"Evaluate the efficacy of Curry leaves (*Murraya koenigi)* incorporated bio gel against early peri-implantitis pathogens" - an in vitro study"

BY

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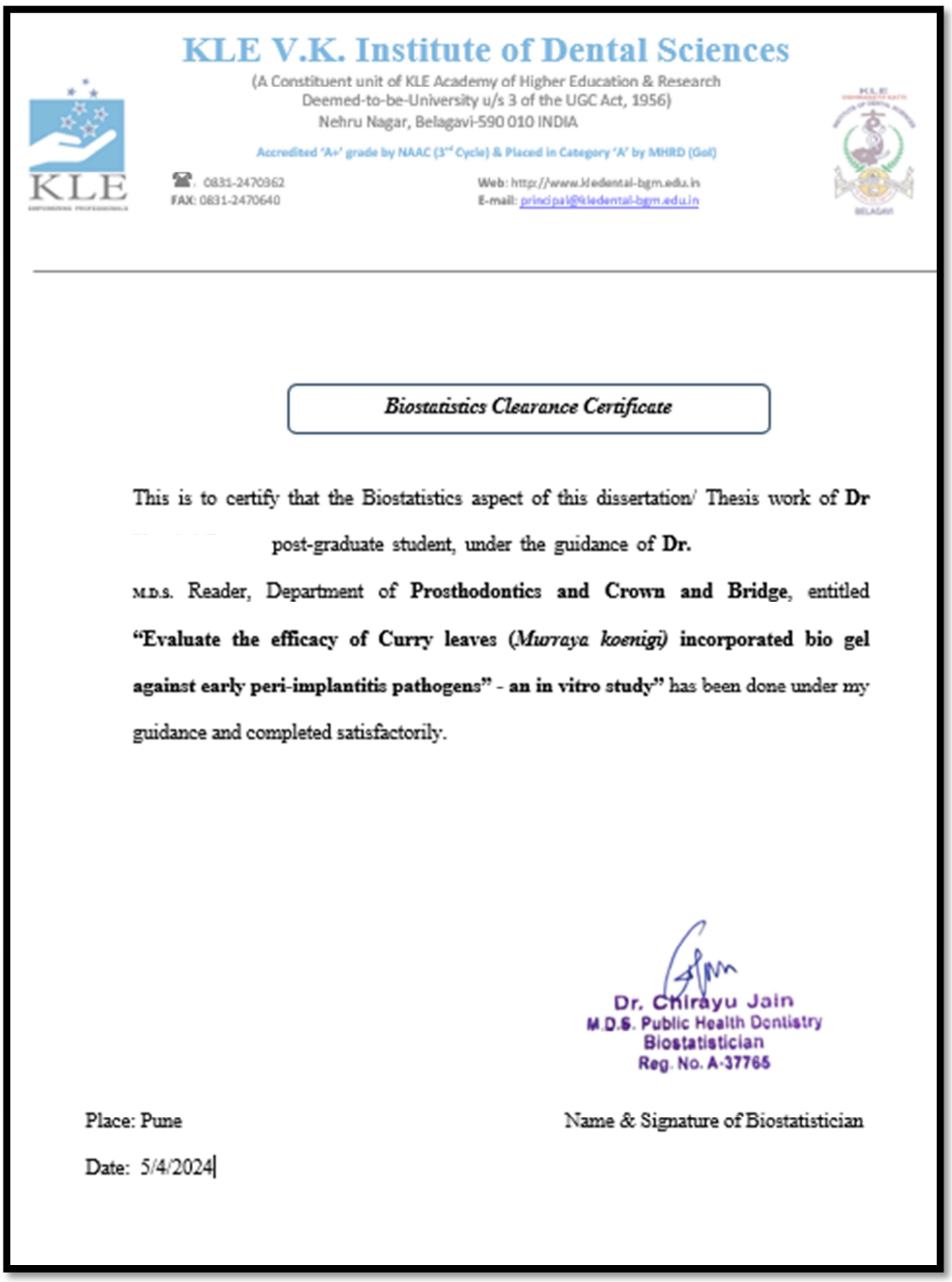

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LIST OF ABBREVIATIONS USED IN THE STUDY

GROUP TE	Titanium experimental group (Curry leaves gel)
GROUP TC	Titanium control group (CHX)
GROUP ZE	Zirconia experimental group (Curry leaves gel)
GROUP ZC	Zirconia control group (CHX)
PB	Probing depth
S.D.	Standard Deviation
C.V.	Coefficient of Variation
S.E.	Standard of error
ANOVA	Analysis of variance
hrs.	Hours
L	Litre
M	Milli
CHX	Chlorhexidine
HA	Hydroalcoholic
CLSI	Clinical Laboratory Standard Institute Guidelines
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration

ABSTRACT

STATEMENT OF PROBLEM

The removal of natural teeth due to caries, trauma, or periodontal conditions needs the replacement in terms of restorative procedure such as removable partial dentures, fixed partial dentures or dental implants. Osseo-integrated implants, exhibit significant longevity and success rates ranging from 83.9% to 98%. However, even after successful Osseo-integration implant might lead to failure because of periimplantitis. It is characterized by inflammation and progressive bone loss around implants. Depending on the severity, the management of peri-implantitis is classified as non-surgical and surgical. Non-surgical management consist of manual debridement and use of adjuvant like chlorhexidine gluconate (CHX), to overcome the side-effects of commercially available 1% CHX gel the herbal remedies can be used, because of their antimicromial and non-cytotoxic properties.

Curry leaf (Murraya koenigii) has demonstrated antibacterial activity against various pathogens, including those associated with early periimplantitis. In particular, its incorporation into biogels, for their site specific action.

The present study aims to formulate and evaluate the antimicrobial activity of Curry leaf (Murraya koenigii) incorporated biogels against early periimplantitis pathogens. By exploring novel formulations that harness the therapeutic potential of herbal extracts in controlled drug delivery systems.

PURPOSE

To formulate and evaluate the efficacy of Curry leaves (*Murraya Koenigii*) incorporated bio gel against *Staphylococcus aureus* and *Porphyromonas gingivalis* in early peri– implantitis pathogens adhered to Titanium and Zirconia discs.

METHODS

A total of 120 discs were fabricated each measuring 10 mm in diameter and 2 mm in width. Out of those 120, 60 were fabricated from commercially available pure Titanium grade 4 and 60 were fabricated from Zirconia. These discs were divided into four groups.

1. GROUP TE – Titanium experimental group (bio gel)
2. GROUP TC – Titanium control group (CHX)
3. GROUP ZE – Zirconia experimental group (bio gel)
4. GROUP ZC – Zirconia control group (CHX)

Authentication of Curry leaves (Murraya Koenigii) was conducted, and an extract was formulated, and prepared in a hydroalcoholic solution. The prepared extract underwent antibacterial assay (MIC and MBC) via serial dilution and agar plate culture. Following the determination of MIC and MBC values, the formulation of the bio gel was done. The gel formulation, based on carbopol, incorporated 3% of the hydroalcoholic extract along with other ingredients. Subsequently, the antibacterial testing of the Titanium and Zirconia discs was carried out in vitro using the disk diffusion method followed by that MTT assay was carried out.

RESULT

The collected data was subjected to statistical analysis using unpaired t-test. There was statistically significant difference between the control and experimental group ($P < 0.05$).

The effectiveness of antibacterial activity of 3% Curry leaves incorporated biogel was measured by disk diffusion test. *S. aureus* showed a zone of inhibition of 26.13 mm on Zirconia discs and of 25.03 mm on Titanium discs.

The results of disc diffusion test for *P.gingivalis* showed zone of inhibition of 19.06 mm for Zirconia and 16.46mm for Titanium.

The MTT assay showed that the 3% curry leaves incorporated biogel is less cytotoxic compared to commercially available 1% CHX.

This results showed that 3% Curry leaves incorporated biogel is as effective as commercially available 1% CHX gel with less cytotoxic effects.

CONCLUSION

This study concludes that incorporating 3% Curry Leaves (Murraya Koenigii) gel exhibits promising antibacterial efficacy against early peri-implant pathogens, comparable to 1% CHX gel. Moreover, Curry Leaves (Murraya Koenigii) gel emerges as a potential alternative to CHX in treating early peri-implant infections, potentially with lower cytotoxicity. The use of disk diffusion tests is highlighted as an affordable and accessible method for assessing antibacterial activity. However, further research is necessary for clinical validation, assessment of long-term effects, and broader evaluation of peri-implant pathogens to enhance the reliability and applicability of these findings.

KEYWORDS

Curry Leaves (Murraya Koenigii), Chlorhexidine gel, peri-implantitis, bio gel, disc diffusion.

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INTRODUCTION

A person's overall health and well-being are significantly impacted by their oral health. Teeth are essential for speaking, maintaining facial appearance, and performing masticatory functions. In the field of dentistry, when a tooth is lost, it's not just about the space it leaves behind. It sets off a cascade of effects that affects various parts of one's life. It interferes with routine activities such as speaking, facial apperarance and chewing, and it can negatively impact an individual's self-esteem and even cause them to stay away from social interactions. The World Health Organization (WHO) estimates that 3.5 billion people worldwide suffer from some kind of oral health issue, and tooth loss is the most common. [1, 2,3]

For the restoration of lost teeth, there are several treatment options available, ranging from advanced methods like dental implants to more traditional choices like fixed and removable dental bridges. Dental implants have become gained popularity due to their predictable results when compared to traditional treatment approaches. Dental implants offer a comprehensive and long-lasting alternative that closely resembles the structure and function of natural teeth, According to studies, dental implants have a remarkable success rate of more than 95% over ten years, outlasting traditional dentures and bridges.[2]

Osseointegration, the process by which dental implants bond with the surrounding bone tissue, It has a crucial role in the success of implant therapy. Osseointegration is necessary for the implant and the bone to have a stable and functional connection. It is necessary to assure the longevity of the implant.[4]

Peri-implantitis is one of the factors that may impact the longevity of implants and osseointegration. It is thought to be the primary cause of long-term implant failure and is characterized by inflammation and bone loss surrounding the

implant. It has been reported to affect around 20% of implant patients. Froum and Rosen (2012) classified peri-implantitis as early, moderate, or advanced based on clinical characteristics and severity.[5] Effective treatment of the previous stage prevents the progression of peri-implantitis into the more difficult-to-treat stages.[6]

According to Froum and Rosen, early periimplantitis is defined as probing depths of ≥4 mm[5], bleeding, with or without suppuration on probing, and bone loss of less than 25% of the implant length. Bacterial pathogens which are frequently associated in the pathophysiology of peri-implantitis are *Staphylococcus aureus (S. aureus)* and *Porphyromonas gingivalis (P. gingivalis).*

Gram-negative, anaerobic *P. gingivalis* is frequently linked to periodontal disorders. It has virulence characteristics that allow it to start tissue destruction surrounding implants and blocks the human immune response. Individuals who have elevated levels of *Porphyromonas gingivalis* are at a higher risk of acquiring new attachment loss due to peri-implantitis.[7]

Gram-positive *S. aureus* bacteria has increased ability to get atttached to implant surfaces and create biofilms. It can be found within an hour following the implantation of dental implants, and in just two weeks, a complex biofilm is forms. It has high affinity for titanium surface. Because of this, it is a key bacteria associated with early peri-implantitis.[8]

Early peri-implantitis is marked by the presence of *P. gingivalis* and *S. aureus,* which emphasizes the critical need for an efficient microbiological management approach. According to Kim and Smeets et al[5]. peri-implantitis management is classified as non-surgical, surface decontamination, and surgical approaches, Rokya F et al. classified it as regenerative and resective.[9,10] Resective therapy concentrates on cleaning implant surfaces to remove causative factors and preserve peri-implant health, regenerative therapy uses membranes, bone grafts and

graft factors to rebuild attachment apparatus. However, the screw-shaped nature of the implant makes total eradication of bacteria difficult and promotes the creation of bacterial biofilms and plaque accumulation, making mechanical debridement alone ineffective. Mechanical debridement is combined with chemical antiseptics such as chlorhexidine (CHX) which can be used as a 0.2% rinse solution or 1% gel shows promising results.[11,12]

Adjuvant therapy reduces anaerobic and aerobic bacteria on the surface of implants more quickly, but long-term usage of chlorhexidine can result in dysgeusia, desquamative gingivitis, allergic responses, and discoloration of teeth, tongue, and restorative materials.[13]. In 1962, Calnan was the first to report this. Dentists encounter difficulties in managing peri-implantitis due to the drawbacks of commercially available CHX solution or gel and the multiple etiologic factors of the condition. Herbal complementary and alternative medicine has been more popular than allopathic care in recent years, which may be related to their natural origin and safety of the drugs, keeping the significance of the antibacterial, antifungal, antiviral, and anti-inflammatory qualities of herbal medicines into account[14] As they come from natural sources like plants and herbs, herbal remedies are more palatable and safer for a wider range of people, even those with underlying medical conditions or sensitivities, as they are more compatible with the human body and less likely to cause negative effects or toxicity than synthetic substances. Furthermore, compared to synthetic medications, herbal therapies are an affordable and convenient option.

Curry leaves (*Murraya koenigii*) stand out among the variety of herbal remedies. Curry leaf, also known as *Morraya koenigii*, is a common herbal product used to flavor curries and chutneys, particularly in southern India. The word Curry came from the Tamil term Kari, which describes hot sauces. In India, it is also

referred to as Kari-Patta. It belongs to the *Rutaceae* family. Curry leaf (*Murraya koenigii*) has antibacterial capabilities because of its complex phytochemical makeup, which contains tannins, alkaloids, flavonoids, and essential oils.[15] According to literature Curry leaves has potent antibacterial qualities against bacteria such as *Porphyromonas gingivalis, Escherichia coli, Staphylococcus aureus,* and *Streptococcus mutans*.[16] There were several formulations of Curry leaves. *(Murraya koenigii)* which have been attempted previously **Gupta et al.**[17] formulated a mouthwash incorporating Curry leaves *(Murraya koenigii)* for reducing the pH of saliva and plaque formulation. Also **Chanchal et al.**[18] **and Nakao et al**.[7] evaluated the antibacterial activity of Curry leaves extract against *S. aureus* and *P. gingivalis*, respectively, all these formulations showed promising results.

Biogels play a crucial role in delivering drugs in continuous and steadily manner at implant site, reducing bacterial load in the treatment of early peri-implantitis. They are comprised of hydrophilic and amphiphilic polymer chains forming a swollen network. Biogels can effectively deliver biologically active molecules through physical cross-linking,[19] by facilitating drug incorporation. Due to their low molecular mass and the ability to adjust their characteristics easily, biogel formulations are highly suitable for developing oral and topical gels for early peri-implantitis treatment.[20]

Following an extensive literature review, the use of gel formulations containing Curry leaves (Murraya koenigii) for managing early peri-implantitis is limited. Hence, this study was conducted to formulate and evaluate the antimicrobial activity of a biogel incorporating Curry leaves (*Murraya koenigii*) against early peri-implantitis pathogens.

NEED FOR THE STUDY

Dental implants have emerged as a promising solution in modern dentistry, offering patients a reliable means to restore missing teeth and perform oral function and aesthetics compaired to the traditional removable and fixed prosthesis. These biocompatible option acts as artificial tooth and provide a stable foundation for prosthetic teeth that closely resemble natural dentition.[21] Even after following standard protocol of surgical and prosthetic rehabilitation implant might lead to failure because of peri-implantitis.

Peri-implantitis is destructive inflammatory process impacting soft and hard tissues surrounding Osseo-integrated implants, resulting in peri-implant pocket formation along with loss of supporting bone.[5,22]

Chlorhexidine gluconate (CHX) serves as a common antimicrobial agent for the resective management of early peri-implantitis.[11,12] It is effective against a wide range of microorganisms including gram-positive and gram-negative bacteria, fungi, yeasts, and viruses. However continuous use of Chlorhexidine (CHX) can result in discoloration of teeth, tongue, and restorative materials, taste alteration, mucous membrane irritation, and occasional allergic reactions.[13] It is prescribed in the form of solution 0.2% and as gel 1%

Given the disadvantages of chlorhexidine and multifactorial aetiology of peri-implantitis and dentists face challenges in its management. Hence, herbal therapies are increasingly sought to address peri-implantitis by leveraging their antimicrobial and anti-inflammatory properties of herbal agents.

Curry leaves (Murraya koenigii) have gathered attention for their potential therapeutic benefits. These tropical to sub-tropical tree leaves has high antioxidant

activity and have demonstrated antibacterial properties against pathogens like *Staphylococcus aureus* and *P. gingivalis*.[15,16]

Biogel helps to localise a drug in a particular region, thereby increasing bioavailability at the same time. Given to the polymeric nature, it is easy to incorporate the herbal agents. It also increases the contact time between drug and mucosa and help in sustained and long term release of drug[19]

While some studies indicate that Zirconia implants may exhibit reduced microbial colonization compared to Titanium, the literature presents conflicting findings on the bacterial colonization between Titanium and Zirconia implants.[23,24]

The incorporation of Curry leaves *(Murraya koenigii*) extract into a customized gel offers an easily accessible and cost-effective solution with antibacterial properties. Therefore, this study aims to formulate and evaluate a biogel incorporating Curry leaves (*Murraya koenigii*) extract for its antimicrobial activity against peri-implantitis pathogens on Zirconia and Titanium implant surface.

REVIEW OF LITERATURE

1. **B. V. Harvey et al.(1984)[25]** conducted a study to assess how chlorhexidine (CHX) affects hamster cheek pouch mucosa. Treatment with 2.0% CHX caused white lesions in all animals, along with increased epithelial thickness and inflammatory cell accumulation. However, 0.2% CHX or saline showed no changes. While CHX didn't affect mucosal permeability to 14C-CHX, 2.0% CHX decreased permeability to H2O and thickened the permeability barrier. The study suggests using CHX cautiously, preferably at concentrations of 0.2% or lower.[12]
2. **Helms et al. (1995)[26]** et al In a review on how chlorhexidine affects taste perception in humans, they noted that various adverse effects like mucosal desquamation, dark staining, increased calculus development, and sensitization. Taste perception impairment is an additional negative effect of regular use of chlorhexidine.[15]
3. **Bonacucina et al (2004)[27]** investigated the gelation properties of Carbopol 971 and 974 in co-solvents that are water-miscible, such as PEG 400 and glycerine.By avoiding neutralization, Carbopol gels were obtained at higher temperatures, enabling the dissolution of poorly soluble drugs. Rheological analysis indicated that Carbopol gels in PEG 400 exhibited satisfactory elastic behaviour, typical of gel-like structures. Mucoadhesive properties were checked by ex vivo analysis, and cutaneous irritation was not noted in a vivo study conducted for PEG 400 and water gels of Carbopol 974 prepared by heating. Paracetamol incorporation demonstrated better release control in PEG 400-Carbopol 971 samples, indicating that these systems could be a good substitute for conventional hydro gels.[14]

4. **Mavrogenis A.F. et al (2009)[4]** highlighted the coordinated participation of different growth factors, cytokines, implant bone tissues, and cell types during the phases of bone repair that entail remodeling and inflammatory development. They concluded that osseointegration should not be viewed solely as a reaction to specific implant materials but rather as a manifestation of the inherent regenerative capacity of bone. In order to facilitate osseointegration a direct structural and functional connection between living bone and implant surfaces and enable early implant loading, the ultimate goal is to establish controlled, directed, and quick peri-implant bone healing. Maintaining proper oral hygiene is crucial for enhancing osseointegration and preventing peri-implantitis.[4]

5. **Ningappa M.(2010)[28]** Murraya koenigii L. Curry leaves contain an antioxidant protein called APC (antioxidant protein from curry leaves). In an article published on this protein's potent antibacterial property described how a monomeric protein with a molecular mass of 35 kDa that was isolated from the curry leaves exhibits strong antibacterial activity, against every human pathogenic strain examined, the protein known as APC (antioxidant protein from curry leaves) showed strong antibacterial activity. Salmonella typhi, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, and Klebsiella pneumoniae were all efficiently suppressed by APC. The inhibition is similar to that of streptomycin, gentamycin, and chloramphenicol, three common commercial antibiotics. With MIC values ranging from 13 to 24 lg/ml, which are equivalent to the MIC values of most antibiotics, APC suppressed the growth of bacteria. Ribonuclease/deoxyribonuclease and protease activities are absent from APC.

6. **Al-Radha(2011)[24]** An investigation on the surface characteristics of dental implant materials made of zirconia and titanium and how they affect bacterial adherence was carried out. This in-vitro study compared the bacterial affinities for titanium and zirconia materials while examining their respective physicochemical properties. A variety of disc samples with varying surface conditions were used, such as polished titanium as a control, polished titanium partially stabilized zirconia, titanium blasted with zirconia, and titanium blasted with zirconia followed by acid etching. Using profilometry and scanning electron microscopy, surface morphology was evaluated. In addition, the chemical composition, surface microhardness, contact angle, and surface free energy were examined. For six hours, the disc samples were exposed to the microorganisms *Streptococcus mitis* and *Prevotella nigrescens*, either before or after being coated with human saliva. Fluorescence microscopy pictures were used to quantify the amount of bacterial coverage on the surfaces. The research findings indicate that zirconia material outperformed titanium in terms of its ability to decrease bacterial adhesion, especially following the application of a saliva pellicle coating.[18]

7. **Bortoluzzi M. et al (2012)[29]** A study was carried out to investigate the relationship between tooth loss, chewing ability, and quality of life. This observational study took place at a single center, where data were gathered through clinical examinations and questionnaires aimed at capturing sociodemographic details, chewing ability (measured by the Index of Chewing Ability, ICA), and quality of the life (measured using the Oral Health Impact Profile, OHIP-14). Among partially edentulous patients, common issues reported included difficulties in chewing, psychological distress associated with dental problems, and dissatisfaction with appearance due to issues with teeth,

mouth, or dentures.The study found that within the subscales or domains of the OHIP-14, patients with impaired chewing abilities experienced heightened levels of psychological discomfort and disability, along with functional limitations, physical pain, and disability. The conclusion drawn was that oral health status could significantly impact the Oral Health Impact Profile, and implants with prosthetic devices emerged as the most effective treatment option for replacing natural teeth up to the present time.[29]

8. **Gerspach I.H, et al (2012)[30]** A study examined gaseous ozone's impact on peri-implantitis, focusing on its effectiveness against bacteria and its effects on cellular response. Titanium and zirconia materials were tested in vitro. The objective was to assess the antibacterial efficacy of ozone on bacteria adhering to different surfaces as well as its effect on the adherence of osteoblast-like MG-63 cells. Adherence to Streptococcus sanguinis DSM20068 and Porphyromonas gingivalis ATCC33277 was tested on saliva-coated titanium (SLA and polished) and zirconia (acid-etched and polished) disks. For six and twenty-four seconds, gaseous ozone (140 ppm; 33 mL/s) was administered. After resuspension, the bacteria were grown. Analysis was done on the attachment, form, spreading, and proliferation of MG-63 cells. SEM was used to analyze cell morphology and surface topography. The maximum bacterial adhesion was seen on titanium SLA, with a 50–75% reduction on other surfaces. On zirconia substrates, S. sanguinis exhibited a reduction of over 90%, whereas P. gingivalis was eradicated from all surfaces in less than 24 seconds. Ozone treatment had no effect on osteoblastic adhesion of cells and proliferation, nor did it change surface features.According to the study, because smoothed the titanium and acid-etched/polished zirconia have a lesser potential for colonization, they could be good choices for implant abutments. All adhering bacteria on both surfaces

were selectively eliminated by gaseous ozone without affecting the function of osteoblastic cells.

9. **Persson R and Renvert S (2014)[31]** An article about a group of microorganisms connected to peri-implantitis was released. Microbiological samples were taken from 47 people with healthy implants and one implant from each of the 166 participants in this study who had been diagnosed with peri-implantitis. The samples were checked using DNA checkerboard hybridization, targeting 78 species. The study concluded that a bacterial cluster, which includes *S. aureus, P. gingivalis, and T. forsythia,* is associated with peri-implantitis.

10. **Smeets R. et al (2014)[11]** In their review article on peri-implantitis, the authors concluded that despite numerous approaches described for their definition, etiology, prevention, and treatment, there is no universally ideal therapy because there aren't enough long-term, prospective, randomized follow-up studies. Studies with varying designs, populations, and materials used often suffer from small sample sizes and short follow-up periods. Therefore, prevention emerges as the most crucial strategy, emphasizing appropriate treatment planning, gentle implant insertion techniques, and regular professional cleaning. Risk factors that need to be closely watched include smoking and a history of periodontitis. Usually, non-surgical treatments consist of air polishing and mechanical cleaning, with the addition of antibiotics and antiseptic rinses for temporary bacterial control. Other alternatives include laser and photodynamic therapy, although there is still a lack of long-term efficacy data.[11]

11. **Al Harbi H et al.(2016)[16]** A study was carried out to assess the ability of Curry leaves (Murraya koenigii) extracts to inhibit the growth of various bacterial strains of both ethanolic and methanolic extracts.The findings revealed that both extracts exhibited efficacy against *Staphylococcus, Escherichia coli,*

Streptococcus, and *Proteus strains*, comparable to that of antibiotics like Gentamycin and Amikacin.

12. **Sivaraman K et al. (2017)[23]** The authors highlighted the limitations of titanium-based implant systems, including hypersensitivity reactions, biocompatibility issues, and aesthetic concerns, prompting the search for more suitable materials. Zirconia has emerged as a promising alternative due to its exceptional visual, biological, and artistic qualities. The purpose of the review was to evaluate zirconia implants' suitability for prosthetic rehabilitation. The scientists concluded that zirconia implants have similar osseointegration to titanium but also provide benefits including better soft-tissue response, biological compatibility, and aesthetics. They did, however, emphasize the necessity of additional comparative, long-term clinical trials to confirm zirconia's viability as a titanium implant substitute.[23]

13. **Gupta R. et al (2017)[32]** A review article was published focusing on dental implants, where the authors elucidated that modern dentistry aims to restore patients to a normal profile, function, comfort, aesthetics, speech, and health, irrespective of stomatognathic system atrophy, disease, or injury. With people living longer, coupled with a population already facing dental issues, implant dentistry is poised to remain vital for generations of dentists. Dental implants are increasingly favoured for single-tooth replacement, especially in posterior regions, avoiding the need to compromise adjacent teeth and potentially compromising oral hygiene. The acceptance of implant dentistry by organized dentistry is now widespread, and the ongoing trend suggests continued expansion in its use until it becomes the primary option for all tooth replacements, supporting both fixed and removable prostheses routinely in restorative practices.[24]

14. **Jamadar M. J. et al. (2017)[20]** released a study on the formulation and evaluation of herbal gel. This publication provided instructions on how to use Carbopol 934 to produce a gel. After carefully weighing the carbopol 934, it was dissolved in 50 milliliters of pure water in a beaker. After setting the beaker aside for 30 minutes to allow the carbopol to swell, stir the mixture with a mechanical or lab stirrer set to 1200 rpm for 30 minutes. Take the necessary amount of Extract and 5 milliliters of propylene glycol. Transfer 5 milliliters of propylene glycol to a separate beaker, then accurately weigh out and mix in methyl and propyl paraben. Ultimately, 1 gram of extract was disseminated by Carbopol, and preservation of solutions were added while being continuously stirred. Lastly, the volume was increased to 100 ml by incorporating the remaining distilled water, and dropwise additions of triethanolamine were made to the formulations to get the desired consistency and skin pH (6.8–7). They came to the conclusion that creating and developing an appropriate drug delivery system requires careful selection of both polymers and medications.[25]
15. **Hallstrom H. et al(2017)[33]** A study examined the impact of a chlorhexidine-containing brush-on gel on peri-implant mucositis in adults. It involved 38 participants aged 48–87, with peri-implant mucositis, across three private clinics. Using a double-blind controlled design, participants were randomly assigned to either a test group (using 0.2% chlorhexidine digluconate gel) or a control group. After 12 weeks of intervention, they found that incorporating the chlorhexidine gel with oral hygiene instructions and mechanical debridement improved clinical parameters around implants in the short term.
16. **Varghese A et al (2018)[34]** carried out to evaluate the effectiveness of 3% Curry leaves (Murraya koenigii) extract mouthwash for lowering gingivitis and plaque. The results revealed that the mouthwash with Curry leaves (Murraya

koenigii) extract incorporated is just as effective as mouthwash with 0.2% chlorhexidine that is available in stores.

17. **Cai Z. et al(2019)[35]** Researchers investigated the impact of combining photodynamic therapy (PDT) with antiseptics on *Staphylococcus aureus* biofilm on titanium surfaces. Biofilm was grown on polished and sandblasted large-grit acid-etched (SLA) titanium disks. The disks were treated with various combinations of PBS, chlorhexidine (CHX), hydrogen peroxide (H_2O_2), and PDT. Results showed that combining antiseptics with PDT was more effective in eliminating S. aureus on both polished and SLA titanium disks compared to individual treatments, suggesting a promising approach for peri-implantitis treatment.

18. **Chirayath RB et al (2019)[36]** Researchers developed a carbopol hydrogel incorporating Mangifera indica leaf extract and evaluated its antibacterial efficacy against *Staphylococcus aureus*. Given the limitations of current antibiotic treatments against drug-resistant strains, there's a need for new antibacterial compounds. The study found that mango leaf extract exhibited significant anti-staphylococcal activity without mutagenicity, containing beneficial phytochemicals. The developed MLEC hydrogel showed promising antibacterial activity against *S. aureus* in both in vitro and ex vivo porcine skin models, suggesting its potential as an alternative to existing topical antibiotics for drug-resistant staphylococcal infections.

19. **Katariya et al. (2019)[18]** The study explored the antimicrobial properties of curry leaves, focusing on *Murraya koenigii*, a commonly used leaf-spice. *Murraya koenigii*, from the Rutaceae family, is valued for its aromatic volatile oils. Its leaves, with a slightly bitter taste, are rich in nutrients and bioactive compounds like koenigin and koenimbine, offering various health benefits

including antimicrobial effects. The research underscores the therapeutic potential of plant-based remedies, advocating for their integration into dentistry to mitigate the side effects associated with synthetic medications like chlorhexidine.

20. **Nakao R. et al (2020)[37]** conducted a study on the impact of topically administering propolis and curry leaves on chronic periodontitis. They highlighted the association between periodontitis and various periodontopathic bacteria, including *P. gingivalis, T. denticola, T. forsythia, A. actinomycetemcomitans, F. nucleatum,* and *P. intermedia. P. gingivalis,* recognized as a keystone pathogen, plays a pivotal role in periodontal disease development by instigating inflammation and evading the host immune system through virulence factors such as LPS, fimbriae, gingipains, and outer membrane vesicles. Moreover, *P. gingivalis, T. denticola,* and *T. forsythia* constitute the red complex bacteria, predominantly associated with periodontitis

21. **Varma S. and Zope S.(2020)[14]** wrote a piece on periodontal care in a natural approach and stated that the most common cause of tooth loss is periodontal disease, which is one of the most common dental conditions affecting adults globally. Periodontal disorders have been treated with a variety of antimicrobials and chemotherapeutic drugs, including triclosan, cetylpyridinium chloride, and chlorhexidine. The multiple origin and intricate disease course of periodontitis present a challenge for dentists treating the condition. In order to combat periodontal disorders, herbal therapy has been tried for its antibacterial, anti-inflammatory, and other positive properties. This eventually caused herbal supplementary and alternative medicine to become more and more popular.

22. **Rokaya F et al (2020)[9]** released an article titled Peri-implantitis Update: Risk Indicators, Diagnosis, and Treatment, in which they stated that because the etiologies and clinical characteristics of peri-implant infections are similar to those of periodontal diseases, comparable management strategies have been used. Treatment results, however, differ. Treatment for mucositis is more dependable than that for peri-implantitis, which is challenging to treat with variable results. As a result, early supportive therapy lowers the chance that peri-implantitis would develop. Different peri-implantitis therapies are categorized as

I. Nonsurgical
   a. Mechanical method
   b. Antiseptics
   c. Antibiotics

II. Surface Decontamination
   a. Chemical Methods
   b. Lasers

III. Surgical Treatment.
   a. Air Abrasive
   b. Resective surgery
   c. Regenerative surgery

23. **Nafea J. et al (2020)[38]** published a review on formulation of antibacterial mouthwash from local herbs. In that they stated that Several different types of bacteria can be found in the oral cavity. The mouth is a refuge for microbial development and colonization because of its wet environment and an availability of nutrients. Therefore, maintaining good dental hygiene is essential to reducing the amount of colonizing bacteria and shielding the mouth and body

from their harmful effects. They came to the conclusion that mouthwash made of herbs worked best to combat the germs in the red complex.

24. **Zadeh R. et al (2020)**[39] carried out a study comparing the biofilm formation on materials used in implant-supported prosthesis manufacturing. Researchers compared the formation of biofilms on various materials used in implant-supported dental prostheses and found that bacteria colonize zirconia to a similar or lesser extent than titanium. These findings imply that the use of zirconia restorations may lead to a comparable or decreased incidence of peri-implantitis and peri-mucositis cases.

25. **Leelanarathiwat K. et al(2020)**[40] The study investigated the antibacterial effects of blue high-power light-emitting diode-activated flavin mononucleotide against *Staphylococcus aureus* biofilm on a sandblasted and etched surface. Peri-implantitis, a significant cause of dental implant failure, is often linked to *S. aureus*, despite it not being a typical periodontal pathogen. Its strong affinity to titanium implant surfaces and presence on human skin increase the risk of infection during surgical procedures.

26. **Romya Nakao (2021)**[7] conducted study investigated the antibacterial properties of curry leaf, clove, and cinnamon extracts against *Porphyomonas gingivalis,* a key bacteria in periodontal disease. Curry leaf extract demonstrated the strongest inhibitory activity, even after heat treatment. Unlike clove and cinnamon, curry leaf extract didn't enhance biofilm production at below MIC value. It had a higher MIC against *P. gingivalis* compared to other oral bacteria and effectively killed *P. gingivalis* within 30 minutes. High-speed atomic force microscopy revealed that curry leaf extract induced abnormal membrane vesicle formation on bacterial surfaces, leading to membrane depolarization. These

findings suggest the potential therapeutic use of curry leaf extract for periodontal diseases.

27. **Zhong S. et al (2022)[21]** The study examined the impact of dental implant restoration on clinical efficacy, masticatory function, and patient comfort in individuals with dentition defects. Data from 90 patients treated at Yuyao People's Hospital of Zhejiang Province were analyzed retrospectively. Patients were divided into two groups: the control group received traditional fixed partial denture (FPD) restoration, while the observation group underwent dental implant restoration. Results showed that dental implant restoration significantly improved treatment effectiveness, masticatory function, tooth-related indexes, and patient comfort compared to traditional FPD restoration.

28. **Dumitriu A. S. et al(2023)[12]** published a review evaluating the efficacy of treating peri-implant mucositis with mechanical therapy in conjunction with chlorhexidine. The purpose of the current study was to demonstrate how CHX affects patients with peri-implant mucositis' reaction to nonsurgical treatment. The studies demonstrated a positive influence on the reduction of bacterial plaque accumulation and mucosal inflammation, as evidenced by reduced bleeding on probing, despite certain limitations, including varying follow-up periods, a limited number of participants, and the use of different concentrations of CHX. However, a complete removal of inflammation did not occur in all cases.

29. **Nicholson J. (2023)**[41] A review article was published on Titanium Alloys for Dental Implants, examining their usage since around 1981. The main alloys discussed are commercially pure titanium (cpTi) and Ti-6Al-4V, both yielding clinical success rates of up to 99% at 10 years. These alloys demonstrate biocompatibility with bone and gingival tissues, facilitating osseointegration.

Despite investigations into novel titanium alloys developed for orthopedics, they offer minimal advantages for dental implants. The review concludes that cpTi and Ti-6Al-4V remain highly satisfactory materials for dental implants, suggesting limited room for improvement in dentistry, and foreseeing their continued use in the future.

30. **Lusan SAL et al (2023)[42]** An academic review explored the predominant bacterial communities found in periimplantitis-affected sites, a condition characterized by infectious inflammation of periimplant tissue with bacterial origins. The review synthesized data from 25 studies, revealing Gram-negative anaerobic species such as *Prevotella, Streptococcus, Fusobacterium*, and *Treponema* as the most commonly identified bacteria. The findings underscored the prevalence of gram-negative anaerobic species in periimplantitis sites, implicating their role in both periimplantitis and periodontal disease pathogenesis.

MATERIALS AND METHODS

SOURCE OF DATA:

This study was conducted in-

- KAHER's KLE VKIDS Department of Prosthodontics and Crown & Bridge
- KAHER's Dr. Prabhakar Kore's Basic science research center (BSRC)
- Department of Microbiology, JNMC, Belagavi.
- Department of Pharmaceutics, KLE College of Pharmacy, Belagavi.
- Authentication of *Murraya koenigii* from ICMR Belgavi

METHOD OF COLLECTION OF DATA:

INCLUSION CRITERIA:

- Even disks with a diameter 10 mm and a width of 2 mm (ASTM B348).
- Optimal Surface Roughness of 0 - 5 μm was included in the study

EXCLUSION CRITERIA:

- Discs with surface irregularities
- Optimal Surface Roughness above 5 μm was excluded from the study.

MATERIALS USED IN THE STUDY

Materials	Description	Manufacturer
Titanium alloy	TYPE V (Ti-6Al-4V alloy)	Special Metals, Mumbai, India
Zirconium disc	ISO 13356:2015	Special Metals, Mumbai, India
Hydro-alcoholic solvent	Absolute Ethanol	Analytical CSS reagent
Mueller hinton agar	Culture media (M173 500G)	Hi-media,India
BHI blood agar	Culture media (M210-500G)	Hi-media,India
Carbopol gel base	Carbopol 940 (GRM2033)	Hi- media,India
Distilled water	-	Anchiale technology, India
Murraya koenigii incorporated Bio Gel	Leaves of *Murraya koenigii*	Novel
Chlorhexidine Gel	Hexi gel 1%	ICPA Health products

ARMAMENTARIUM	DESCRIPTION	MANUFACTURER
Incubator	Model No KIS6	kemi elektrotechnik ltd India
Weighing machine	Unibloc weighing balance, AV series	Shimadzu Balances, Japan
Profilometer	Contact prophylometer Surtronic S128	Taylor Hobson, Brazil
Water bath	Rectangular water bath Model LSC-70	Labline,Mumbai,India
Anaerobic Jar	Mcintosh Fildes' Jar	Raut Scientific, Pune, India

SPECIMEN PREPARATION -

A total of 120 discs of commercially available pure Titanium grade 4 and Zirconia were fabricated of diameter 10 mm and a width of 2 mm (ASTM B348). The discs were subdivided into four groups as control and experimental.

1. GROUP TE – Titanium experimental group (bio gel)
2. GROUP TC – Titanium control group (CHX)
3. GROUP ZE – Zirconia experimental group (bio gel)
4. GROUP ZC – Zirconia control group (CHX)

SURFACE ANALYSIS –

Surface roughness was measured using a contact stylus profilometer (Surtronic S128). In the present study, discs with an ideal surface roughness of 0 to 5 μm were included in the study. Every disc was positioned on a level surface, with the testing side facing up. Three lines on the surface are used by the profilometer to measure surface roughness.Following the mounting of the disk specimen, all absolute surface roughness distances from the center line inside the measuring length was calculated by constructing the diamond point stylus with a lateral length of 4 mm and a cut-off length of 0.8 mm.

FORMULATION OF EXTRACT[22]–

The Curry leaves (*Murraya koenigii*) were cleaned, dried, and ground to powder using motorized grinder. The coarse powder was passed through a 180 no sieve with aperture size of 0.180 mm to form a fine powder. A total of 10gm of Curry leaves (*Murraya koenigii*) powder is mixed in 70% of 100 ml ethanol covered with

aluminum foil and kept in the dark for 24 hours at room temperature. Filter paper (Whitman 1) was used for filtration of extract. The filtered extract was collected and the solvent was evaporated by keeping it in a water bath (labline- model LSC-70) at 70^0c to obtain the final volume of extract [16]

DETERMINATION OF MIC (MINIMUM INHIBITORY CONCENTRATION) [43]

The minimum inhibitory concentration (MIC) is defined as the concentration at which bacterial growth is absent.To assess the antimicrobial properties of the Curry leaves (*Murraya koenigii*) an in vitro study was carried out using standard strain of *S. aureus* (ATCC 25923) and *P. gingivalis* (ATCC 33277)

According to the CLSI guidelines (Clinical Lab of Standard Institute Guidelines). The bacterial inoculum was standardized by adjusting the opacity 0.5 Mc Farland equivalents to 1×106 to 5×106 colony forming units (cfu/ml). The antimicrobial activity was measured by using the sequential dilution method.

After dissolving 100 mg of extract in 10 milliliters of distilled water, additional dilutions were conducted to obtain the desired final concentrations in BHI broth: 100 mg, 50 mg, 25 mg, 12.5 mg, 6.75 mg, 3.35, and so forth. To the MIC tubes, 10ul of previously prepared bacteria were introduced. For a duration of 24 hours for *S. aureus* and 48 hours for *P. gingivalis*, the MIC tubes were incubated. Subsequently, the dilutions were duplicated onto a 96-well plate, and each well received 20µl of Resazurin dye. The wells were then incubated at 37°C for one to four hours. based on the well's apparent color shift. Metabolically active cells reduce the blue-colored Rizazurin dye into a pink-colored formazan product through cellular enzyme activity. Hence pink colour represents presence of bacteria and blue represents absence bacterial growth

DETERMINATION OF MBC (MINIMUM BACTERICIDAL CONCENTRATION) [43]

The solution from the MIC tubes was sub-cultured onto BHI agar and incubated for 48 hours in an anaerobic jar for *Porphyromonas gingivalis*. Similarly, *Staphylococcus aureus* was sub-cultured onto BHI agar and incubated for 24 hours in an incubator (Absence of growth indicates a bactericidal effect.)

Based on the values obtained by MIC and MBC 3% of hydroalcoholic extract was incorporated in the Bio gel.

FORMULATION OF BIO GEL

A weighed quantity of Carbopol 940 (GRM 2033)(1 gram) was mixed in 100ml of distilled water with the help of a magnetic stirrer (SESW and NSAW MSB158) for 2 hours. Carbopol was Soaked for 24 hours for complete hydration of Carbopol. 3% of Curry leaves (Murraya koenigi) extract was added to the soaked carbopol with constant stirring with magnetic stirrer for ½ hour. 5% glycerine (molychem), 0.5% sodium benzoate and 0.01% methylparaben(Rasayan) were added as preservatives in above mixture. The pH of the final product is adjusted to 7.0 by adding Triethanolamine QS. After that, the gel was kept at room temperature in an airtight container.

DETERMINATION OF CYTOTOXICITY OF CURRY LEAVES (*Murraya koenigii*) INCORPORATED BIOGEL[44,45]

The MTT test was applied to calculate the in vitro cytotoxic effect of Curry leaves (Murraya koenigi) incorporated bio gel. For evaluating cell viability and proliferation in cell culture, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a frequently used colorimetric method. The basic idea behind the MTT assay is that mitochondrial dehydrogenases found inside the cells convert the yellow tetrazolium salt into a purple formazan product. The cytotoxicity of the mouse fibroblast cell line L-929 is examined in this work. A 96-well micro titer plate with 5000 cells per well was seeded, and the media from Dulbecco's Modified Eagle Medium (DMEM) was added to bring the final volume to 150 µl. After an overnight incubation period, the plate containing different concentration of Curry leaf (Murraya koenigii) biogel was created and treated with cells ranging from 500 µg to 15.65 ug. After that, the gel was kept in an incubator filled with CO2 with 5% CO2 for a full day at 37^0C. After a day, 20 µl of the 5 mg/ml MTT reagent was applied to the wells. The plate was placed in the incubator for four hours after being covered with aluminum foil. (As a result of MTT reagent photosensitivity). After carefully removing the supernatant to prevent any disruption to the Formazan crystals, 100 µl of DMSO was added to dissolve them. 570 nm was the wavelength used to calculate the optical density (OD).The test was run three times, and the average of the three readings is what was obtained.

. **Formula**

$$\text{Surviving cells \%} = \frac{\text{Mean optical density of test component}}{\text{Mean optical density of control (untreated cell)}} \times 100$$

SETTING UP THE Mcintosh Fildes' Jar –

An anaerobic environment was produced using a catalyst and substances like sodium borohydrate, citric acid, and hydrogen carbonate. After the culture media was put inside the jar, the air was squeezed out and either unmixed hydrogen or a mixture of 10% CO2 and 90% H2 was added. When hydrogen and catalyst were combined, water was formed. This is measured by the manometer as a decrease in the jar's internal pressure. The jar is filled to atmospheric pressure with hydrogen by pumping in more gas. The jar was incubated at the desired temperature conditions.

DISC-DIFFUSION TEST[46]-

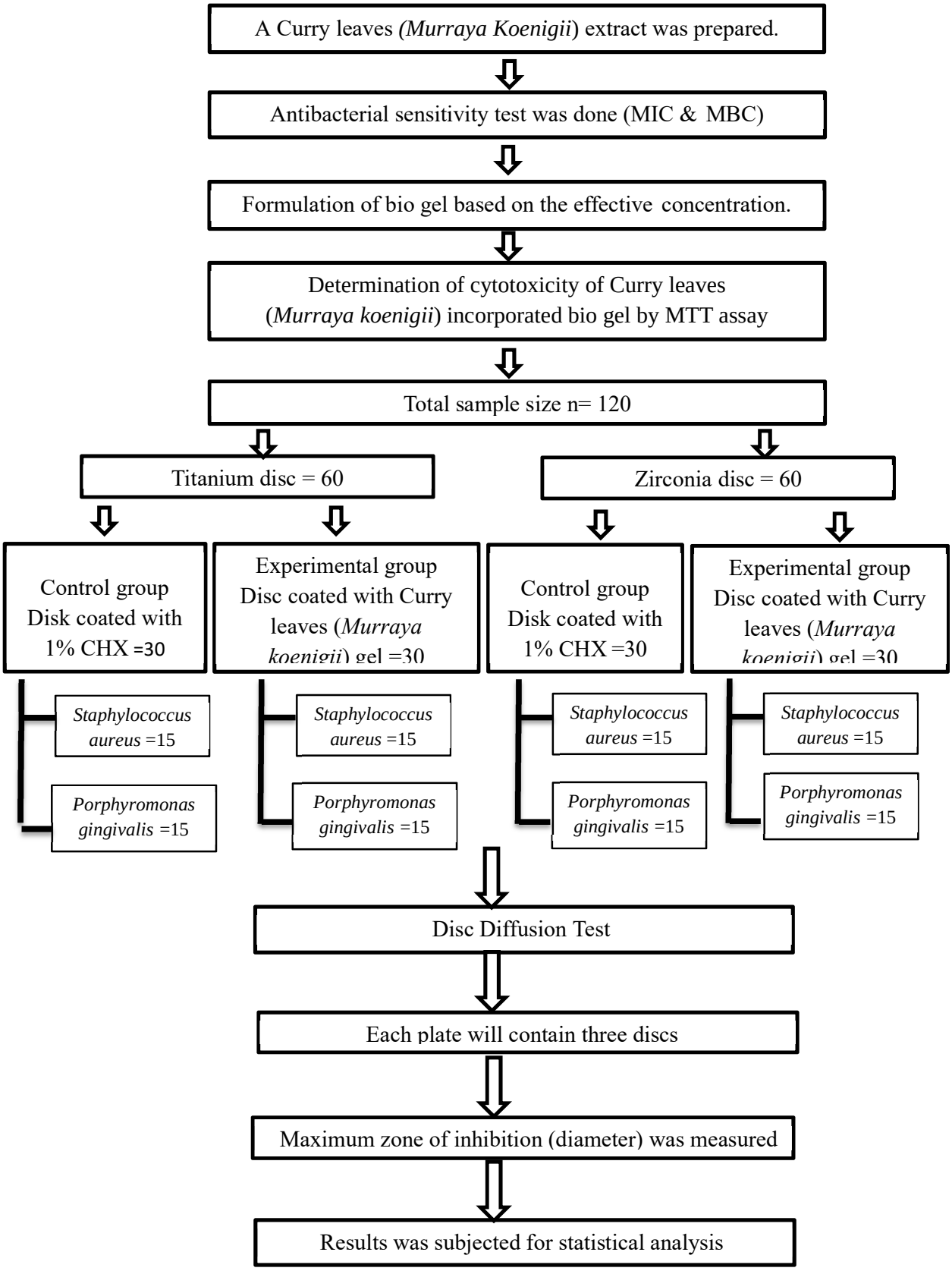
The disk-diffusion agar method was used to test the antibacterial effectiveness of Curry leaves (*Murraya Koenigii*) incorporated bio gel against *Staphylococcus aureus* and *Porphyromonas gingivalis* The bacteria were inoculated using lawn culture on agar plates on muller Hilton agar for *S. aureus* and blood agar for *P. gingivalis.* The sterile Titanium discs (ASTM B348 grade 5) and Zirconia discs (ISO 13356) were coated as:

1. *Experimental group-* Disc coated with 3% Curry leaves (*Murraya koenigii*) incorporated bio-gel
2. *Negative control*- Uncoated disk
3. *Positive control-* Disc coated with 1% Chlorhexidine gel

In one agar plate, 3 disks were cultured.

The plates were incubated for 24 hours in aerobic conditions for *Staphylococcus aureus* and 48 hours in an anaerobic jar for *Porphyromonas gingivalis* at 37 °C. When the antibacterial activity is present, no colonies will grow. That whole area is called the zone of inhibition. The effectivity was measured by length of the diameter of the zone of inhibition.

METHODOLOGY



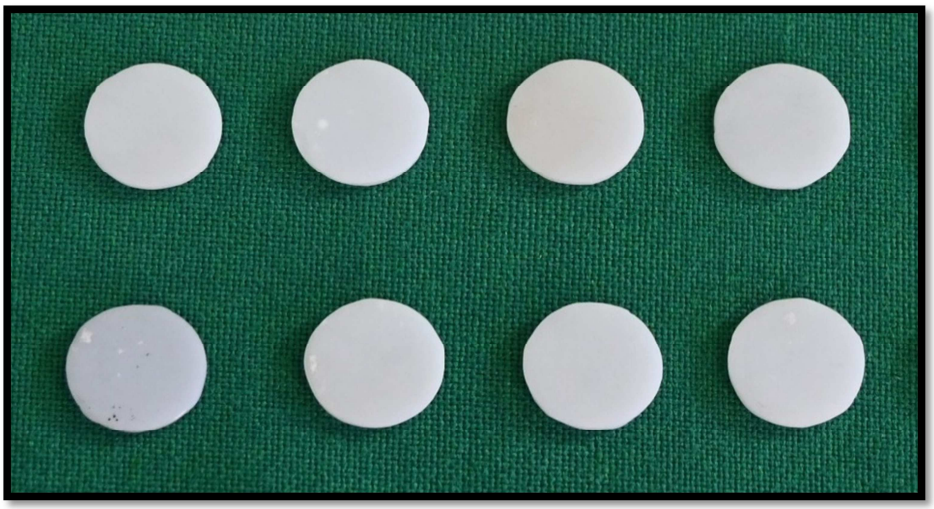


Fig 1: Zirconia Discs

Fig 2: Titanium Discs

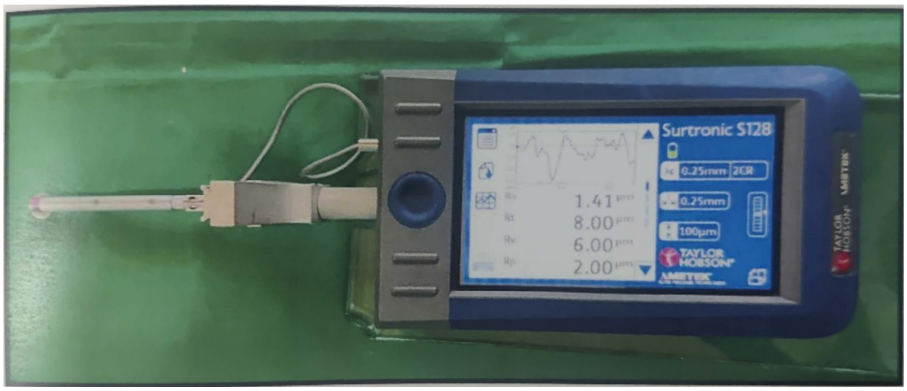


Fig 3: Profilometer.

Fig 4: Powdered Curry Leaves *(Murraya koenigii)*

Fig.5 Hydroalcoholic solution

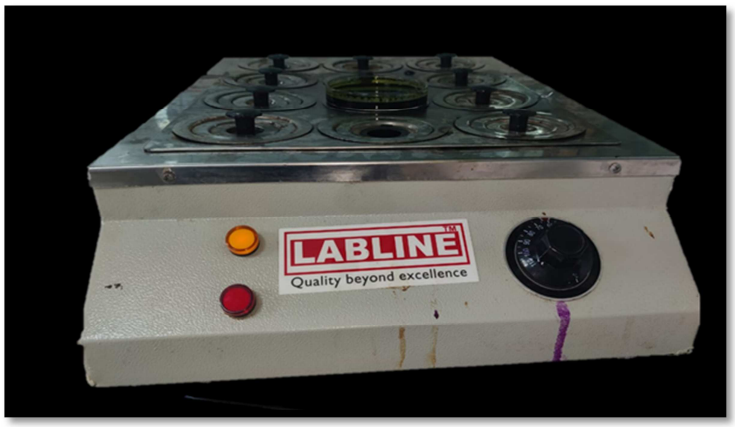


Fig 6. Evaporation of solvent by water bath

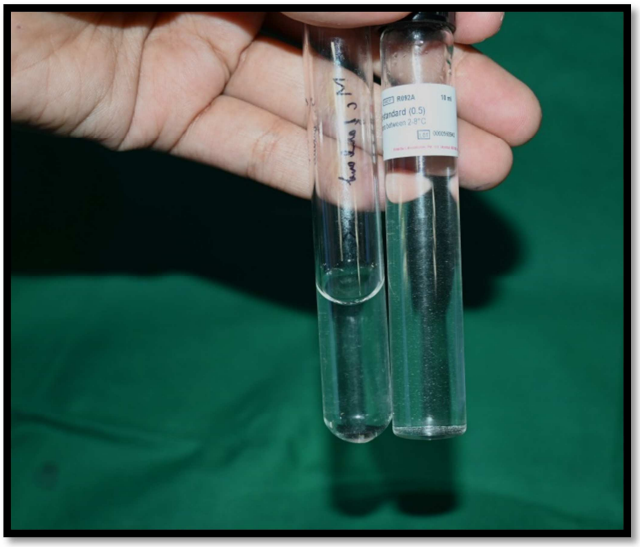


Fig 7: Standardization of bacterial inoculum by adjusting the opacity of 0.5 Mc Farland

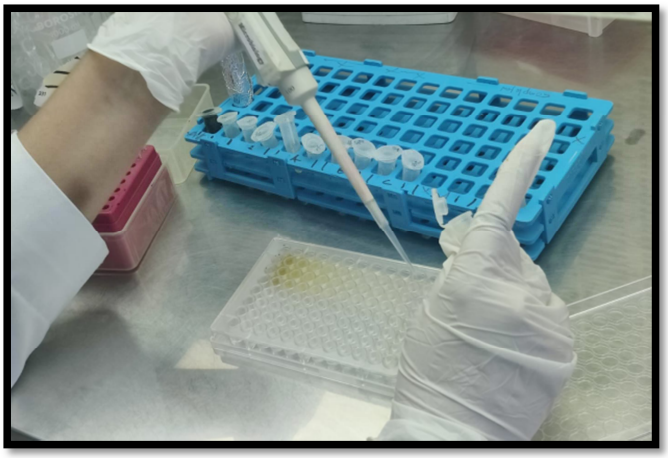


Fig 8: Serial dilution done in 96 well plate for MIC

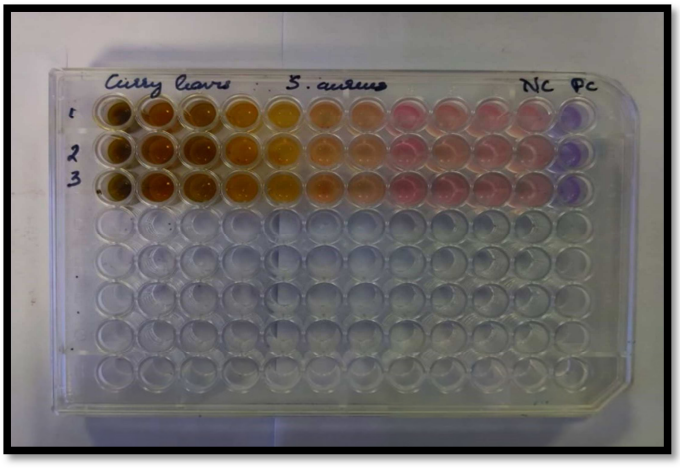


Fig.9: MIC values assessed by serial dilution in 96 well plate for *S.aureus*

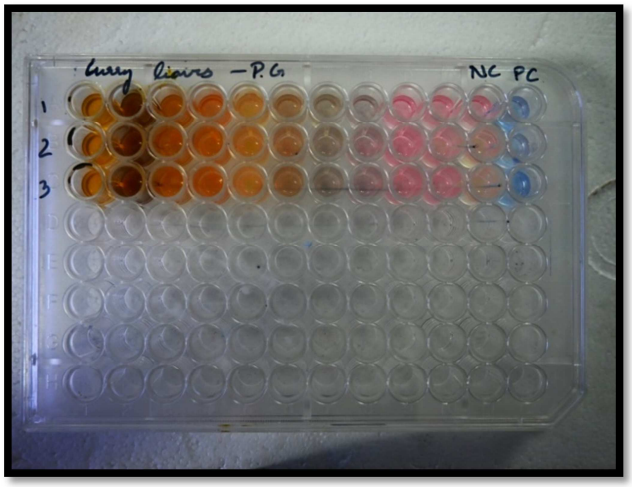


Fig.10: MIC values assessed by serial dilution in 96 well plate for *P.gingivalis*

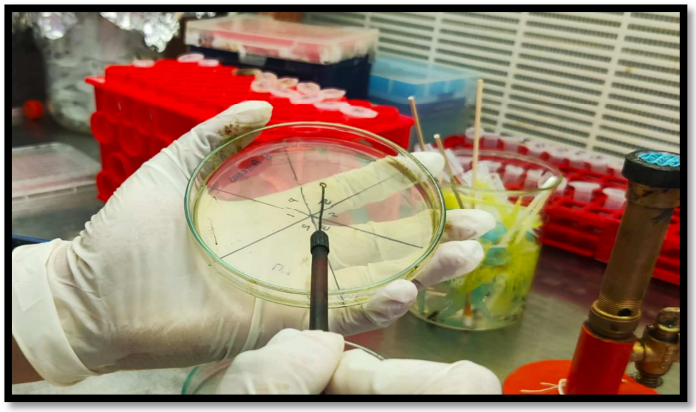


Fig.11: Streaking for MBC evaluation

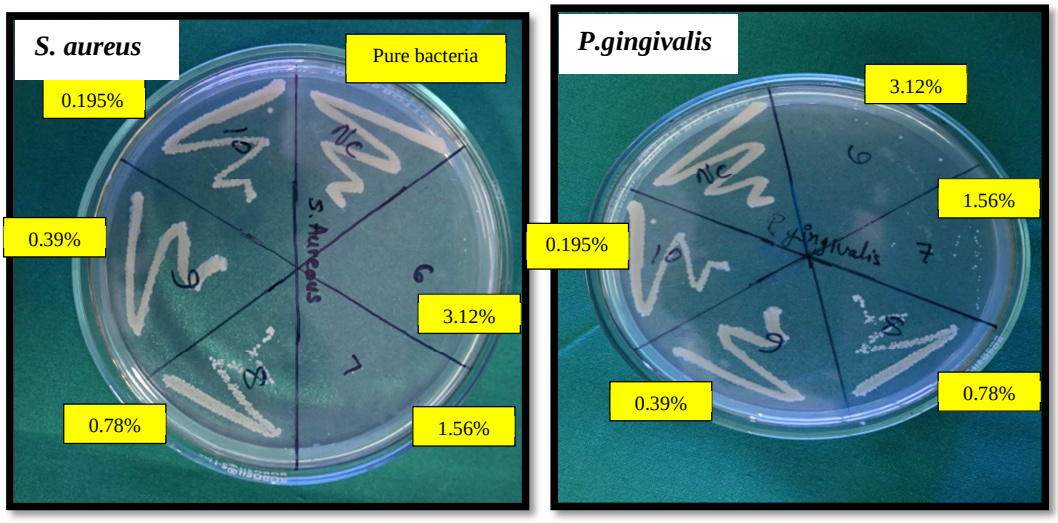


Fig 12 :MBC results of *S. aureus* and *P.gingivalis* for both bacteria growth is absent at a conc. of 3.12% hence it is selected as MBC value for the gel preparation

Fig 13: Materials: Carbopol, Glycerine, Sodium benzoate

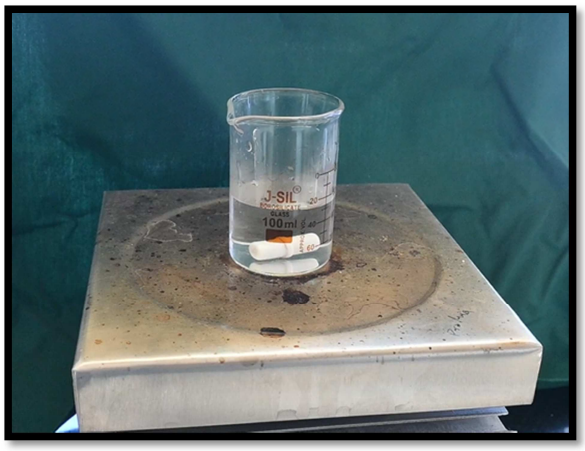


Fig 14: Magnetic stirrer – Carbopol with Distilled water

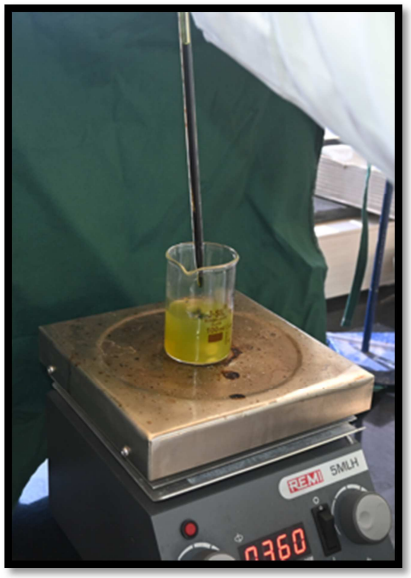


Fig 15: Magnetic stirrer –3% extract added.

Fig 16: Adjusted pH is verified by digital meter

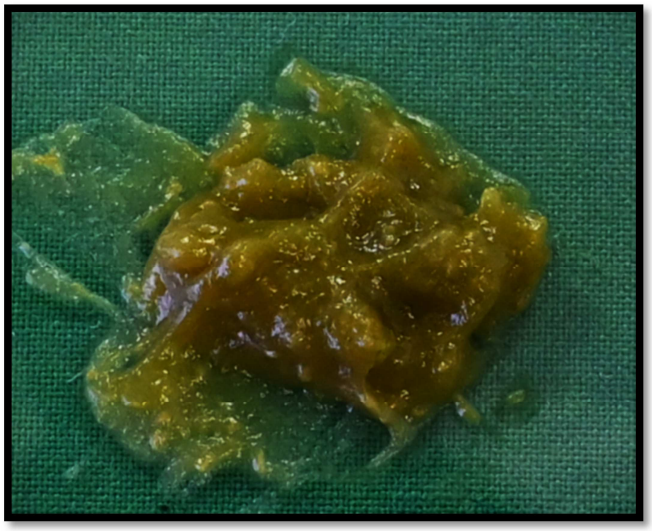


Fig 17: 3% Curry leaves (*Murraya koenigii*) gel

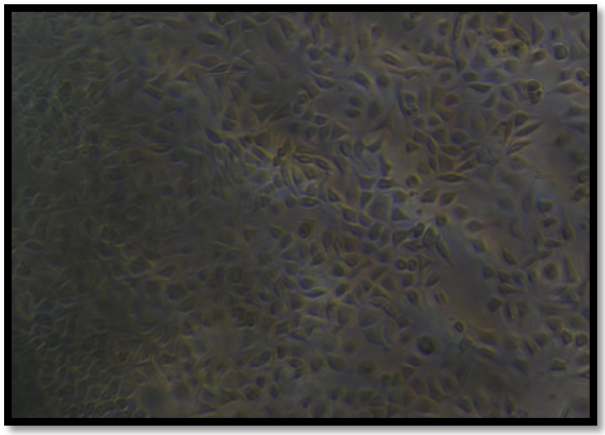


Fig 18: Viable L929 cell line

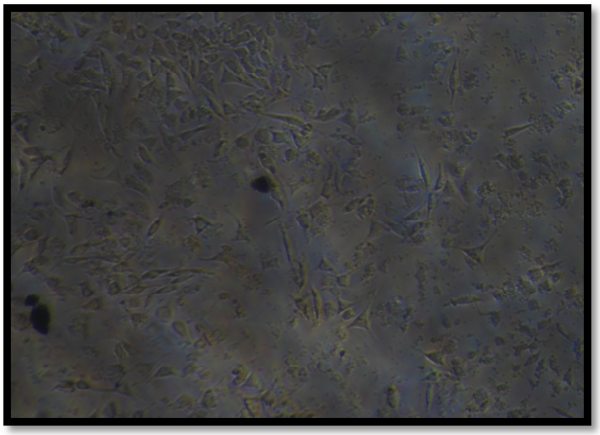


Fig 19: Results of MTT assay for CHX

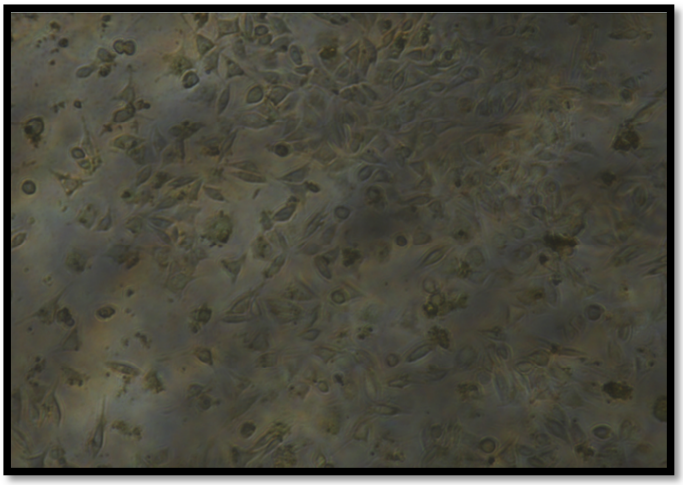


Fig 20: Results of MTT assay for Curry Leaves

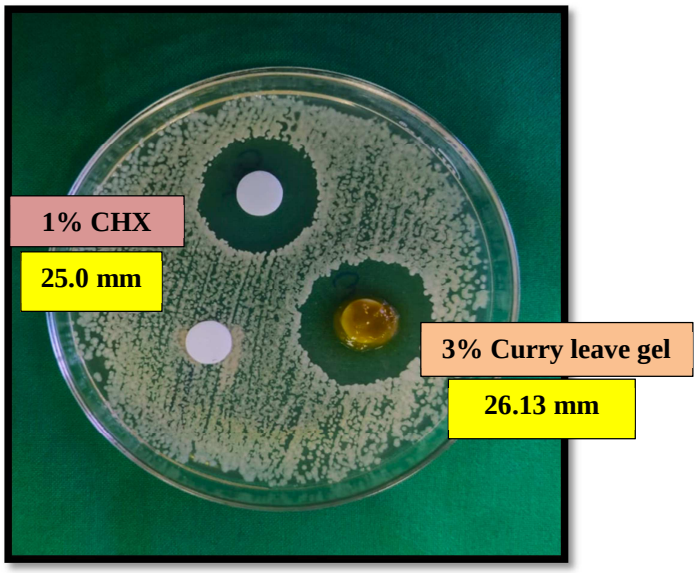


Fig 21: Disc diffusion Zirconia *Staphylococcus aureus*

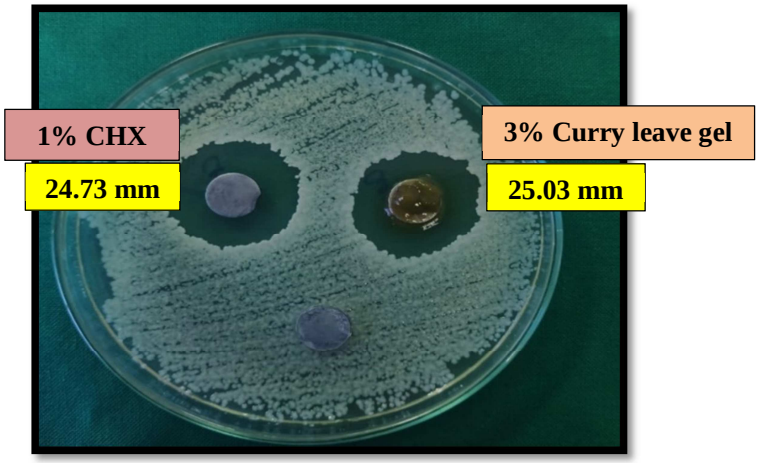


Fig 22: Disc diffusion test for Titanium *Staphylococcus aureus*

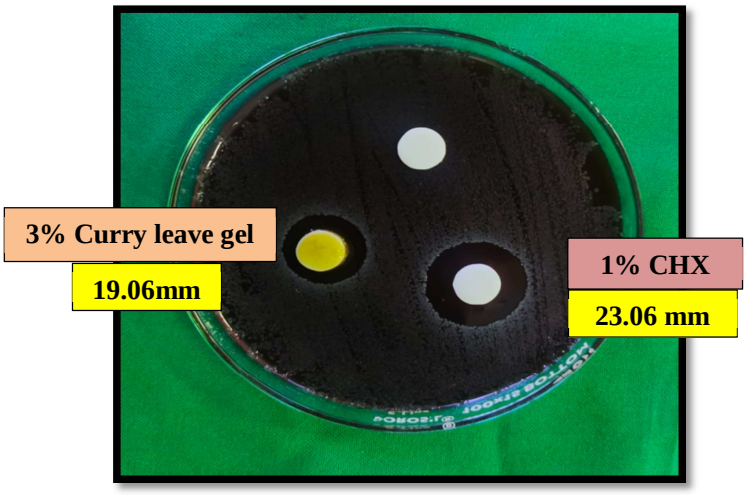


Fig 23: Disc diffusion test Zirconia *Porphyromonas gingivalis*

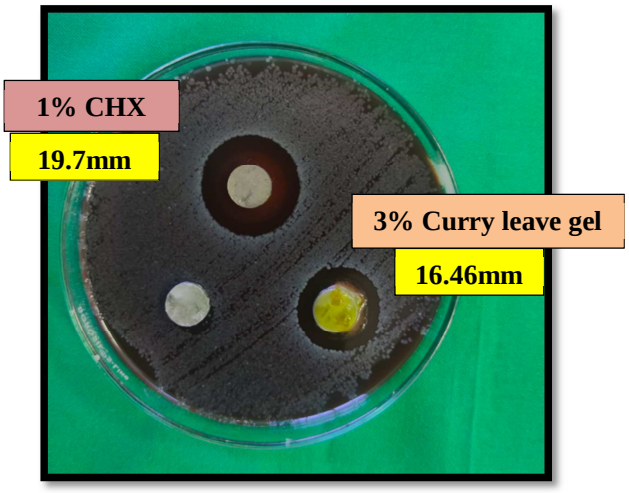


Fig 24: Disc diffusion test Titanium *Porphyromonas gingivalis*

RESULTS

The results of the present study are based on the descriptive statistical measures like Mean, Standard deviation were calculated in order to compare the two groups i.e the Control group 1% chlorhexidine gel with the Experimental group [3% Curry leaves (Murraya koenigii)] incorporated bio gel (table 3 and 4)

Zone of inhibition (mm) was measured to demonstrate antimicrobial activity of the 3% Curry Leaves (Murraya koenigii) incorporated bio gel and 1% CHX .

Comparison between two gels Murraya koenigii biogel and chlorhexidine against two microorganism (*S.aureus* and *P. gingivalis*) using two different implant materials (Titanium and Zirconia) was done by using unpaired T-test.

1. Staphylococcus Aureus

The study determined the mean and standard deviation values of the zone of inhibition for the experimental group, which consisted of 3% Curry Leaves (Murraya koenigii) gel. The results indicated mean values of 25.13 ± 1.18 for Titanium (table 5 and graph 5) and 24.73 ± 1.03 for Zirconia.(table 6 and graph 6).These values were compared with the control group, which used 1% CHX gel. Statistical analysis revealed no significant difference ($P > 0.05$) between the experimental and control groups, suggesting that both formulations possess similar antibacterial efficacy.

Furthermore, when comparing the two implant materials (Titanium and Zirconia) to assess their influence on antimicrobial efficacy against *S.aureus*, the analysis showed that Zirconia material has wider zone of inhibition compared to that of Titanium but there is no statistically significant difference.(table 7 and graph 7)

Based on these findings, it can be concluded that both the experimental and control groups exhibit comparable antibacterial effectiveness, and the choice of implant material does not significantly affect antimicrobial efficacy against *S. aureus*

2. Porphyromonus Gingivalis

The results of the study show that the mean values and standard deviations for the experimental group (3% Curry Leaves gel) were 19.733 ± 0.96 for Zirconia and 16.46 ± 1.50 for Titanium. For , the control group (1% CHX gel), had mean values of 23.06 ± 1.16 for Zirconia and 19.06 ± 1.66 for Titanium.(table 8 and 9) Statistical analysis revealed a significant difference ($P < 0.05$) between the experimental and control groups, indicating that 1% CHX exhibited greater antibacterial efficacy against *P. gingivalis* compared to the experimental group with 3% Curry Leaves gel.

Additionally, when assessing the impact of material on antibacterial activity against *P. gingivalis,* (table 10) the results displayed a statistically significant difference. Zirconia exhibited lower bacterial growth compared to Titanium. This suggests that Zirconia may possess superior antibacterial properties against *P. gingivalis* when compared to Titanium. These findings underscore the significance of both the antimicrobial agent and the implant material influences the effectiveness of antibacterial treatment against specific pathogens in peri-implantitis.

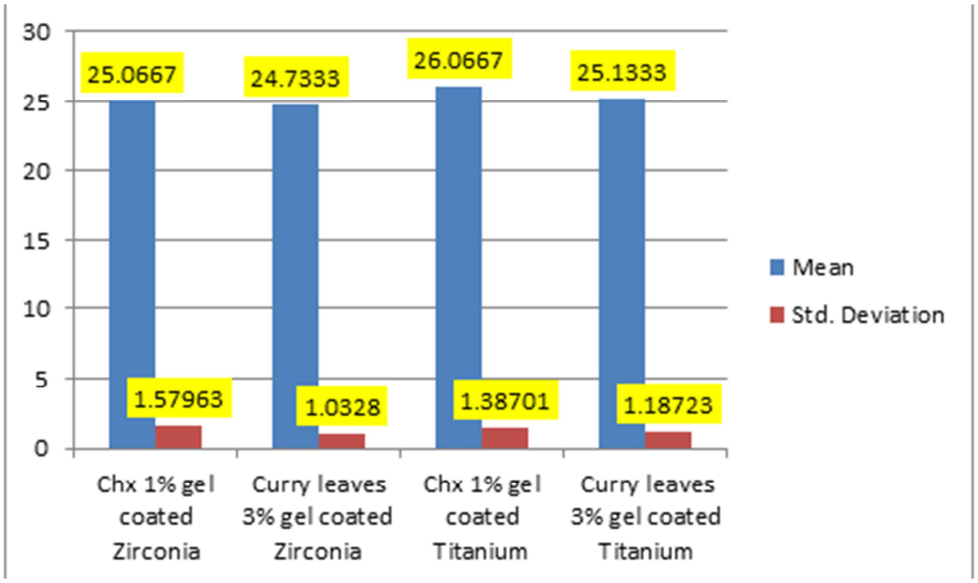
3. **MTT Assay**

The MTT test was applied to calculate the in vitro cytotoxic effect of Curry leaves (Murraya koenigi) incorporated bio gel. For evaluating cell viability and proliferation in cell culture, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a frequently used colorimetric method. The results showed that ICso value of Curry leaves gel and chlorhexidine gel against L929-23.37 is 442.9 and 199.3 ug/ml respectively.In comparison to CHX gel, Curry extract gel shows low cytotoxic effects.

Table 3 Summary of Comparison of efficacy of chlorhexidine 1% gel coated and Curry leaves 3% gel coated against *S. Aureus* on two materials (Titanium and Zirconia)

Groups	N	Mean	Std. Deviation
CHX1% gel coated Zirconia	15	25.0667	1.57963
Curry leaves 3% gel coated Zirconia	15	24.7333	1.03280
CHX1% gel coated Titanium	15	26.0667	1.38701
Curry leaves 3% gel coated Titanium	15	25.1333	1.18723

Graph 3 Summary of Comparison of efficacy of chlorhexidine 1% gel coated and Curry leaves 3% gel coated against *S. Aureus* on two materials (Titanium and Zirconia)



This table and graph demonstrates the mean and standard deviation of zone of inhibition for 1% CHX and 3% Curry Leaves incorporated gel on titanium and Zirconia for *S.aureus*

Table 4 Summary of Comparison of efficacy of chlorhexidine 1% gel coated and Curry leaves 3% gel coated against *P. gingivalis* on two materials (Titanium and Zirconia)

Groups	N	Mean	Std. Deviation
Chx 1% gel coated Zirconia	15	23.0667	1.16292
Curry leaves 3% gel coated Zirconia	15	19.7333	.96115
Chx 1% gel coated Titanium	15	19.0667	1.66762
Curry leaves 3% gel coated Titanium	15	16.4667	1.50555

Graph 4 Summary of Comparison of efficacy of chlorhexidine 1% gel coated and Curry leaves 3% gel coated against *P. gingivalis* on two materials (Titanium and Zirconia)

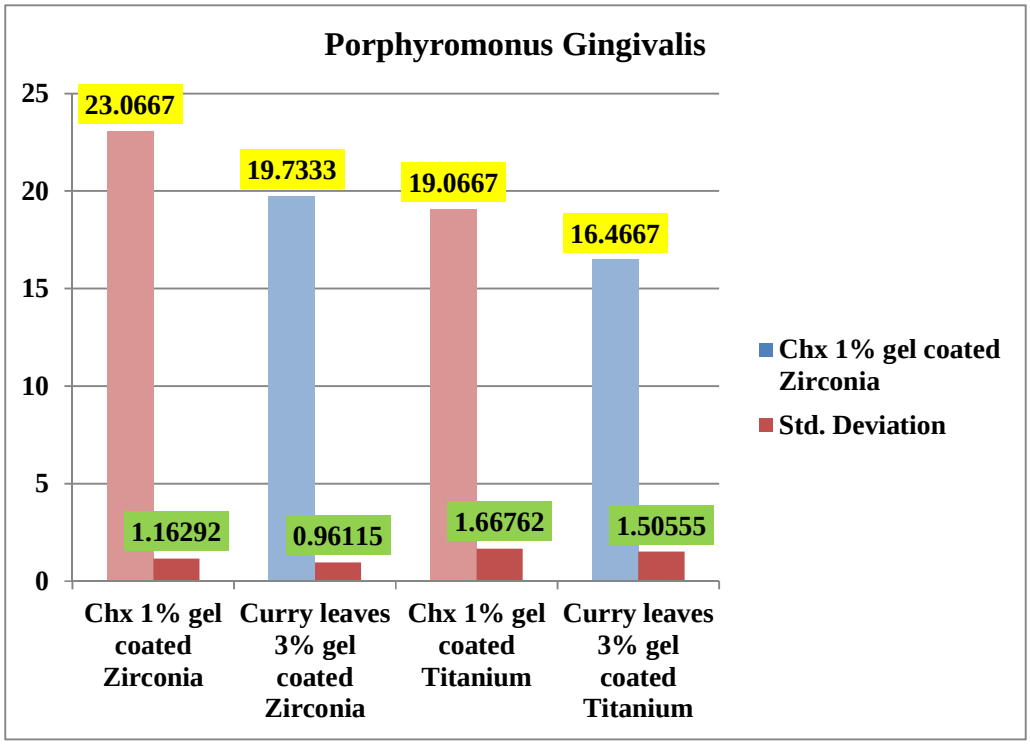


Table 5 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Titanium surface for *S. aureus*

	Groups	N	Mean	Std. Deviation	t value	P value
Titanium	CHX 1% gel coated	15	26.0667	1.38701	1.980	0.058
	Curry leaves 3% gel coated	15	25.1333	1.18723		

P> 0.05

On pairwise comparison of these two groups the results were showed no statistically significant difference between 3% Curry Leaves incorporated gel and 1% CHX and concluded similar antibacterial efficacy between both groups

Graph 5 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Titanium surface for *S. aureus*

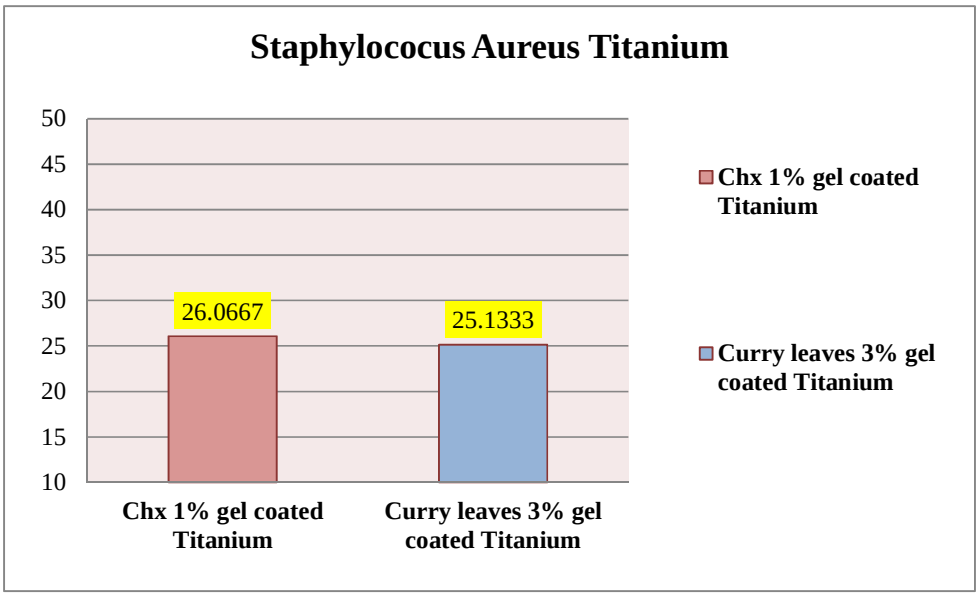


Table 6 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX on Zirconia surface for *S. aureus*

	Groups	N	Mean	Std. Deviation	t value	P value
Zirconia	Chx 1% gel coated	15	25.0667	1.57963	0.684	0.500
	Curry leaves 3% gel coated	15	24.7333	1.03280		

P> 0.05

On pairwise comparison of these two groups the results showed no statistically significant difference on Zirconia between 3% Curry leaves incorporated gel and 1% CHX and concluded similar antibacterial efficacy between both groups

Graph 6 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Zirconia surface for *S. aureus*

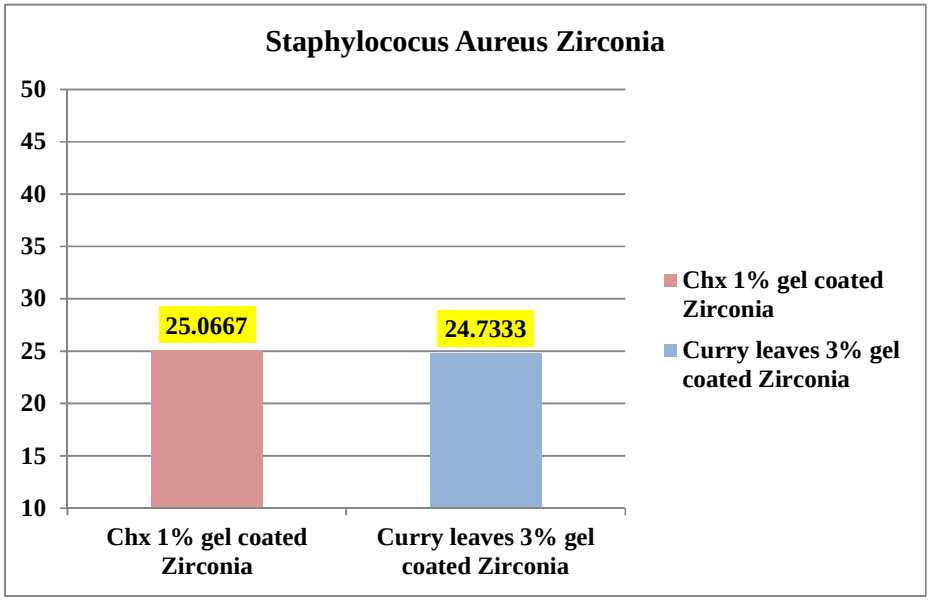


Table 7 Pairwise comparison for effect of material on antibacterial efficacy of two groups 3% curry leaves incorporated gel on Titanium and Zirconia for *S. aureus*

Groups	N	Mean	Std. Deviation	t value	P value
Zirconia	15	26.0667	1.38701	1.980	0.058
Titanium	15	25.1333	1.18723		

On pairwise comparison of these two groups for *S. aureus* the results showed no statistically significant difference between Zirconia and Titanium on 3% Curry leaves incorporated gel concluded no significant effect on antibacterial efficacy of novel gel

Graph 7 Pairwise comparison for effect of material on antibacterial efficacy of two groups 3% curry leaves incorporated gel on Titanium and Zirconia for *S. aureus*

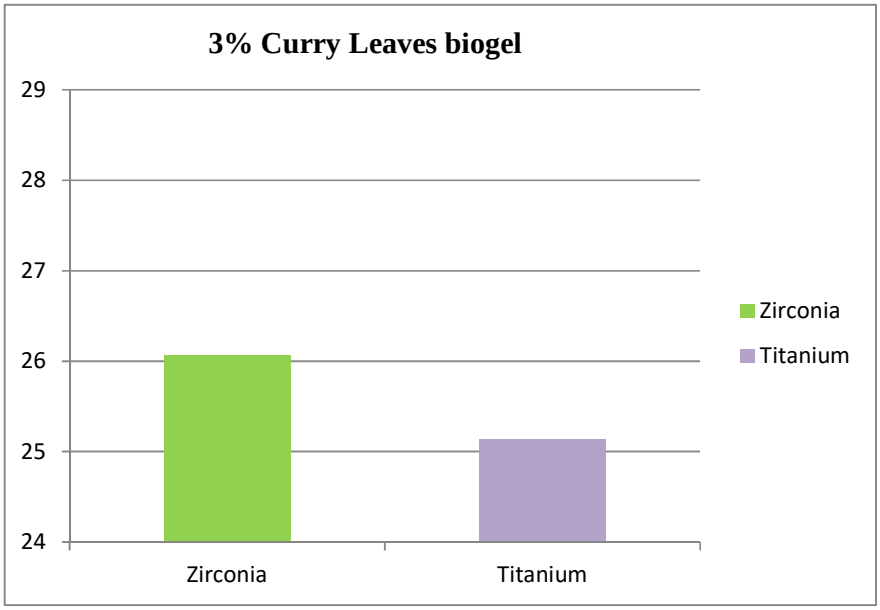


Table 8 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Titanium surface for *P.gingivalis*

	Groups	N	Mean	Std. Deviation	t value	**P value**
Titanium	CHX 1% gel coated	15	19.0667	1.66762	4.482	**<0.001***
	Curry leaves 3% gel coated	15	16.4667	1.50555		

P < 0.05

On pairwise comparison of these two groups the results were showed statistically significant difference between 3% Curry leaves incorporated gel and 1% CHX and concluded that for *P.gingivalis* 1% CHX gel has better antibacterial efficacy between both groups than 3% Curry leaves incorporated gel

Graph 8 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Titanium surface for *P.gingivalis*

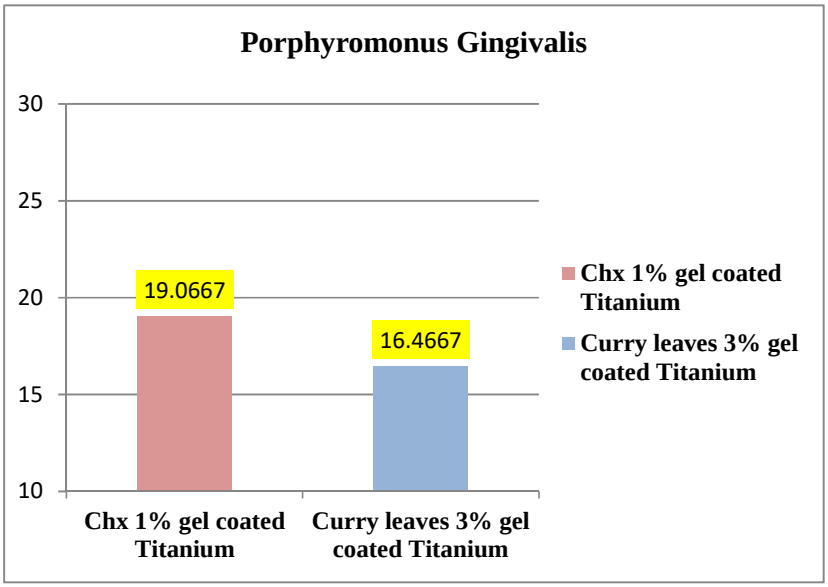


Table 9 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Zirconia surface for *P.gingivalis.*

	Groups	N	Mean	Std. Deviation	t value	P value
Zirconia	CHX 1% gel coated	15	23.0667	1.16292	8.557	**<0.001***
	Curry leaves 3% gel coated	15	19.7333	.96115		

P < 0.05

On pairwise comparison of these two groups the results were showed statistically significant difference between 3% Curry leaves incorporated gel and 1% CHX and concluded that for P.gingivalis 1% CHX gel has better antibacterial efficacy between both groups than 3% Curry leaves incorporated gel

Graph 9 Pairwise comparison of two groups (3% Curry leaves incorporated gel and 1% CHX) on Zirconia surface for *P.gingivalis*.

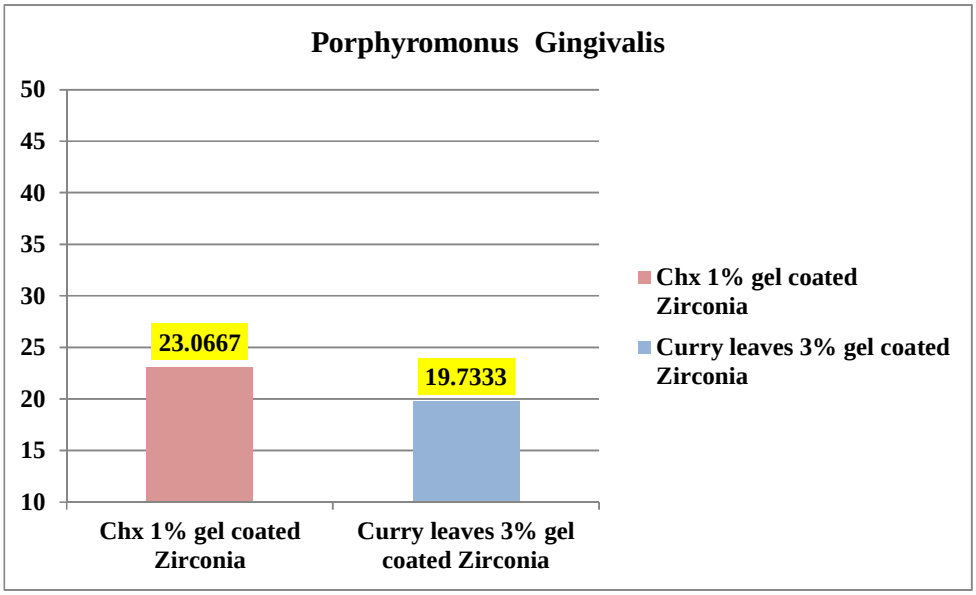


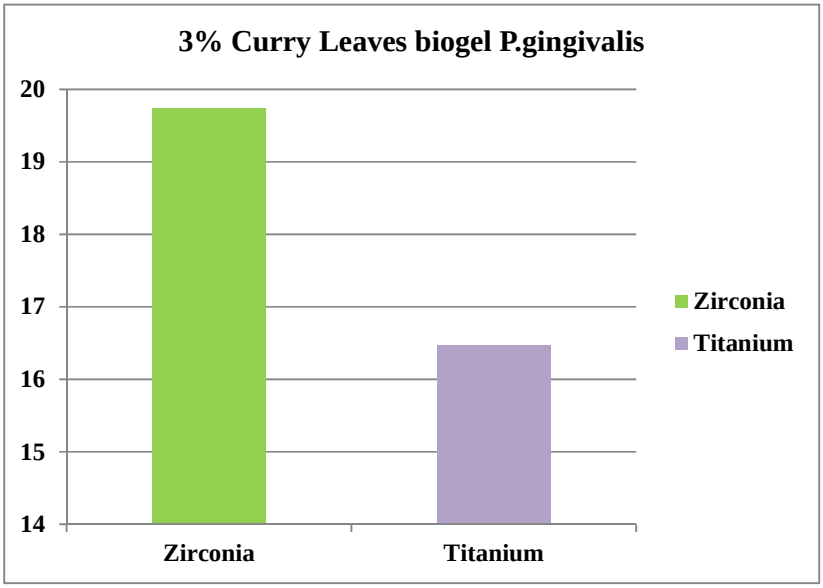
Table 10 Pairwise comparison for effect of material on antibacterial efficacy of two groups 3% curry leaves incorporated gel on Titanium and Zirconia for *P.gingivalis*

Groups	N	Mean	Std. Deviation	t value	P value
Zirconia	15	19.7333	.96115	4.482	<0.001*
Titanium	15	16.4667	1.50555		

P< 0.05

On pairwise comparison of these two groups for P.gingivalis the results showed statistically significant difference between Zirconia and Titanium on 3% Curry leaves incorporated gel concluded that choice of material influences effect on antibacterial efficacy of novel gel

Graph 10 Pairwise comparison for effect of material on antibacterial efficacy of two groups 3% curry leaves incorporated gel on Titanium and Zirconia for *P.gingivalis*



DISCUSSION

Replacing the missing teeth, osseointegrated implants are widely employed treatment modality known for their favourable long-term prognosis and a survival rate typically ranging between 93.3 to 98%.[47] There is a fair chance of failure even after following the standard surgical protocol in particular clinical setting. These failures could be loss of osseointegration, implant and screw fracture, screw loosening and biologic complications such as peri-implantitis.[5]

Peri-implantitis based on clinical features, and severity, it is classified as early, moderate and advanced by Froum and Rosen in 2012.[5]

Reducing the microbial burden and inflammation surrounding the peri-implant mucosa is the main goal of treating early peri-implantitis in order to protect the surrounding bone. In order manage peri-implantitis, therapy consist of mechanical debridment, periodontal maintenance therapy, and home care.[48] Mechanical debridement techniques including plastic scaler tips, air abrasive and water jet spray are used to reduce the microbial burden. **Karring et al**[49] thorough removal of submucosal bacterial load by using ultrasonic device or carbon curettes, is not sufficient for completely decontaminating the implants surface with presence of pockets combined with implant threads. **Renvert et al.**[11] **Liran Levin**[46] the chemical dis-infectants like chlorhexidine mouthwash and gel can gain the supplemental advantages, which are considered to be gold standard for the management of early peri-implantitis.

The use of adjunct therapy leads to more rapid suppression of both anaerobic and aerobic bacteria on the implant surface. However, prolonged use of Chlorhexidine may result in discoloration of teeth, tongue, and restorative materials, as well as

conditions such as altered taste sensation (dysgeusia), desquamative gingivitis, burning of the mucous membranes, and allergic reactions.[13,25,26] To overcome all these dis-advantages, herbal medicines emerges as a promising alternative. Herbal remedies offer a natural, well-tolerated approach, minimizing the risk of adverse reactions common with pharmaceuticals. Their cost-effectiveness and accessibility make herbal treatments attractive for long-term management of early peri-implantitis.

The aim of present study was to formulate and evaluate the antibacterial efficacy of Curry leaves incorporated biogel for the treatment of early peri-implantitis pathogens *S.aureus and P.gingivalis* on Titanium and Zirconia disc. (implant surface)

In the present study, carbopol serves as a carrier for formulating the biogel incorporating 3% Curry leaves (Murrya koenigii) extract, leading to their ability to incorporate a biologically active drug molecules through physical cross-linking within the polymer chains, resulting in the formation of a swollen network.[27] **Nurul Ain et al.[51]** have discussed the bioadhesive property of Carbopol, which refers to the ability of these gels to effectively adhere to biological surfaces such as mucous membranes. This property facilitates the localization of the drug in a specific region, thereby enhancing its bioavailability. Additionally, it prolongs the contact time between the drug and the mucosa, leading to sustained and prolonged drug release.

In the present study, 3% Curry leaves (Murrya koenigii) biogel formation was carried out. The concentration of the Curry leaves (Murrya koenigii) was determined based on the MIC and MBC values of *S aureus and P.gingivalis* 1.56 and 3.12 respectively. In order to formulate the gel effective against both bacteria the final concentration of 3% is selected for novel gel preparation. Similarly **Gupta et al [17] and Varghese et al** [34] formulated a mouthwash at a concentration 2.5% and 3%

respectively and showed significant results. The antibacterial effectiveness of 1% CHX and 3% gel containing Curry Leaves (*Murraya koenigii*) was evaluated using the disk diffusion test was used for *S.aureus and P.gingivalis.* As this method is cost-effective, simple to execute, and easy to interpret, making it a more practical choice for evaluating antimicrobial formulations[52]

The results of Disk diffusion study the study for *S. aureus,* showed that the 3% gel incorporating Curry Leaves (Murraya koenigii) is as effective as 1% CHX, displaying similar zones of inhibition of 26 mm (fig 21 and 22, tables 5 and 6). These results align with a study conducted by **Chanchal Katariya[18]**, which compared CHX and Curry leaf extract using the agar diffusion method, yielding similar outcomes. Additionally, another study by **Al Harbi et al[16]** indicated that the ethanolic extract of *Murraya koenigii* exhibited lower activity compared to CHX against *S. aureus,* forming a zone of inhibition of 15mm.

The results of Disk diffusion study for *P.gingivalis,* the 3% Curry Leaves (Murraya koenigii) incorporated gel demonstrates effective antibacterial activity against *P.gingivalis* by forming a zone of inhibition of 16 mm (fig 23 and 24, table 6 and 7). **Nakao et al.[7]** the antibacterial efficacy of Curry Leaves (*Murraya koenigii)* against *P. gingivalis* occurs through vesicle formation on bacterial surfaces, leading to membrane depolarization and rupture and found out that Curry leaf extract (CLE) efficiently eradicated *P. gingivalis* within 30 minutes of exposure.

However, upon comparison of 3% Curry leaves (*Murraya koenigii*) biogel with the 1% CHX gel, 3% Curry leaves (*Murraya koenigii*) gel exhibits lesser zone of inhibition of 16 mm compared to 19 mm zone for CHX. This findings are consistent with the results of **Varghese et al.,[34]** where they compared chlorhexidine

gluconate with a 3% Curry Leaves *(Murraya koenigii*) mouthwash for *P. gingivalis*, showing reduced periodontal and gingival index, but compared to CHX it is less effective. These outcomes might be attributed to the lower concentration of Curry Leaves (*Murraya koenigii*) in the gel; at higher concentrations, this gel may yield more promising results.

The biogel antibacterial activity was evaluated on Titanium and Zirconia discs .The results showed that on Zirconia discs, antibacterial action was better compared to Titanium discs for both *S.aureus* and *P.gingivalis* (fig 21, 22, 23 and 24 Tables 6 and 10). These results could because of the fact that The surface topography of the biomaterial is a significant factor in cellular adhesion. Similar results were seen in study by **Al-Radha[24]** et al. they compared bacterial adherence on two varieties of Zirconia surfaces and on Titanium surface in vivo tests, and found that both Zirconia surfaces contained noticeably fewer bacteria than Titanium surface. **Irmgard Hauser-Gerspach et al.** [53] and **Franco et al**.[54] offer insights into Zirconia, confirming that its smoother surface texture results in lower bacterial adhesion compared to Titanium. Their findings suggest that Zirconia could be a favorable material for implant abutments, exhibiting reduced colonization potential

The surface characteristics of dental implants such as surface roughness and surface energy, can also play a crucial role in bacterial adhesion, influencing the development of peri-implantitis, hence the surface roughness values for both Titanium discs and Zirconia discs I,e (0-5μm) were standardized in the present study..

The commercially available chlorhexidine's bactericidal activity is by precipitation or coagulation of the cytoplasm, induced by protein cross-linking. But continuous use of CHX is associated with numerous disadvantages like staining,

changes in the mucosal permeability. according to a study by **Rebstein et al**.[13] even when CHX concentration was reduced to 0.0025%, staining still occurred in the teeth. **HELMS et al.**[26] also demonstrated further side effects of CHX and indicated that the use of 0.12% chlorhexidine gluconate can lead to taste alterations, specifically by decreasing the perception of saltiness and bitterness. The solutions containing sodium chloride may be perceived as bitter after chlorhexidine rinsing. **Harvey**[25] Colleagues noted that 2% chlorhexidine used topically can cause hyperplasia and a reduction in mucosal permeability. They also mentioned that P-chloraniline (PCA), which has been demonstrated to have mutagenic and carcinogenic qualities, is a component of all formulations containing chlorhexidine.

So one of the aim of the present study was to create a non-surgical topical treatment option that is safe and free from adverse effects, aiming to enhance treatment standards for patients.

The cytotoxicity test for Curry leaves (Murraya koenigii) was carried out by using MTT assay on L929 mouse fibroblast cells. The MTT test was applied to calculate the in vitro cytotoxic effect of Curry leaves (Murraya koenigi) incorporated bio gel. For evaluating cell viability and proliferation in cell culture, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a frequently used colorimetric method. The results showed that ICso value of Curry leaves gel and chlorhexidine gel against L929-23.37 is 442.9 and 199.3 ug/ml respectively.In comparison to CHX gel, Curry extract gel shows low cytotoxie effects. (fig 18 and 19)

Similar results are stated by study conducted by **Umesh Varma**[44] on herbal extracts with the aim that herbal extracts are less cytotoxic compared to synthetic

agents. In his study, he compared CHX and Neem extract and concluded that CHX above 1% concentration has shown a toxic effect on hGFs (human gingival fibroblast) at 1min, 5min, and 15 min of time exposure. However, Neem Extract did not show adverse side effects on the fibroblasts even after the use of 50% concentration of Neem Extract. Resulting in overallnless toxic effect in comparison with CHX.[50]

The outcome of this in vitro study by using 3% Curry Leaves (Murraya koenigii) incorporated biogel has shown promising and reliable results and it can be a effective alternative for management of early peri-implantitis.

CLINICAL IMPLICATIONS

According to present study the reduced cytotoxicity of the Curry leaf extract(Murraya koenigi) based gel suggests a higher safety profile for clinical use compared to CHX gel. This is particularly advantageous in situations where repeated or prolonged application of the gel is necessary, such as in the management of early peri-implantitis.

The Curry leaf (Murraya koenigi) extract-based gel demonstrates comparable bactericidal properties to CHX gel. This indicates that it can effectively combat oral pathogens and contribute to the control of infections, including peri-implantitis, without compromising safety.

Overall, the use of Curry leaf (Murraya koenigi) extract-based herbal gel presents promising clinical implications, offering a safer yet equally effective alternative to CHX gel for the management of early peri-implantitis. Further research and clinical trials are warranted to validate these findings and explore the full potential of Curry leaf (Murraya koenigi) extract in dental care.

SCOPE OF STUDY

According to the findings of this study, a notable decrease in microbial colony forming units occurred following the application of the novel 3% Curry Leaves (*Murraya koenigii)* incorporated biogel against the targeted strains. It is essential to explore the impact of this herbal biogel on various strains of microorganisms present in the oral cavity.

Future investigations could involve assessing antimicrobial efficacy at higher concentrations of Curry leaves (Murraya koenigii) gel and for longer durations, as microbial growth may vary over time. Additionally, the long-term effects of the novel 3% Curry Leaves (Murraya koenigii) incorporated biogel should be documented.

This study lays the groundwork for further research into the antimicrobial effectiveness of different formulations and combinations of phytopharmaceutical extracts from Curry leaves (Murraya koenigii), as well as the optimization of pharmaceutical compositions to achieve desired antimicrobial properties over time. Furthermore, future studies could explore the long-term effects of herbal preparations, evaluating the biogel's osteogenic properties, and include additional in vivo parameters under varied conditions with long-term monitoring.

LIMITATIONS OF THE STUDY

The limitations of the study include:

1. The study primarily relies on laboratory experiments such as disk diffusion tests, which may not fully replicate real clinical conditions.
2. Single Concentration Evaluation: The study only assesses the antibacterial efficacy of a single concentration (3% Curry Leaves gel), which may not capture potential variations in effectiveness at different concentrations.
3. Limited Scope of Tested Pathogens: The study evaluates the efficacy against only two specific bacteria (*S. aureus* and *P. gingivalis*), potentially overlooking the effects on other relevant peri-implant pathogens.
4. Short-Term Evaluation: The study's assessment of cytotoxicity and antibacterial efficacy may not reflect long-term effects or consider potential changes in effectiveness over time.

CONCLUSION

Based on the discussions and findings in the present study, The study demonstrates that 3% Curry Leaves (*Murraya koenigi*) gel exhibits promising antibacterial efficacy against early peri-implant pathogens, comparable to conventional 1% CHX gel with less cytotoxicic potential which are associated with standard drug.

The antibacterial properties of curry leaves (*Murraya koenigi*) coupled with the biogel's matrix provides promising avenue for treatment of early peri-implantitis

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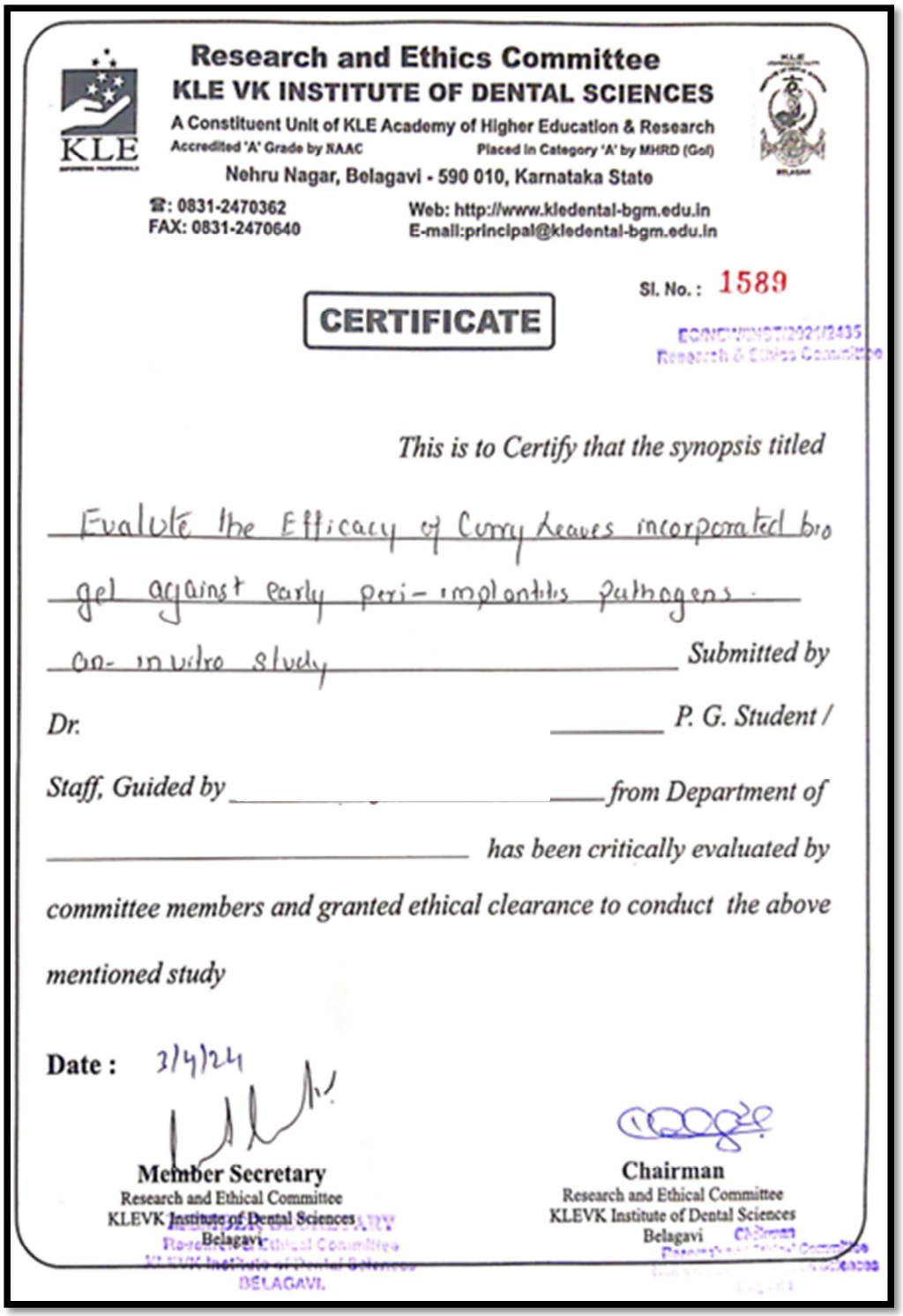
ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE



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E-mail:principal@kledental-bgm.edu.in

Sl. No.: 1589

CERTIFICATE

This is to Certify that the synopsis titled

Evalute the Efficacy of Curry leaves incorporated bio gel against early peri-implantitis pathogens - an- in vitro study *Submitted by*

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

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

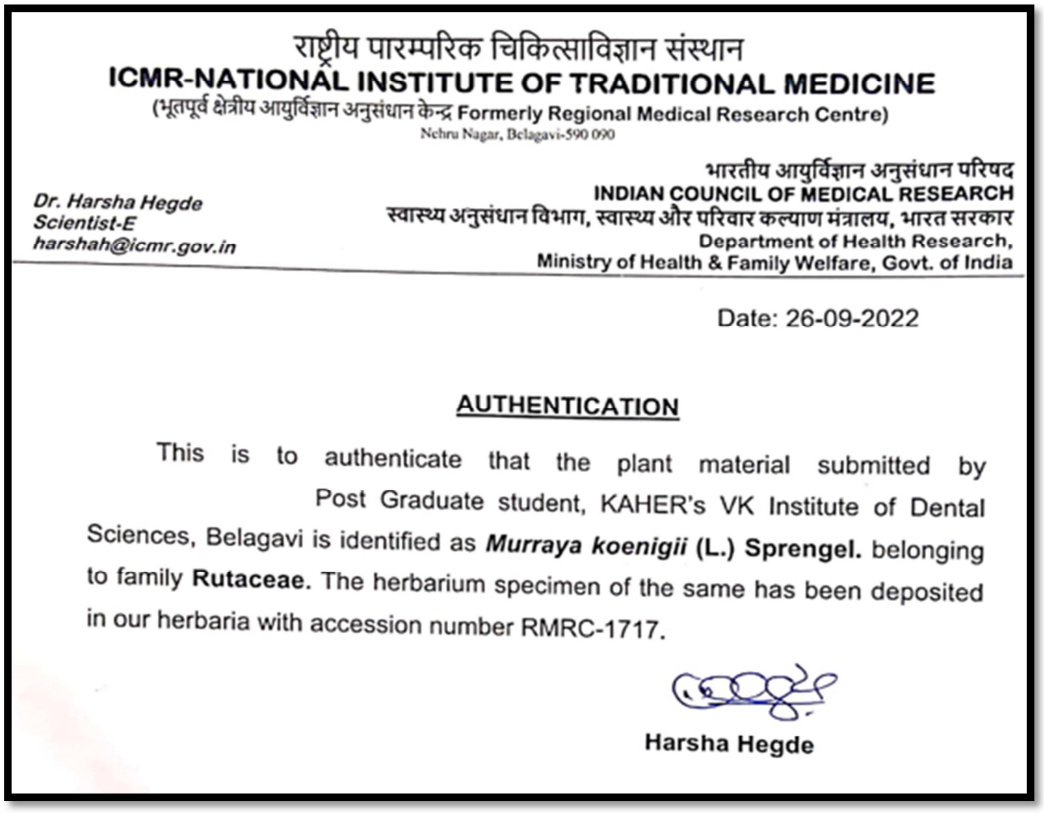
Date : 3/4/24

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

CURRY LEAVES AUTHENTICATION CERTIFICATE



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Department of Health Research,
Ministry of Health & Family Welfare, Govt. of India

Date: 26-09-2022

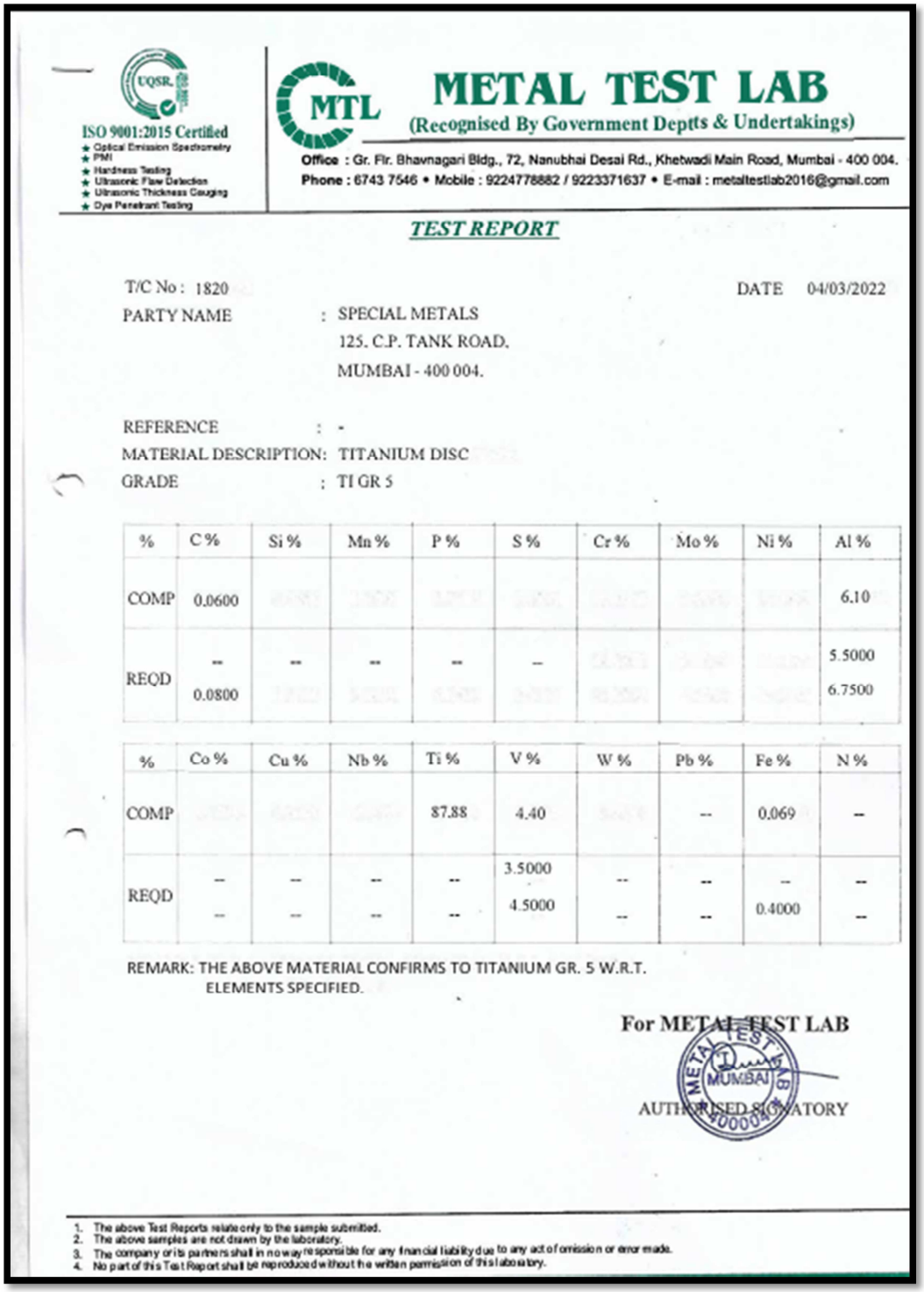
AUTHENTICATION

This is to authenticate that the plant material submitted by Post Graduate student, KAHER's VK Institute of Dental Sciences, Belagavi is identified as ***Murraya koenigii* (L.) Sprengel.** belonging to family **Rutaceae.** The herbarium specimen of the same has been deposited in our herbaria with accession number RMRC-1717.

Harsha Hegde

ANNEXURE III

CERTIFICATE OF GRADE V TITANIUM DISC



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Office : Gr. Flr. Bhavnagari Bldg., 72, Nanubhai Desai Rd., Khetwadi Main Road, Mumbai - 400 004.
Phone : 6743 7546 • Mobile : 9224778882 / 9223371637 • E-mail : metaltestlab2016@gmail.com

TEST REPORT

T/C No : 1820 DATE 04/03/2022

PARTY NAME : SPECIAL METALS
125. C.P. TANK ROAD.
MUMBAI - 400 004.

REFERENCE : -
MATERIAL DESCRIPTION: TITANIUM DISC
GRADE : TI GR 5

%	C %	Si %	Mn %	P %	S %	Cr %	Mo %	Ni %	Al %
COMP	0.0600								6.10
REQD	-- 0.0800	--	--	--	--				5.5000 6.7500

%	Co %	Cu %	Nb %	Ti %	V %	W %	Pb %	Fe %	N %
COMP				87.88	4.40		--	0.069	--
REQD	-- --	-- --	-- --	-- --	3.5000 4.5000	-- --	-- --	-- 0.4000	-- --

REMARK: THE ABOVE MATERIAL CONFIRMS TO TITANIUM GR. 5 W.R.T. ELEMENTS SPECIFIED.

For METAL TEST LAB

AUTHORISED SIGNATORY

1. The above Test Reports relate only to the sample submitted.
2. The above samples are not drawn by the laboratory.
3. The company or its partners shall in no way responsible for any financial liability due to any act of omission or error made.
4. No part of this Test Report shall be reproduced without the written permission of this laboratory.

ANNEXURE IV

RESULTS OF DISC DIFFUSION

***S. aureus* Zirconia (all measurments are in mm)**

Sr no	CHX 1% gel coated	Curry leaves 3% gel coated	Non coated
1	25	25	-
2	26	26	-
3	25	27	-
4	23	25	-
5	26	25	-
6	25	26	-
7	22	24	-
8	27	27	-
9	28	28	-
10	23	24	-
11	24	25	-
12	23	25	-
13	24	26	-
14	25	25	-
15	25	27	-

***S. aureus* Titanium**

Sr no	CHX 1% gel coated	Curry leaves 3% gel coated	Non coated
1	23	24	
2	24	26	
3	24	25	
4	25	25	
5	24	26	
6	25	24	
7	26	27	
8	27	26	
9	25	23	
10	24	223	
11	26	25	
12	25	26	
13	24	25	
14	25	24	
15	24	24	

***P. gingivalis* Titanium (all measurement are in mm)**

Sr no	CHX 1% gel coated	Curry leaves 3% gel coated	Non coated
1	23	19	-
2	24	18	--
3	22	18	-
4	22	18	-
5	25	23	-
6	24	18	-
7	23	19	-
8	23	19	-
9	25	23	-
10	23	19	-
11	22	18	-
12	23	19	-
13	24	18	-
14	22	18	-
15	21	19	-
	Mean 23.06	Mean 19.06	

***P.gingivalis* Zirconia**

Sr no	Chx 1% gel coated	Curry leaves 3% gel coated	Non-coated
1	20	16	-
2	20	18	-
3	20	16	-
4	19	16	-
5	21	17	-
6	18	15	-
7	20	17	-
8	19	14	-
9	20	18	-
10	18	14	-
11	19	15	-
12	20	17	-
13	21	19	-
14	21	18	-
15	20	17	-
	Mean 19.73	Mean 18.6	