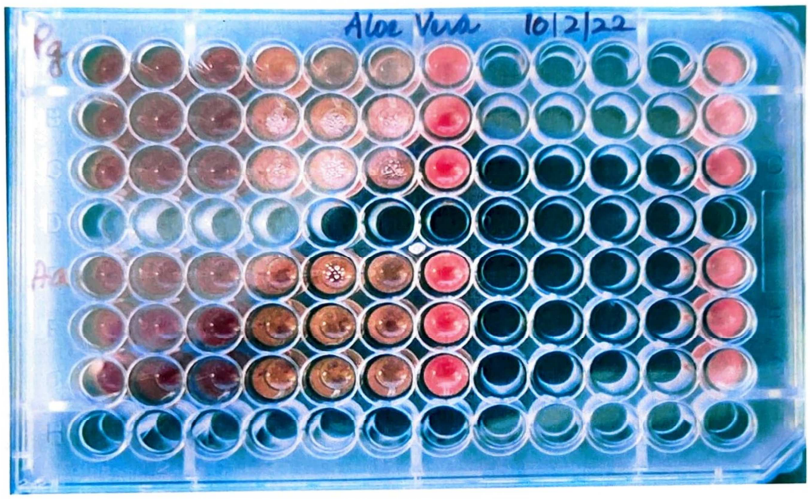
Sl.No.	*Porphyromonas gingivalis*				*Aggregatibacter actinomycetemcomitans*			
	MIC		MBC		MIC		MBC	
Aloe vera (extract)	1.25	1.25	2.5	2.5	1.25	1.25	2.5	2.5
	1.25		2.5		1.25		2.5	
	1.25		2.5		1.25		2.5	
Neem (extract)	0.156	0.208	0.312	0.312	2.5	2.5	5	5
	0.156		0.312		2.5		5	
	0.312		0.312		2.5		5	

All values are expressed in mg/ml against tested organism

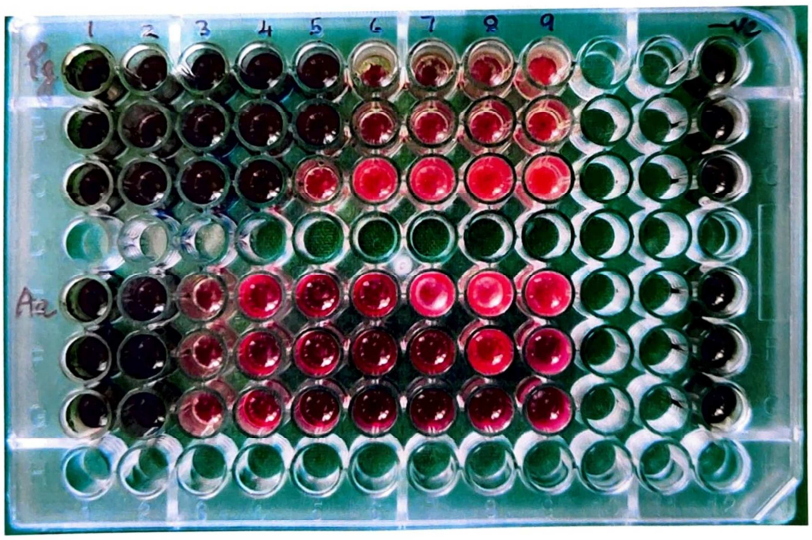
Remarks
The results are satisfactory and relevant references have been followed.

Minimum Inhibitory Concentration:

Aloe vera

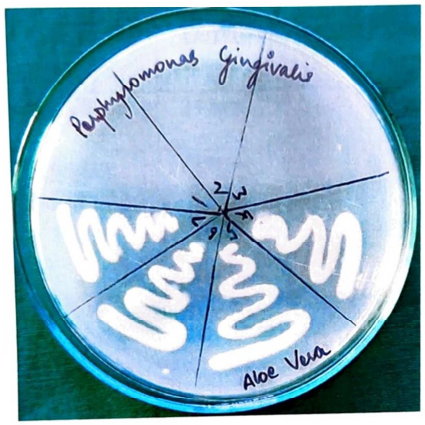


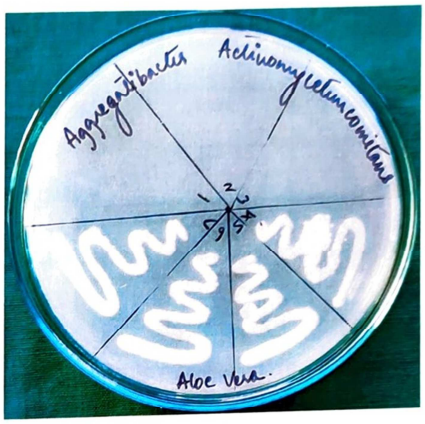
Neem



Minimum Bactericidal Concentration:

Aloe vera



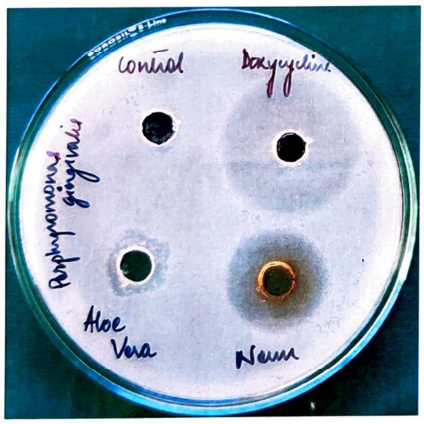


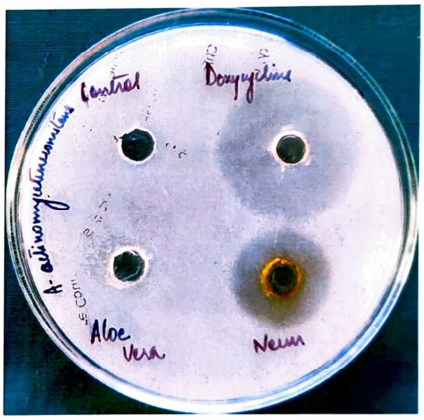
Neem

Agar well Diffusion Assay:

Groups	*Porphyromonas gingivalis*			*Aggregatibacter actinomycetemcomitans*		
Doxycycline	34 mm	32mm	35mm	34 mm	34mm	32mm
Aloe vera	13 mm	10mm	0	10 mm	0	0
Neem	26 mm	25mm	22mm	24 mm	22mm	24mm

mm: millimeter; NZI: No Zone of Inhibition





Research Assistant.

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)

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Date : 24.12.2022 Serial No. : 132

PLAGIARISM CHECK REPORT

Name of the Applicant
UG / PG / Ph.D / Staff : POSTGRADUATE STUDENT
Batch & Year : 2020-23
Department : PERIODONTICS

The soft copy of Research Work / Manuscript by . .... entitled
"TO EVALUATE AND COMPARE THE ANTIMICROBIAL EFFICACY OF ALOEVERA AND NEEM GEL WITH DOXYCYCLINE GEL AGAINST PORPHYROMONAS GINGIVALIS AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS- AN INVITRO STUDY"
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 .....4.....%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

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

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
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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 the Biostatistics aspect of the Dissertation/Research work of **Post Graduate Student**, under the guidance of M.D.S, **Professor, Department of Periodontics, entitled "To Evaluate and Compare the Antibacterial Efficacy of Aloe Vera and Neem Gel with Doxycycline Gel against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* - An in-vitro study"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 11/10/22

11/10/2022
Name & Signature of Biostatistician

DEPARTMENT OF EPIDEMIOLOGY & BIOSTATISTICS-MANERI'S BELAGAVI