"COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF A NOVEL HERBAL GEL CONTAINING ORANGE PEEL EXTRACT AND GINGER EXTRACT WITH CHLORHEXIDINE GEL ON PERI-IMPLANTITIS PATHOGENS – AN VITRO STUDY

BY

REG. NO- IM0220004

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LIST OF ABBREVIATIONS USED IN THE STUDY

P.gingivalis	Porphyromonas gingivalis
P.intermedia	Prevotella intermedia
PB	Probing depth
S.D.	Standard Deviation
hrs.	Hours
L	Litre
M	milli
CHX	Chlorhexidine
AQ	Aqueous
ALC	Alcoholic
PBS	Phosphate Buffer Solution
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
DMEM	Dulbecco's Modified Eagle Medium
CO_2	Carbon dioxide
BHI	Brain Heart Infusion Agar

ABSTRACT

STATEMENT OF PROBLEM

The partially edentulous conditions can be treated with conventional removable prosthesis, fixed restorations or dental implants. Dental implants are the most recommended treatment modality due to their success rate. Although it may lead to complications in few cases, as they are prone to bacterial colonization which may result in bone destruction and implant failure. The management of peri-implant disease aim to reduce bacterial adherence while leaving the implant surface intact and improving the prognosis of the implant.

PURPOSE

The aim of this present study is to evaluate the efficacy of novel herbal gel containing aqueous orange peel extract and alcoholic ginger extract when compared with chlorhexidine gel against periimplantitis pathogens.

METHODS

A total of 68 discs of commercially available pure Titanium grade 5 fabricated of diameter 10 mm and a width of 2 mm. The discs were subdivided into groups as control(chlorhexidine) and experimental (herbal gel). The extracts used was alcoholic ginger extract and aqueous orange peel extract. The prepared extract was subjected to antibacterial assay (MIC and MBC) using serum dilution and disk diffusion method to check the zone of inhibition. Once the MIC and MBC values were achieved the formulation of the herbal gel was carried out. The gel formulation was carbopol based Once the herbal gel was formulated, the Titanium discs were subjected to antibacterial testing in vitro using disk diffusion method.

RESULT

The collected data was subjected to statistical analysis using Kolmogorov Smirnov test and parametric tests. Herbal gel showed comparable results with chlorhexidine gel on day 4.

CONCLUSION

This present study showed that herbal gel showed antibacterial activity against early peri implant pathogens in Titanium implant material. On the other hand when compared to the Chlorhexidine (CHX) gel control group, the experimental group(herbal gel) showed comparable zone of inhibition on P.intermedia and *P.gingivalis*.

KEYWORDS

Chlorhexidine gel, disc diffusion, ginger,orange peel, Porphyromonas gingivalis, Prevotella intermedia , peri-implantitis,,

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INTRODUCTION

The main purpose of any dental treatment is to restore a patient's natural form, function, comfort, aesthetics, speech, and health by eliminating a disease process from a tooth or replacing teeth with a prosthesis. However, the more number of teeth a patient is lacking, the more difficult the process gets. Predictable success in the rehabilitation of many tough clinical situation is now a reality as a result of ongoing research, different diagnostic tools, treatment planning, implant designs, sophisticated materials, and methodologies that can be used.[1] There are various treatment options to rehabilitate the missing teeth such as fixed partial dentures, removable partial denture and implants.[1]

Dental implants can be defined as any artificial, biocompatible material which is partially or completely inserted or grafted into the body for therapeutic, diagnostic, prosthetic, or experimental purposes. It has become most popular and common treatment plan for replacing missing tooth or teeth in completely or partially edentulous patients with high success rate (95-98%) and patient acceptability .[2] The higher success rate depends on the case selection, planning, placement, and fabrication of missing teeth and other related structures, maintenance of restoration(s). There are recent advances in the field of implant dentistry in terms of design, implant material, and placement techniques which are giving predictable success in restoring the teeth.[3]

Titanium and its alloys was known to be the most popularly used material for dental implant, orthopedics and osteosynthesis applications.[4] These materials has become more significant and suitable for biomedical application because they inherit excellent biocompatible properties. In the year 1969, Branemark et al noticed that

there was direct contact between titanium and bone. This phenomenon was referred as osseointegration.[4] It was dependent on the choice of implant design, processing, material used and the type of bone which received the implant.[4] For dental applications, Titanium grade IV and V are used because of their biological properties and thus promoting osseointegration and biocompatibility.[4-5]

A subperiosteal form, a blade form, a ramus frame, and an endosseous form are the four primary forms of dental implant designs that are accessible today and that are used in the field of implant dentistry**.** However, endosseous implants are the most frequently used implants in dentistry today.

In comparison to more traditional treatment methods, such as fixed or removable dentures, implant therapy has several benefits. The survival and success rates that have been reported by several implant investigators often surpass the survival and success rates of conventional dental therapy. Both the concept and the practise of dentistry have been irrevocably altered as a result of the success and predictability of osseointegrated dental implants. Patient's expectations from a therapy plays a possible part to their ultimate satisfaction with the treatment results; the ever-increasing awareness among patients has increased the patient's expectation of the treatment.[5]

The introduction of the dental implant caused a paradigm change in the traditionally used therapies; as a result, patients treated with dental implants have improved treatment results and quality of life. Even while dental implants have a higher success rate than traditional methods, there are still a number of things that might cause an implant to fail. In recent years, the use of dental implants as an essential therapeutic modality for the reconstruction of the dental system has seen a

major growth. Concurrent with this increase in the use of dental implants, however, the prevalence of peri-implantitis has also grown.[6]

An inflammation and destruction of soft and hard tissues around dental implants is referred to as mucositis and peri-implantitis. This condition is analogous to gingivitis and periodontitis, which affect the periodontium of natural teeth. As a result, transitions are often seamless and cannot be clinically distinguished in the same way. Inflammation of the soft tissue around an implant that is caused by bacteria and may be reversed; symptoms include reddening, swelling, and bleeding when the gums are probed. This condition is referred to as mucositis.

Peri-implantitis is a disease that affects the hard and soft tissues that surround implants. It is a degenerative condition that cannot be reversed, and its symptoms include bone resorption, impaired osseointegration, increased pocket development, and purulence.[6-7]

There are different types of micro-organisms that affects the periodontium around implant. It is mainly classified into Red complex and orange complex microorganisms .Red complex includes *Porphyromonas gingivalis ,Tannerella forsythia and Treponema denticola*; Orange complex includes *Prevotella intermedia and fusobacterium nucleatum* and others include *Aggregatibacter actinomycetemcomitans ,Eikenella corrodens,Parvimonas micra and Camylobacter rectus* .According to Paulo et al ,there are 5 main Gram negative anerobes that are seen in peri implantitis which are *Porphyromonas gingivalis ,Treponema denticola* and *Tannerella forsythia* which belongs to red complex and *Prevotella intermedia ,Camylobacter rectus* which belongs to orange complex .Gram-positive aerobic

bacteria such as *Staphylococcus aureus* is also a part of the early colonizing bacterias.[8-9]

Porphyromonas gingivalis is one of main periodontal pathogen which has severe virulence factors and causes periodontal destruction. Another most commonly seen organism which is present in peri implantitis is Prevotella intermedia which affects host immune response and periodontal destruction .[10]

Peri-implant contamination will be morequick in patients who are partly dentate and have an active periodontal disease. Patients with an active periodontal disease will show signs of peri-implant sulcus colonisation by these bacteria as early as the first month after the implant's interface to its prosthetic half. In many cases, the bacterial communities that are found in the peri-implant space are very different from those that are found in the sulcus of the teeth that are adjacent.[11]

Due to the similarities, the same treatment protocols that are utilised in the management of gingivitis and periodontitis have been utilised in the management of peri-implant mucositis and peri-implantitis respectively. Both non-surgical and surgical treatments are available for the management of peri-implantitis. Non surgical treatment includes scaling and root planning , air abrasive system, ultra sonic devices and lasers, topical application of therapeutic gels, irrigation solutions have also been suggested as a way to improve the results of nonsurgical debridement and/or rinses, as well as in a variety of formulated combinations. Whereas surgical treatment includes flap surgery, apically repositioning flaps, regenerative techniques etc. In most of the periodontitis cases, that the main therapy consists of non-surgical application of mechanical debridement and the application of topical chlorhexidine to disrupt the biofilm and bacterial load reduction, if required further surgical procedures and

adjuvant treatments are advocated. Application of topical chlorhexidine, are sometimes necessary (CHX) as a adjunct therapy .[12]

In the management of peri-implantitis, chlorhexidine gluconate, also known as CHX, is frequently prescribed as a non-surgical antimicrobial agent. CHX is effective against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, yeasts, and several different viruses. It is typically recommended to be used as a rinsing solution (0.2%) or in the form of a gel topical application (1%). Although, Chlorhexidine has side effects such as discoloration of teeth, tongue and restorative materials, dysgeusia, desquamative gingivitis, burning of mucous membrane and sometimes allergic reactions. Accidental contact with the tympanum can result in ototoxicity, and damage to the conjunctiva can be permanent if it is scratched or punctured. Contact sensitivity to CHX was first reported by Calnan (1962). CHX is known to elicit allergic contact dermatitis, including connubial contact dermatitis, generally after prolonged and repeated application (Krautheim et al. 2004). It can also cause contact urticaria, photosensitivity, fixed drug eruption and occupational asthma. peri-implant contamination will be more quick in patients who are partly dentate and have an active periodontal disease.

In general, despite the fact that sensitivity to CHX is very uncommon, this potential problem should be kept in mind whenever the drug is being administered. Hence, its use should be limited to a maximum of 3 weeks.[12-13]

When treating peri-implant infection, the objective of the therapy is to decrease the amount of bacteria present, alter the bacterial make-up of the biofilm, and enhance the cleanability of the implants that are involved. Herbal compounds are an alternative technique to treat the infection. Unlike the commonly available CHX

gel, which may cause unpleasant side effects and is harmful, herbal compounds are safe to use and do not cause any adverse reactions. Ginger extract and orange peel extract have shown antibacterial properties against Porhyromonas gingivalis and Prevotella intermedia.

Zingiber officinale, more commonly known as ginger, is a member of the Zingiberaceae family. The pungent chemicals found in ginger, namely gingerols, in its Rhizomes offer properties that are advantageous to one's health, including those that are antioxidant, anti-inflammatory, antibacterial, and antifungal, anti-emetic.[14]

Orange peel (Citrus Reticulata), contains substances likes tannins, saponins, flavonoids, terpenoids, cardiac glycosides, alkaloids and phenols they exhibit antimicrobial and antioxidant properties. [15]

So, this study was conducted to compare the synergistic effect of aqueous orange peel extract and alcoholic extract of ginger with chlorhexidine against Prophyromonas gingivalis and Prevotella intermedia.

NEED FOR THE STUDY

Implant therapy has several benefits over traditional treatment choices such as fixed or removable prosthesis for replacing missing tooth or teeth , as it is preferred method of treatment in complex cases where the conventional treatment have limitations . The survival and success rates that have been reported often surpass the success rates of standard conventional dental therapy, provided patient maintains good oral hygiene practices.

Dental implants have a survival rate of 89% or higher after 10–15 years but, the infection incidence for dental implants, that is peri-implantitis, may reach as high as 14% limiting the success of the dental implants. In peri implantitis there a destructive inflammatory process that affects the soft and hard tissues around osseointegrated implants. Patients who smoke or have poor dental health, are more likely to develop peri-implantitis in the first year after implantation. In the treatment of peri-implantitis, biomaterial treatments have been employed to administer antibiotics.

There are both non-surgical and surgical approaches to the treatment of peri-implantitis; however, the non-surgical therapeutic method is the main line of treatment. As a kind of non-surgical antibacterial therapy for the treatment of peri-implantitis, chlorhexidine gluconate, often known as CHX, is frequently recommended. CHX is effective against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, yeasts, and numerous different viruses. It is recommended to be used as a gel (1% concentration) or a rinse solution (0.2%). As a potential adverse effect, chlorhexidine may cause discoloration of the

tongue, teeth, and restorative materials, as well as alterations in taste, burning of the mucous membranes, and occasionally allergic responses.

In recent years, an increased amount of focus has been placed on the utilisation of herbal anti-microbial agents such as neem, tulsi, aloe vera, ginger, turmeric, chamomile, tea tree, eucalyptus, orange peel extract, etc., in order to combat a variety of organisms, including Porphyromonas gingivalis, Provotella intermedia, Staphylococcus aureus, etc.

Anti-inflammatory, anti-rhinoviral, anti-bacterial, anti-protozoal, and anti-fungal properties have been attributed to ginger, along with anti-emetic, anti-rhinoviral, and anti-protozoal properties.

Extracts and oils made from citrus peels have been shown to possess antibacterial and antioxidant capabilities, as well as a high concentration of phenolic components, which may include various flavonoids.

This research was carried out to examine the synergistic impact of alcoholic extract of ginger and aqueous orange peel extract with chlorhexidine against Prophyromonas gingivalis and Prevotella intermedia.

HYPOTHESIS

NULL HYPOTHESIS:

There is no difference between anti-microbial efficacy of herbal gel containing orange peel and ginger extract and standardized chlorhexidine gel on peri-implantitis pathogens.

RESEARCH HYPOTHESIS:

There is difference between anti-microbial efficacy of herbal gel containing orange peel extract and ginger extract and standardized chlorhexidine gel on peri-implantitis pathogens.

AIM AND OBJECTIVES

AIM OF THE STUDY:

"To evaluate anti-microbial efficacy between novel herbal gel containing orange peel extract and ginger extract with chlorhexidine gel against peri-implantitis pathogens."

OBJECTIVES:

PRIMARY OBJECTIVES:

- To evaluate Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC) of aqueous orange peel extract and alcoholic ginger extract.
- To evaluate anti-microbial efficacy of a novel herbal gel containing aqueous orange peel extract and alcoholic ginger extract against Porphyromonas gingivalis and Prevotella intermedia.

SECONDARY OBJECTIVES:

- To evaluate anti-microbial efficacy of 1% chlorhexidine gel against Porphyromonas gingivalis and Prevotella intermedia.
- Comparison and evaluation of anti-microbial efficacy of herbal gel with 1% chlorhexidine gel.
- To evaluate the cytotoxicity of the formulated herbal gel

REVIEW OF LITERATURE

1. Study was conducted by **Sennerby L (1991)** to examine key implant factors that determine the bone-metal interface reactions that occur around a Titanium screw. At the ultrastructural level, interfacial reactions to experimental Titanium implants are studied. Tissue reactions to CP Titanium versus Titanium-baluminum-4Vanadium are examined, and relevant surface characteristics and surface structure for achieving reliable osseointegration, as well as probable bonding processes over the bone-to-Titanium interface, are outlined. This article indicates that elements linked to the implant alone do not dictate the bone-metallic interfacial responses, but that other factors such as surgical technique and loading circumstances are equally significant for establishing a reliable osseointegration.[18]
2. A study was conducted by A**. Mombelli (1993**) to evaluate peri-implantitis microbiology and antimicrobial treatment. Gram-negative anaerobic rods made up 41% of the microbes cultivated from failed implants. Fusobacterium species and Prevotella intermedia were frequently found at high levels among these species. The micro organisms in the successful implants had very low cultivable numbers, and the majority of them were gram-positive cocci.[10]
3. A study was conducted by **Gerald mcdonnell (1999)** discussed antibacterial activity of Chlorhexidine. It is bactericidal and fungicidal, however it does not kill or limit the growth of bacterial spores or mycobacteria. It has a low order of effectiveness against viruses, however it is effective in destroying cysts of Acanthamoeba species at high doses.[19]

4. Study conducted by **Gintaras Juodzbalys (2003)** ,developed an acid-etched implant surface that was comparable to the surface that was produced by sandblasting and acid etching, and compared it to the surfaces of a number of other screw-type implants that are commercially available. It was decided to utilise an electron microscope to examine all of the etched surfaces, and digital pictures were produced for further visual analysis and description. The surface that was created by a combination of hydrochloric and sulfuric acid was the one that was most comparable to the sandblasted and acid-etched surface.[20]
5. Study conducted by **Lindhe J Meyle (2008)** was done to identify the reasons for peri-implant mucositis and peri-implantitis, as well as the treatment for these conditions. A history of periodontitis, dental cement, a lack of proper oral care, and smoking cigarettes are all considered to be risk factors. In order to get rid of the plaque and complete the therapy for peri-implant mucositis, patients will need to undergo mechanical debridement. Beneficial results may be achieved after using mechanical non-surgical therapy for the peri-implant mucositis lesions. The treatment of peri-implantitis Non-surgical mechanical cleaning of the implant surface, with or without an adjuvant antibacterial therapy, is unsuccessful in the treatment of peri-implantitis.[22]
6. **Miri Park(2008)**,studied the antibacterial property of ginger. Ginger, also known as Zingiber officinale Roscoe, has a long history of use both in culinary and medical contexts. Particularly, it has gingerol-related components, and those components have been shown to exhibit antibacterial and antifungal effects, in addition to a variety of medicinal qualities. It has not yet been determined which components of ginger are responsible for ginger's ability to prevent the development of oral bacteria in the human oral cavity that are

related with periodontitis. According to this research, both ethanol and n-hexane extracts of ginger displayed antibacterial activity against a variety of bacterial strains. There are three anaerobic Gram-negative bacteria that are responsible for periodontal illnesses. These bacteria include Porphyromonas gingivalis ATCC 53978, Porphyromonas endodontalis ATCC 35406, and Prevotella intermedia ATCC 25611. In conclusion, two highly alkylated gingerols known as [10]-gingerol and [12]-gingerol were able to successfully suppress the development of these oral pathogens within a minimum inhibitory concentration (MIC) range of 6–30 g/mL. At a minimum bactericidal concentration (MBC) range of 4–20 g/ mL.[33]

7. **Stefan Renvert (2008)** conducted a research search with the goal of evaluating the effectiveness of various non-surgical treatment options for peri-implant mucositis and peri-implantitis. It was noted that mechanical therapy that does not include surgery may be effective in the treatment of peri-implant mucositis lesions. In addition, the use of antibacterial mouth rinses in conjunction with mechanical treatment enhanced the final results of such mucositis lesions. The use of chlorhexidine had very little of an impact on the clinical and microbiological features. On the other hand, it has been established that the use of adjunct local or systemic antibiotics may reduce the amount of bleeding that occurs during the probing process as well as the probing depths.[21]
8. A review was done by **Norowski Jr PA et al (2009),** which stated that dental implants have a survival rate of 89% or higher after 10–15 years, although the infection incidence for dental implants, known as peri-implantitis, may reach as high as 14%. Patients who smoke or have poor dental health, as well as

those with calcium-phosphate-coated or surface-roughened implants, are more likely to develop peri-implantitis in the first year after implantation, when the incidence of the condition is also at its maximum. In order to treat peri-implantitis, biomaterial treatments have been employed that distribute antibiotics via the use of fibres, gels, and beads..[16]

9. A research was done by **Krisztina Ungvari (2010)** in which three different cleaning solutions were assessed. In order to cure commercially pure (grade 4) machined titanium discs, 3% hydrogen peroxide was used for five minutes, saturated citric acid (pH 14.1) was used for one minute, or chlorhexidine gel was employed (5 min). For the purpose of investigating human epithelial cell attachment (observation lasting 24 hours) and proliferation, the dimethylthiazolyl-diphenyltetrazolium bromide (MTT) and bicinchoninic acid (BCA) protein content tests were used (72-h observation). These compounds do not have any effect on the surface of the titanium. In opposed to chlorhexidine gel, washing with hydrogen peroxide only marginally increases the formation of human epithelial cells.[23]
10. A research as done by **Salah Sakka (2012)** to discover the key reasons of early and late implant failure since it is essential in the area of oral implantology to have a comprehensive awareness of this inescapable clinical phenomenon. Lack of primary stability, surgical stress, and infection were the three key culprits in the majority of cases of premature implant failure. Early symptoms of infection may signal a much more dangerous result than the same problems occurring later, which may predict a much less critical prognosis due to the disruption of the basic bone healing process. It would

seem that occlusal overload and peri-implantitis are the two variables that contribute to late failure the most often.[24]

11. **Rafael Gómez-de Diego(2014)**conducted a literature evaluation of the most recent scientific research in order to conduct an analysis of the clinical indications and exclusion criteria for dental implants in patients with medical conditions. The following criteria were used for inclusion in the study, a minimum of ten patients were treated, consensus papers, reviewed studies, and meta-analysis were done in humans treated with dental implants, and the illness diagnosis was included. There were a total of 64 items located, however only 16 of them satisfied the requirements to be included. It does not seem that systemic cardiac illnesses, diabetic endocrine pathologies, or metabolic disorders that are under control provide a complete or partial barrier to the placing dental implants. Both chronic tobacco use and radiation to the head and neck have been linked to an increased risk of dental implant failure. Patients who are having treatment with biphosphonates for osteoporosis are at an increased risk of experiencing bone necrosis following oral surgery. This is particularly true if the medicines are given intravenously or if they are combined with specific concurrent medications.[28]
12. Study conducted by **Hyo-Sook Ryu (2015)** was done to determine, on contact with CHX, the ability of modified titanium surfaces to release chlorhexidine That after four titanium surfaces were prepared, each sample was treated for two hours with either whole saliva or phosphate-buffered saline, depending on the kind of saliva used (PBS). In order to study the antibacterial activity of CHX-adsorbed discs, a disc diffusion test was carried out using Streptococcus gordonii as the test organism. This study reveals that

alterations to the surface of titanium have a significant influence on the release of chlorohydroxamic acid (CHX), and that SLA and RBM may offer effective CHX absorption capacity in the saliva-filled oral cavity.[25]

13. **Paula Juliana Pérez-Chaparro (2016)** within the scope of this systematic review, it analysed the existing weight of evidence of the microbiological profile that is linked with Peri-implantitis. There were a total of 799 titles discovered, and 11 papers were selected for inclusion in this review. In order to get all of the information, a preset form was used. The study results of this systematic review indicate that there is "Moderate Evidence" to support the association of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia with the aetiology of peri-implantitis, while there is "Some Evidence" to support the correlation of Prevotella intermedia and Campylobacter rectus with the aetiology of peri-implantitis.[8]
14. **Nisha Mahato (2016)** conducted a systematic review in which evaluation of the efficacy of both surgical and non-surgical treatments for peri-implantitis was done. PubMed was the source of the data that was utilised. This database only allowed searches of articles written in the English language during the months of January 2010 and June 2015. Each and every Randomized Controlled Trial that was conducted on human subjects to describe the therapies for peri-implantitis with a follow-up that lasted at least six months were eligible for inclusion. Both the eligibility and the quality of the data were evaluated, and then two different reviewers collected the data. The process of extracting data consisted of identifying the intervention's kind, intensity, provider, and location. Twenty different published works were taken into consideration (10 involving surgical and 10 involving non-surgical mechanical

procedure). Mechanical surface debridement using carbon or titanium currettes, laser light, and antibiotics are involved in the non-surgical approach, while the surgical approach involves implantoplasty, elevation of mucoperiosteal flap, and withdrawal of peri-inflammatory granulation tissue, followed by surface decontamination and bone grafting. According to the findings of this research, non-surgical treatment has a greater propensity to eliminate just the local irritant from the peri-implantitis surface, with or without the use of certain adjuvant therapies, drugs, or devices. Therefore, non-surgical treatment is ineffective in the treatment of osseous defects. The remaining sub-gingival deposits may be removed by surgical treatment, which also helps to reduce the size of the peri-implantitis pocket. This can be done in conjunction with an osseous resective or regenerative approach. Surgical therapy in conjunction with osseous resective or regenerative method revealed the favourable outcomes for the treatment of peri-implantitis, despite the fact that there is no particular advice for the treatment of peri-implantitis.[29]

15. The study was conducted by **Mankar et al (2017),** to evaluate in vitro anti-microbial activity of orange peel extract, 0.2%chlorhexidine (CHX) and saline against dental biofilm-forming bacteria and their effect on plaque structure under SEM. Organisms such as Porphyromonas gingivalis, Provotella intermedia, Aggregatibacter actinomycetemcomitans were selected. The study concluded that CHX and orange peel extract exhibited good anti-microbial activity against biofilm organism, at 75%,50%,25%,10% and 5% concentrations. The plaque samples that were treated with orange peel extract had a somewhat shrunken and coarse appearance, which indicated the extract's potential to break the biofilm that forms on plaque.[15]

16. The study was conducted by **Aziz et al (2017)**, compared the effects of water-based and alcohol-based ginger extracts on the periodontal pathogen Porphyromonas gingivalis. The minimal inhibitory concentration (MIC) of alcoholic ginger extract that inhibits Porphyromonas gingivalis growth was determined to be 30% (0.3g/ml) concentration by the research. The MIC of aqueous ginger extract was determined to be 50% (0.5g/ml) concentration. The minimum bactericidal concentration (MBC) of alcoholic ginger extract was 60% (0.6 g/ml), whereas the MBC of aqueous ginger extract was 80% (0.8 g/ml). The alcoholic ginger extract was more effective in killing Porphyromonas gingivalis than the aqueous ginger extract. However, the alcoholic extract was more active than the aqueous extract, and Porphyromonas gingivalis was more sensitive to both extracts than Aggregatibacter actinomycetemcomitans was. Bacteriostatic and bactericidal activity against Porphyromonas gingivalis was shown by both extracts..[17]
17. A systematic review was done by **Lafaurie GI et al (2017)** ,to assess the microbiological profiles of peri-implantitis ,periodontitis and healthy implants based on studies that evaluated microbial biofilms and entire microbiomes to establish their similarities and differences . Porphyromonas gingivalis and especially Provotella intermedia/nigrescens were often found at peri-implantitis region. Peri-implantitis sites were also colonized by uncultivable asaccharolytic anaerobic gram -positive rods and gram-negative rods, which were not easily seen in teeth with periodontitis or healthy implants.[9]
18. **Ferdinan Pasaribu(2018)** conducted a study to determine and investigate the efficacy of citrus peel extract in inhibiting the development of several periodontal pathogenic bacteria in vitro.Percolation with ethanol, n-hexane,

and ethyl acetate was used to extract citrus peel for further processing citrus peel with a solvent in order to obtain a thick extract. After diluting the extract, the final concentrations were as follows: 25%, 12%, 6.25%, and 3.125%. Each concentration was put through a series of tests to determine whether or not it has antibacterial activity against A. actinomycetemcomintans, P. gingivalis, and F. nucleatum. It was shown that the extract was efficient by observing the inhibitory zone on the BHI broth medium.Citrus peel extract of ethanol at a concentration of 25% has the strongest antibacterial efficacy against the three bacteria when compared to solvents. The diameter of the inhibition zone against A. actinomycetemcomintans is 16.05 mm, while the diameter of the inhibition zone against P. gingivalis and F. nucleatum is 12.75 mm and 11.67 mm, respectively. There are statistically significant differences between the citrus peel extract with concentrations of 25%, 12%, 6,25%, and 3,125% (p 0.05).Citrus peel extract has an antimicrobial effects, as shown by its ability to prevent the development of bacteria such as A. actinomycetemcomintans, P. gingivalis, and F. nucleatum.[34]

19. A study was done to evaluate antibacterial activity on chlorhexidine on implant dentistry. Chlorhexidine is of value in both the prevention and management of peri-implantitis. Thus, the use of a chemical plaque-inhibitory mouthwash (0.2%) and gel (1%) which has various clinical applications in dentistry especially in dental implantology.[13]
20. In this particular investigation conducted by **Priscilla R. Varges (2019),** a Continuous shear rheometry was used in order to investigate the impact that a variety of physical and chemical factors had on the homogeneity of the Carbopol940 and 941 gels. The impact of factors such as polymer

concentration, temperature, neutralisation, and the use of a variety of solvents and neutralizers have on the basic characteristics of Carbopol gels was investigated via the use of small strain testing. In the first approximation, gels might be described as linear viscoelastic and elastic solids. There was no correlation between the kind of solvent or neutralizer that was utilised and the creep compliance, which rose linearly with concentration.[26]

21. **Rosa-Maria Diaz-Sanchez(2020)** Examine a variety of surface treatments for implants with TiIV and TiV for in vitro and in vivo research, respectively. An in vitro investigation was carried out, which consisted of four study groups: treated and untreated TiIV titanium discs (TiIVT and TiIVNT), as well as treated and untreated TiV titanium discs (TiIVT and TiIVNT)titanium discs (TiVT and TiVNT). To achieve the desired change in surface roughness, the surface was treated with a grit blasting treatment followed by alumina and double acid passivation. The chemical makeup of the samples' surfaces as well as their surface microstructures were investigated. Cell cultures were used on the titanium discs in order to assess the adherence and proliferation of osteoblasts on the surfaces of the discs. In order to conduct the in vivo investigation, 18 implants were surgically inserted into the tibias of three New Zealand rabbits. These rabbits were split up into three different experimental groups (TiIVT, TiIVNT, and TiVT). It was determined by the use of micro-computed tomography (micro-CT) what the bone density was close to the implants. The findings indicated that there was only a moderate amount of cell adhesion and cell growth in the TiIVT and TiVT cell cultures after 6 and 24 hours. After a period of 48 hours, there were no discernible alterations identified. And in the in vivo micro-CT and histological studies, there were no

statistically significant changes detected between the groups; nevertheless, there was a favourable tendency in bone production in the groups that had a treated surface. After forty-eight hours, the in vitro cell growth cultures produced results that were comparable across all of the groups. In both the in vivo micro-CT and the histology analysis, we did not uncover any changes that were statistically significant.[30]

22. **Ruth Lourenço(2020)** discussed about the importance of phytotherapeutic agents on periodontium and it states that, the tissues that support and surround the teeth are affected when one has periodontal disease. It is brought on by the microbial biofilms that form on the surfaces of the teeth. When combined with other forms of treatment, such as mechanical therapy, antimicrobial drugs have shown to be beneficial. Nevertheless, the usage of these drugs has also been linked to unwanted consequences as well as an increase in the expense of therapy. Due to the many advantages that phytotherapeutic agents provide, it is possible that they will prove to be an effective replacement for these when they are employed as an adjunct to scaling and root planing. In the treatment of periodontal disease, phototherapeutic agents such as curcumin, triphala, neem, Aloe vera, green tea, tulsi, pomegranate, garlic, ginger, cloves, and cinnamon may exert a beneficial effect on the periodontium.[14]
23. **Maulana Yusuf Alkandahri (2020)**, investigated the antibacterial properties of an ethanol extract of Zingiber officinale as well as to analyse its phytochemical composition. Ginger, also known as Zingiber officinale, is harvested fresh from the Manoko Plantation in Bandung, which is located in West Java, Indonesia.The well diffusion technique was used to evaluate antibacterial activity, and phytochemical screening was carried out to evaluate

the potential of the analyse the rhizome of the ginger plant to discover its phytochemical make-up.The findings of the phytochemical analysis indicated the presence of phenolic compounds, as well as alkaloids, flavonoids, tannins, and terpenoids. The results of the bioassay showed a range of possible outcomes for the biological activity of the extract. Antibacterial activity was shown by the extract against all of the bacteria that were examined, with inhibitory diameter zones ranging in size from 2.67 to 12.47 mm mm. The greatest inhibition zone was shown in Staphylococcus aureus at a concentration of 400 mg/ml, whereas the lowest inhibition zone was seen in Pseudomonas aeruginosa at a concentration of 100 mg/ml.The results of our study indicate that an extract of the rhizome of Z. officinale has the capacity to prevent the development of bacteria. This demonstrates that Z. officinale has the potential to be used in the research and development of novel antibacterial drugs.[32]

24. **Woo-Ri Jung (2021)** examined the prevalence of nine representative periodontal pathogens in the saliva samples of periodontally healthy subjects (PH) and patients with periodontitis who underwent periodontal surgery. These periodontal pathogens included Aggregatibacteractinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerellaforsythia (Tf), Treponema denticola (Td), Prevotella intermedia (SPT). DNA copy numbers of Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerellaforsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Fu The PH and SPT groups had significantly higher detection rates of all other infections, with the exception of Aa. When compared to the PH group, the SPT group had statistically significant lower levels of total

bacteria and Fn abundance while simultaneously displaying greater levels of Pg abundance (P 0.05). The examination of the age-specific pathogen distribution showed that the PH group had a considerably high abundance of Tf and Cr but a significantly lower abundance of Aa. The clinical parameters and microbiological profiles of the SPT group and the PH group were quite comparable to one another. Patients who have periodontitis, on the other hand, need supportive treatment in order to stop the disease from returning. Because the number of some bacteria changed with age, it will be necessary for future research to determine the nature of the association between age-related physiological changes and the periodontal bacterial composition.[27]

25. **Seo-young Kim(2022)** investigated whether or not the addition of ginger to dental titanium screws may improve their biocompatibility and biofunctionality. Ginger was loaded in varying quantities on the titanium surface in order to enhance the quantity of coating of the n-hexane-fractionated ginger on the titanium surface and to manage its release in a photo-crosslinkable GelMA hydrogel. After the wettability of the surface was altered by the process of pre-calcification (TNC), ginger hydrogel (GH) was applied to the surface so that the coating stability of the ginger hydrogel could be improved. As a consequence of this, the ginger fraction, which included a significant amount of phenolic compounds, was successful in the process of inhibiting the development of S. mutans and P. gingivalis. The gingerol (GH) demonstrated outstanding antibacterial properties proportional to concentration and released ginger's primary components in a gradual and steady manner.Although there was a slight decrease in bone regeneration with the ginger-loading concentration due to the increased contents of polyphenolic

compounds, it was significantly supplemented through the promotion of osteosis formation by the hydrogel and TNC coating. This was the case despite the fact that there was a slight reduction. It concluded that a GH-TNC coating on a titanium implant is both biosafe and superior in terms of its biofunctionalities. Also suggested to use the proper dosage, since using an excessive concentration of ginger may have a negative impact on the increased biocompatibility in clinical applications.[31]

26. According to **Dr Aritra et al,** Mouthwash containing 4% ethanolic extract of Citrus sinseis (Orange peel), which was manufactured locally, was compared to mouthwash containing 0.2% chlorhexidine, which is available for purchase in the marketplace. The results of this evaluation and comparison were analysed and reported. It was discovered that a mouthwash containing 4% citrus sinesis was just as effective as 0.2% chlorhexidine in lowering the Plaque Index, and it was much more successful in lowering the gingival inflammation and gingival bleeding index.Citrus sinesis 4% mouthwash is an alternative to 0.2% chlorhexidine mouthwash that may be used for a shorter period of time without the risk of experiencing any adverse effects. This mouthwash was effective in lowering plaque levels and calming gingival inflammation.[37]

METHODOLOGY

SOURCE OF DATA/ LABORATORY DETAILS:

This study will be conducted in-

- This study will be conducted in- KAHER's KLE VKIDS Department of Prosthodontics and Crown & Bridge
- Department of Microbiology, JNMC, Belagavi. (To check anti-bacterial property)
- Department of Pharmaceutics, KLE College of Pharmacy, Belagavi – (For formulation of gel)
- KLE Ayurvedic college, Belagavi. (Preparation of extracts)
- Dr. Prabhakar Kore Basic Science Research Center, Belagavi. (To check cytotoxicity)

STUDY DESIGN:

- An in-vitro comparative study.

INCLUSION CRITERIA:

- Grade V titanium discs with identical size and shape with diameter of 10mm and width 2mm.(certified)
- The titanium disc surface should be free of voids.
- Titanium disc with surface roughness value of –5µm

EXCLUSION CRITERIA:

- Titanium discs with gross surface defects and deformities.
- Specimens with inaccurate size, shape and dimensions.
- Titanium discs other than Grade

TABLE 1: MATERIALS UTILIZED IN THE STUDY:

Materials	Description	Manufacturer
Orange peel extract	Powder form	Herbo Nutra, Delhi
Ginger extract	Powder form	KLE Ayurvedic college belgaum
Carbopol gel base	Carbopol 940	OEM manufacturers
1% chlorhexidine gel	Hexi gel 1%	ICPA health products
Mice fibroblasts		NCCS, Pune
Brain Heart Infusion Agar	Culture media	Hi media
DMEM Media		Hi media, Mumbai
Titanium alloy	Type V (Ti-6Al-4V)	Special metals, Mumbai
Ethanol	LOT No:-20151011	Changshu Hongsheng Fine Chemicals Co.Ltd
MTT reagent	LOT No-0000173715	Hi media, Mumbai
Tryphan blue	LOT No- 2024334	Hi media, Mumbai
Distilled water	Batch no -007M15	Rankem Chemicals,Avantor, India

TABLE 2: ARMAMENTARIUM USED IN THE STUDY:

Material	Description	Manufracturer
CO_2 Incubator	-	Eppendoff
Anaerobic jar	LOT no- 14-1016	Hi-media ,Mumbai
Micro pipettes.	Model No:299932	Riveria Glass Pvt, Ltd, Mumbai
Petri dishes	-	Hi-Media, Mumbai
Electric Loop Sterilization	Model: i-therm Al-401	Hi -media, Mumbai
Microscope	TCM400	LABOMED

METHODOLOGY:

DETAILS OF THE PROCEDURES CONDUCTED DURING THE RESEARCH

METHOD OF EXTRACTION:

A) Preparation of aqueous orange peel extract.

Powder form of peel of citrus reticulate (Herbo Nutra) was used as a source of the extract. As a solvent, distilled water that was kept at room temperature was used.The combination of peel powder and solvent was placed in a water bath and heated to an extraction temperature of 40 degrees Celsius for a period of four hours.The liquid extract was sterilised in an autoclave after being filtered using Whatman No.1 filter paper to remove any solid particles that could have been present. The aqueous extract was collected in a vail and stored in a refrigerator.

B) Preparation of alcoholic ginger extract.

The alcoholic ginger extract is prepared by mixing 40mg of ginger powder to 500ml 99.9 ethanol alcohol was added and mixed well. The container was then sealed airtight with cotton and foil to prevent any alcohol from escaping, and it was allowed to sit out at room temperature for twenty-four hours. The contents will be filtered after being concentrated, and this will be accomplished by evaporating the solvent (alcohol) in a hot air furnace at a temperature of 40 degrees Celsius for 24 hours (Nweze and Okafor 2010).

After this procedure, alcoholic ginger extract was collected and was stored in a refrigerator.

To check the antibacterial sensitivity test for aqueous orange peel and alcoholic ginger extract, MIC (minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) was done.

C) Antibacterial sensitivity test MIC and MBC

MIC Test

A standard procedure for testing the MIC was followed. Micro broth dilution method was used to determine the MIC. 9 dilutions of each extract was done with Thioglycolate broth for MIC. In the initial tube, 20microliter of extract was added into the 380microliter of Thioglycolate broth. For dilutions 200microliter of Thioglycolate broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200 microliter of Thioglycolate broth. This was considered as 10^{-1} dilution. So from 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of Thioglycolate broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity.

MBC Test

MBC test was done from the MIC dilutions tubes, first 3 or 5 tubes were plated (which was sensitive in MIC) and then incubated for 24 hrs then next day the colony count was taken.

This test was done to see whether there was bactericostatic or bactericidal effect of the extract (Drug) against the organism. If there is no growth then it shows its bactericidal effect and if there is growth then it has bacteriostatic effect.

After following the above procedure, Minimum inhibitory and bactericidal concentration of extracts against P.gingivalis and P.intermedia was established.

The reported results revealed that, MIC of aqueous orange peel extract on P.gingivalis was 25µg/ml and of P.intermedia was 50µg/ml whereas, MIC of alcoholic ginger extract on P.gingivalis was 0.2µg/ml and of P.intermedia was 0.4µg/ml. Similarly, MBC of aqueous orange peel extract on P.gingivalis was 25µg/ml and of P.intermedia was 50µg/ml and MBC of alcoholic ginger extract on P.gingivalis was 0.2µg/ml and of P.intermedia was 0.8µg/ml.

Combination MIC, MBC obtained was 0.4 % .Herbal gel was formulated using this concentration.

FORMULATION OF HERBAL GEL

TABLE 3: MATERIALS USED IN THE FORMULATION OF GEL

Carbopol 430	3%
Triethanolamine	0.05%
Glycerine	3%
Methyl paraben	0.01%
Propyl parabens	0.05%
Water	100% W/V

PROCEDURE:

After getting the desired concentration herbal gel formulated.

While preparing herbal gel,carbopol 430 was soaked in 50ml of distilled water and was kept for 24 hours for complete soaking. In 25 ml of water, glycerine, methyl paraben, propyl paraben was added and stirred for 30 minutes with aid of magnetic stirrer. Then polymer solution and preservative solution was mixed together for 10 minutes in magnetic stirrer. Orange peel extract and ginger extract was then added to the above mixture solution. So the pH of the above solution was adjusted to 7 to 7.5 with triethanolamine and the final weight for the gel was adjusted with water and kept in air tight container in cool place.

TO CHECK THE ZONE OF INHIBITION OF HERBAL GEL AND CHLORHEXIDINE GEL.

DISC DIFFUSION TEST:

Disc diffusion test was carried out to check the zone of inhibition. Brain heart infusion agar (BHI) was used. Inoculum preparation was done using a loop or swab, transfer the colonies to the plates. Then it was adjusted visually to see the turbidity with broth to equal that of a 0.5 McFarland turbidity standard that was been vortexed. Alternatively, standardize the suspension with a photometric device.

Inoculation of Agar plate:-

1. Within 15 min, the inoculum was adjusted to a McFarland 0.5 turbidity standard, sterile cotton swab was dip into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.

2. Entire surface of agar plate was swabbed three times, rotating plates approximately 60º between streaking to ensure even distribution.
3. Inoculated plate was allowed to stand for atleast 3 minutes but no longer than 15 min before making wells.

Stock solution preparation:-

Stock solution was prepared weighing 10mg of compound and dissolve it in 1ml of DMSO

Addition of compound into plate:-

1. Take hollow tube of 10mm diameter, heat it. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate.
2. Each discs were coated with herbal gel and chlorhexidine gel and placed in each well.

Incubation:-

1. Incubate plates within 15 min of compound application.
2. Invert plates, and stack them no more than five high.
3. Incubate for 18-24 hrs at 37 ºC in incubator.

Reading plates:-

Read plates only if the lawn of growth is confluent or nearly confluent. Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

TO CHECK CYTOTOXICITY OF THE GEL.

MTT solution (stock solution) of 5 mg in 1 ml of PBS was prepared.

Cell culture:

The cell line used for the study was L929, Mouse Fibroblasts (procured from NCCS, Pune). The cell line was maintained in 96 wells micro titer plate containing DMEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC for 48-72 hours.

Cytotoxicity assay was carried out

Cytotoxicity Assay :

Invitro growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells.Supernatant was removed from the plate and fresh DMEM solution was added and treated with10mg/ml gel.only DMEM was used as negative control group. In our study 10ul of gel was added ,to respective wells containing 200µl of the medium. After 24hrs incubation at 37ºC in a humidified atmosphere of 5% Co2, stock solution of MTT was added to each well (20µl, 5mg per ml in sterile PBS) for further 4 hr incubation. Then the supernatant was carefully aspirated. The precipitated crystals of "Formazan blue' were solubilised by adding DMSO (100µl) and optical density was measured at wavelength of 570nm by using LISA plus. It was calculated using the following formula

Formula

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100$$

METHODOLOGY WITH FLOW CHART

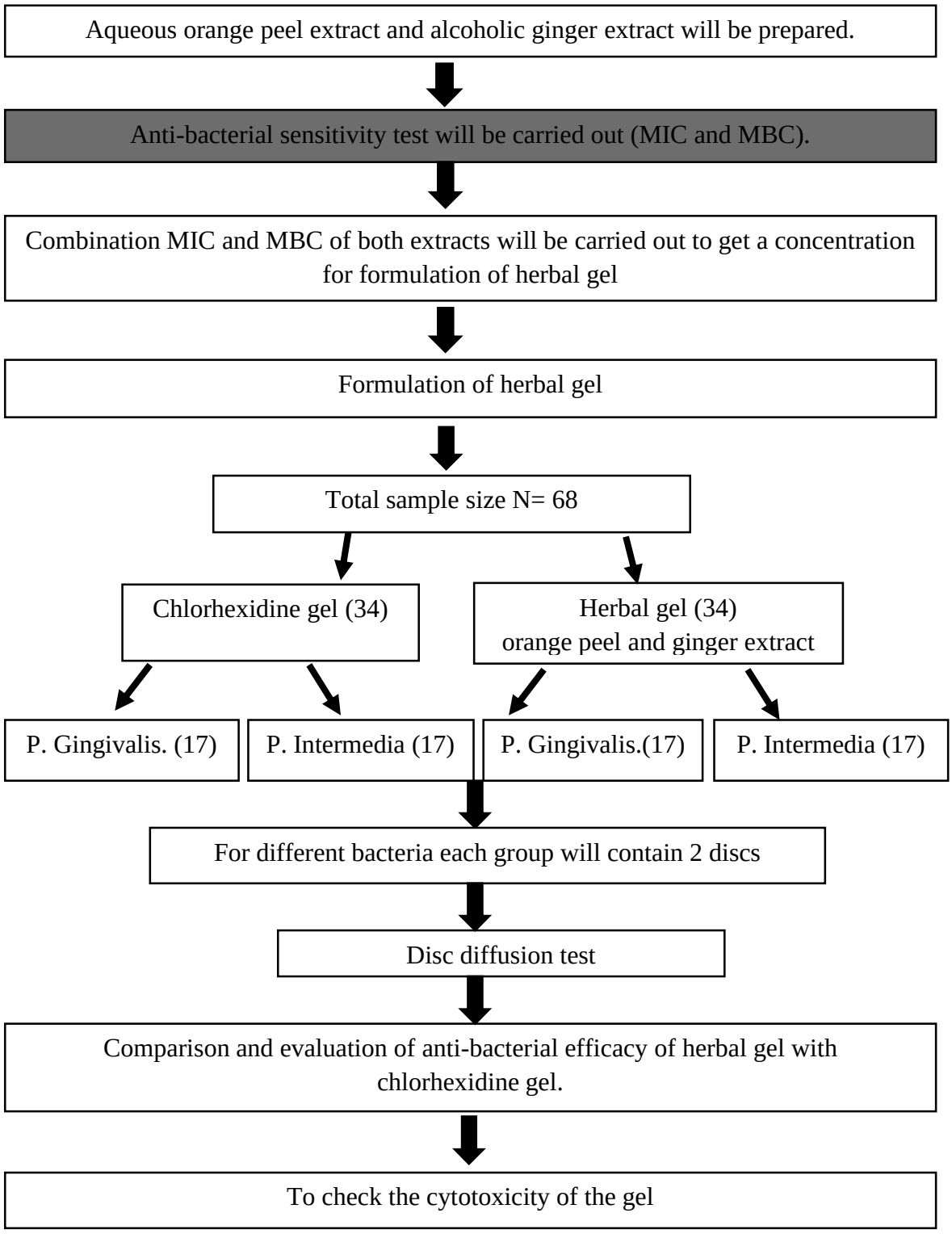

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Figure 1: Prepared Alcoholic ginger extract.

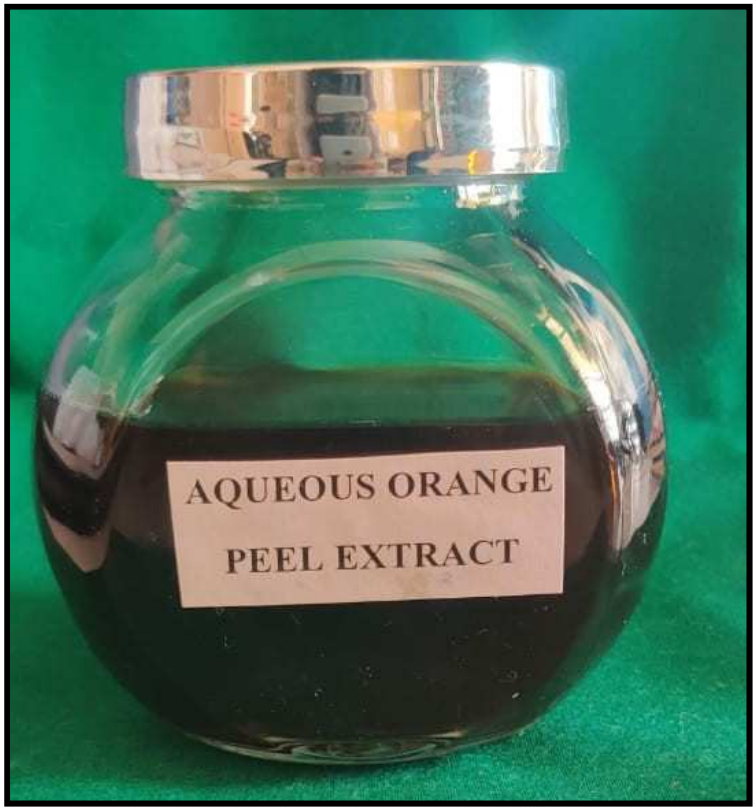


Figure 2: Prepared Aqueous orange peel extract.

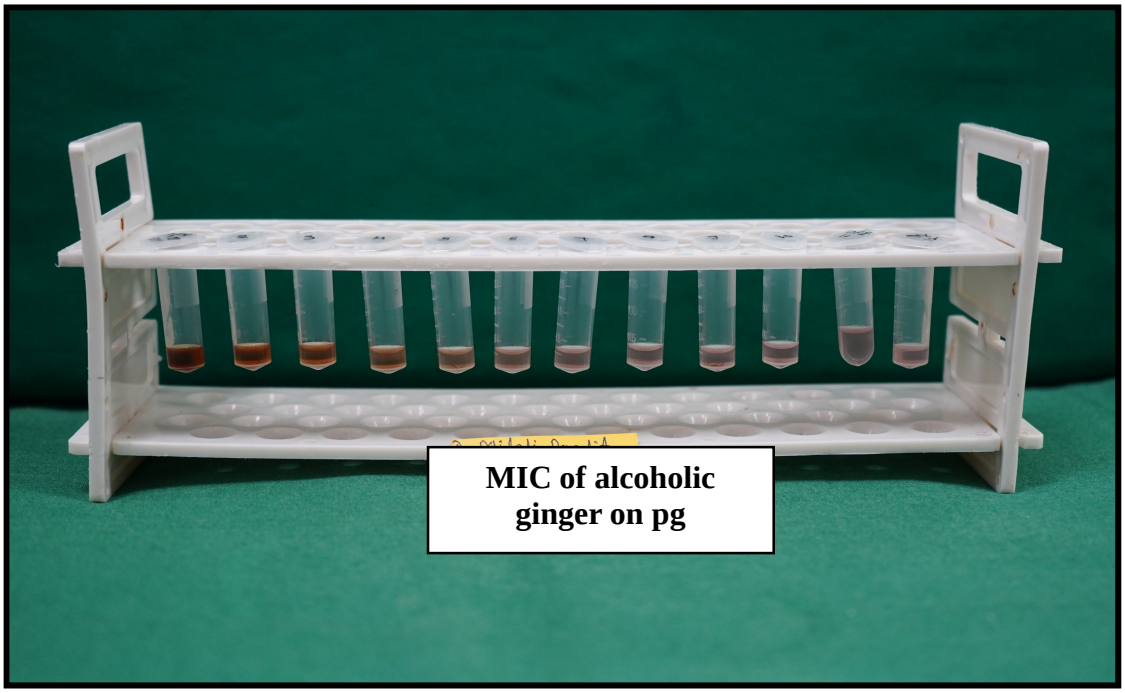


Figure 3: MIC of alcoholic ginger extract on P.gingivalis .

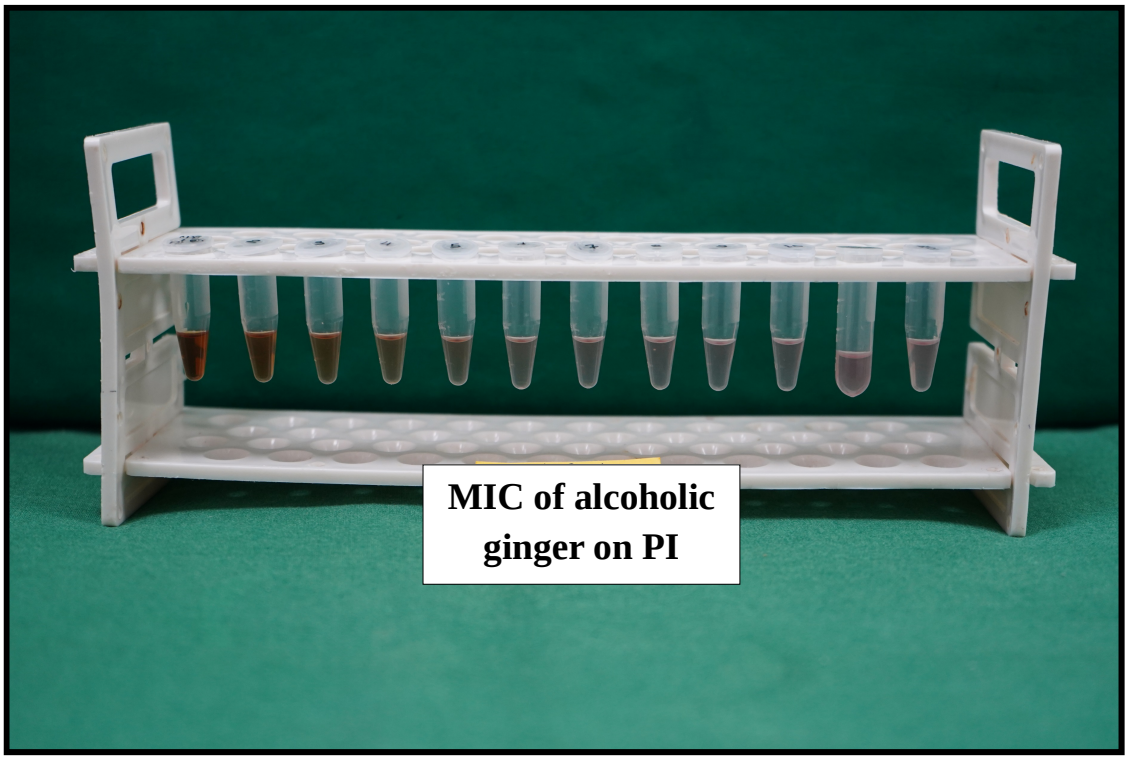


Figure 4: MIC of alcoholic ginger on P.intermedia.

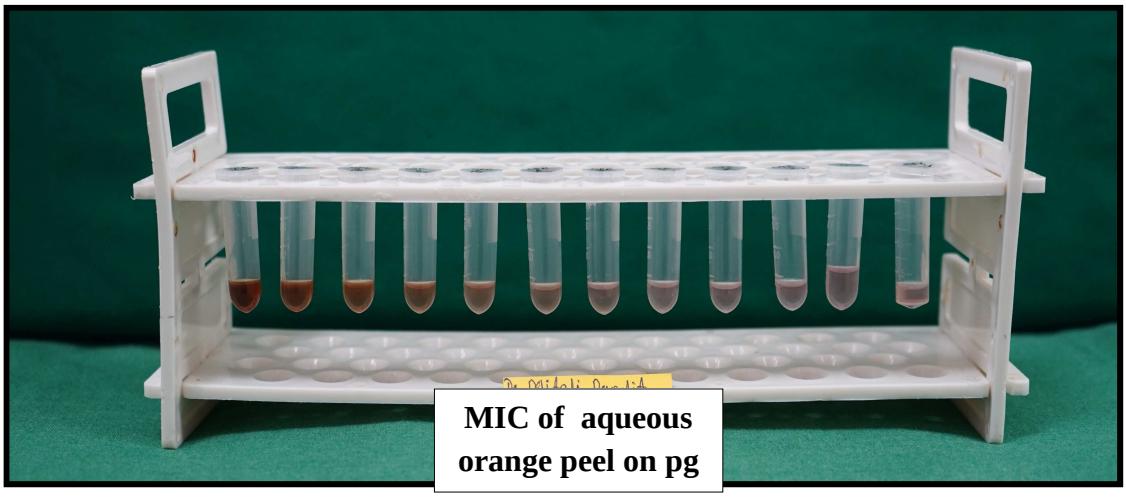


Figure 5: MIC of aqueous orange peel extract on P.gingivalis

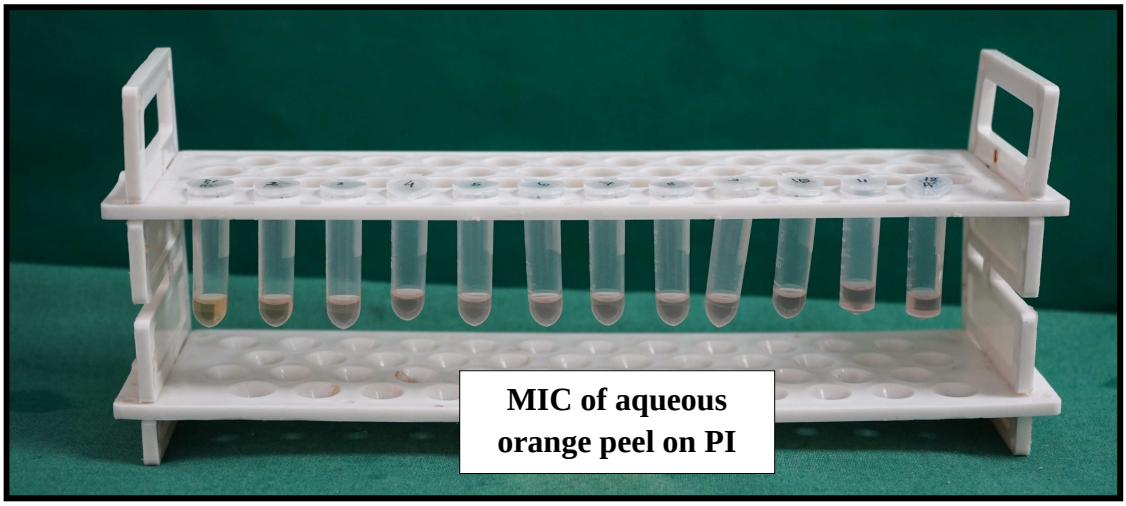


Figure 6: MIC of aqueous orange peel extract on P.intermedia

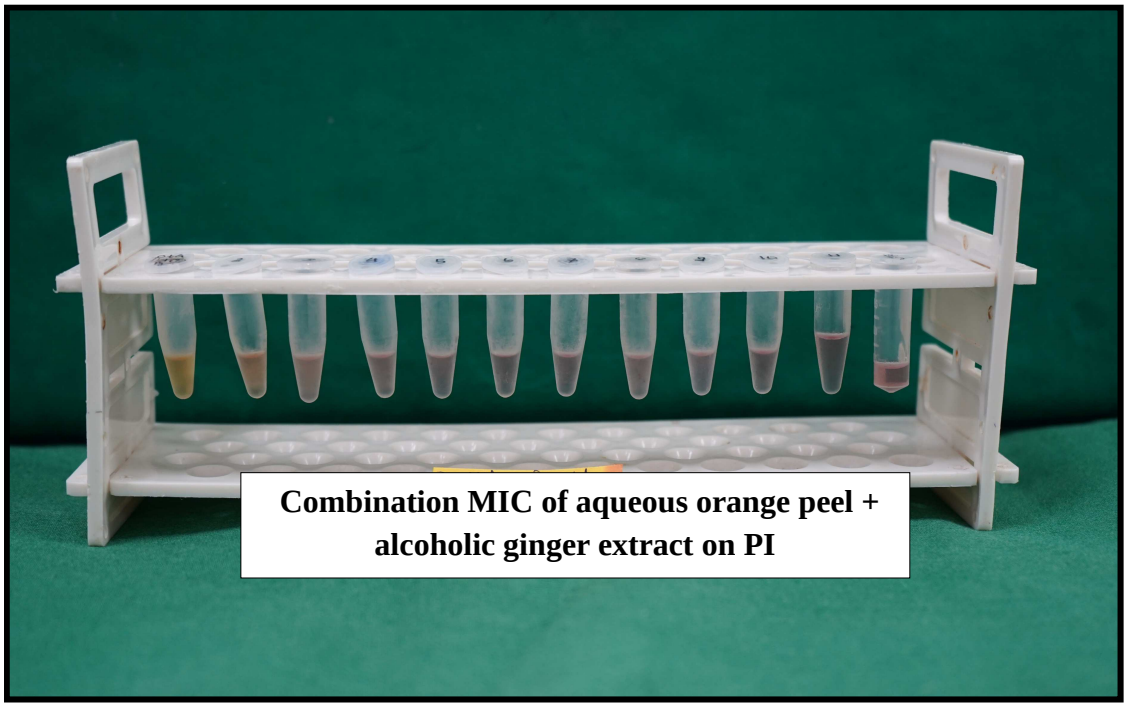


Figure 7: Combination MIC of alcoholic ginger extract and aqueous orange peel extract on P.intermedia.

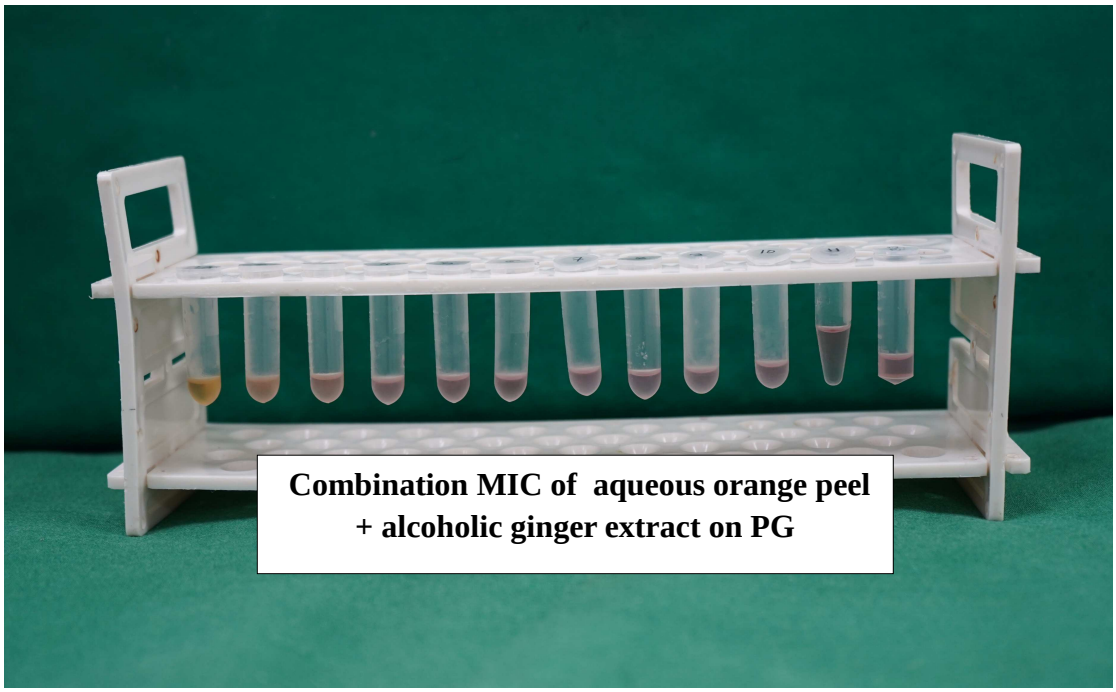


Figure 8: Combination MIC of alcoholic ginger extract and aqueous orange peel extract on P.gingivalis.

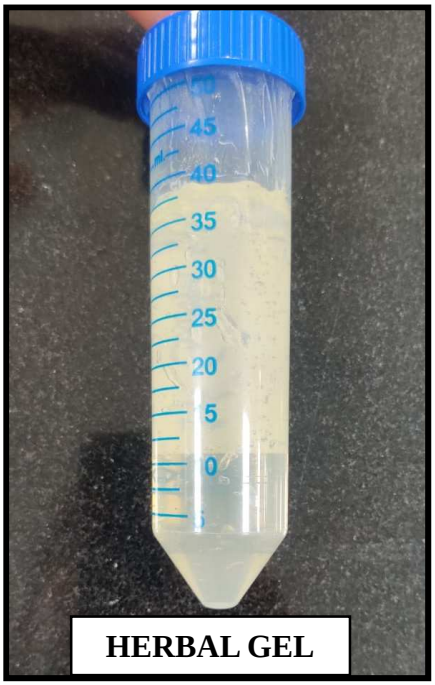


Figure 9: (A) Herbal gel (B) Chlorhexidine gel

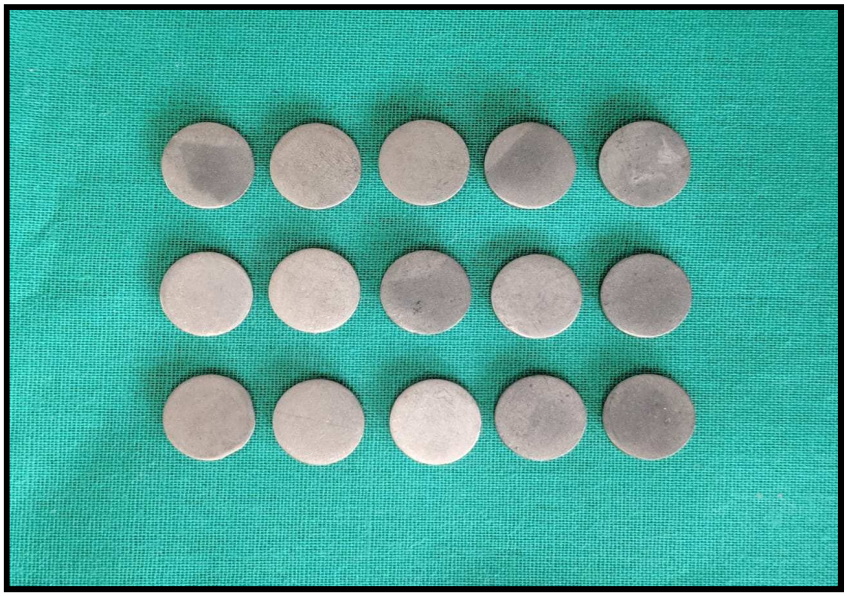


Figure 10: Titanium discs

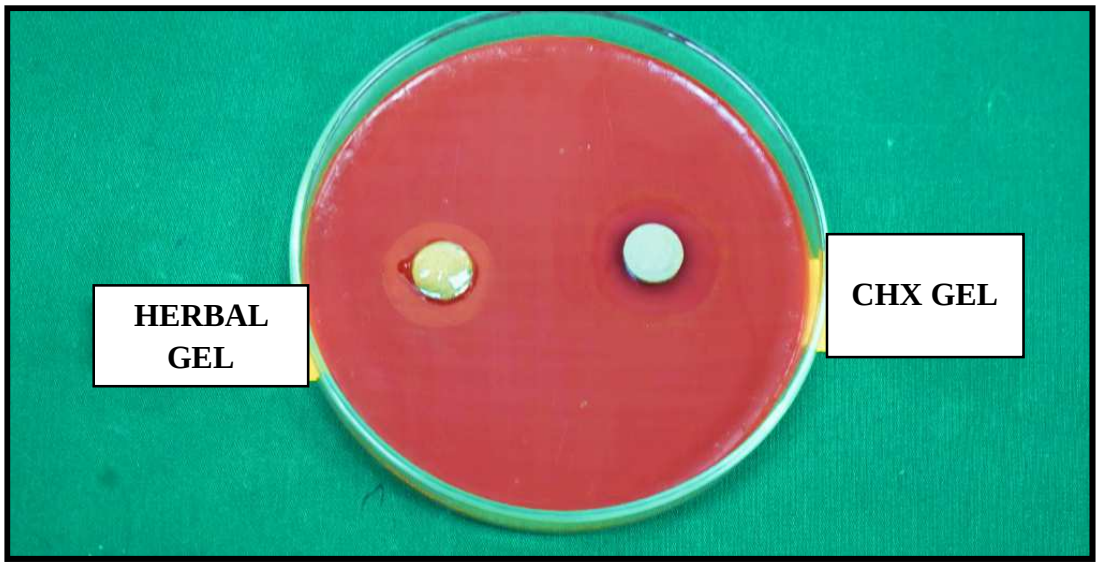


Figure 11: (A) Zone of inhibition on P.gingivalis and chlorhexidine on day 4 by disc diffusion method

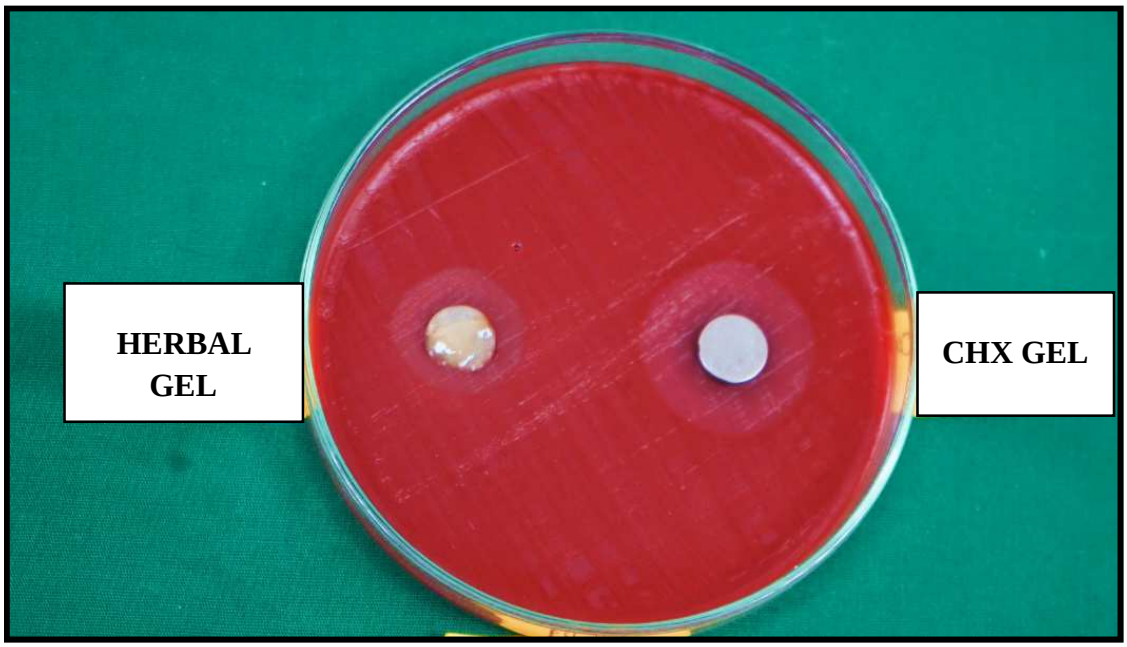


Figure 11: (B) Zone of inhibition on P.gingivalis and chlorhexidine on day 7 by disc diffusion method

Figure 11: (C) Zone of inhibition on P.gingivalis and chlorhexidine on day 10 by disc diffusion method

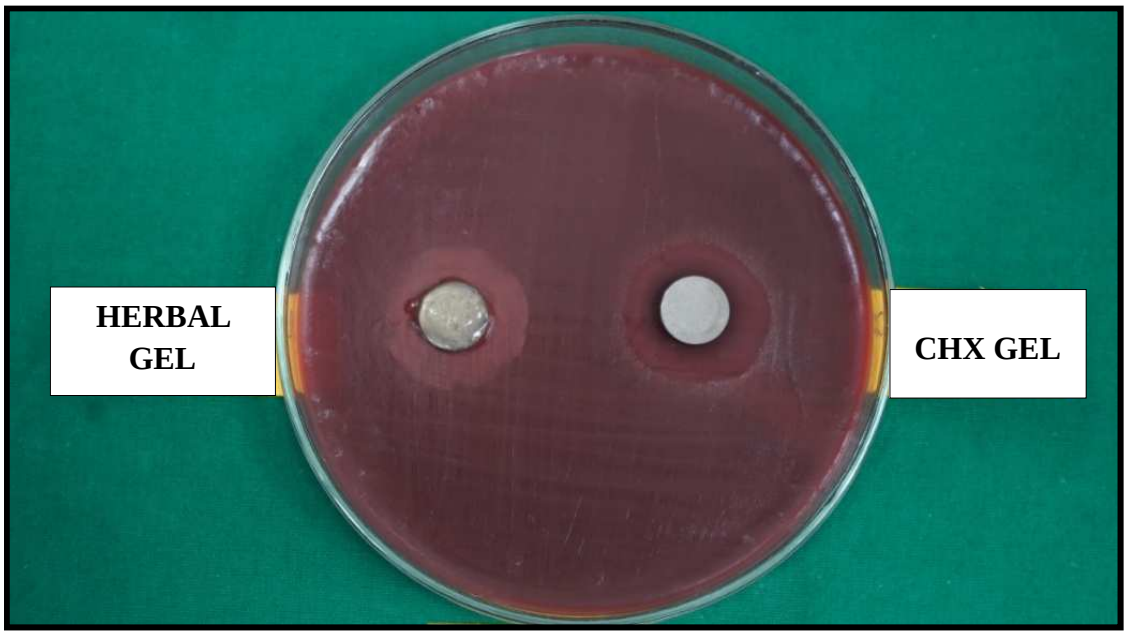


Figure 12: (A) Zone of inhibition on P.intermedia and chlorhexidine on day 4 by disc diffusion method

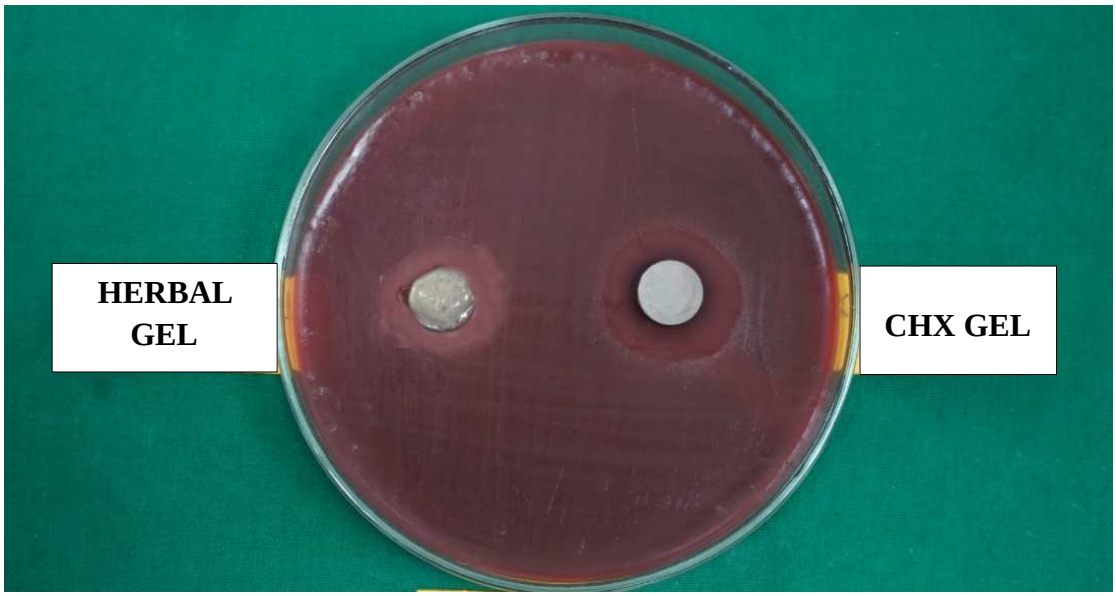


Figure 12: (B) Zone of inhibition on P.intermedia and chlorhexidine on day 7 by disc diffusion method

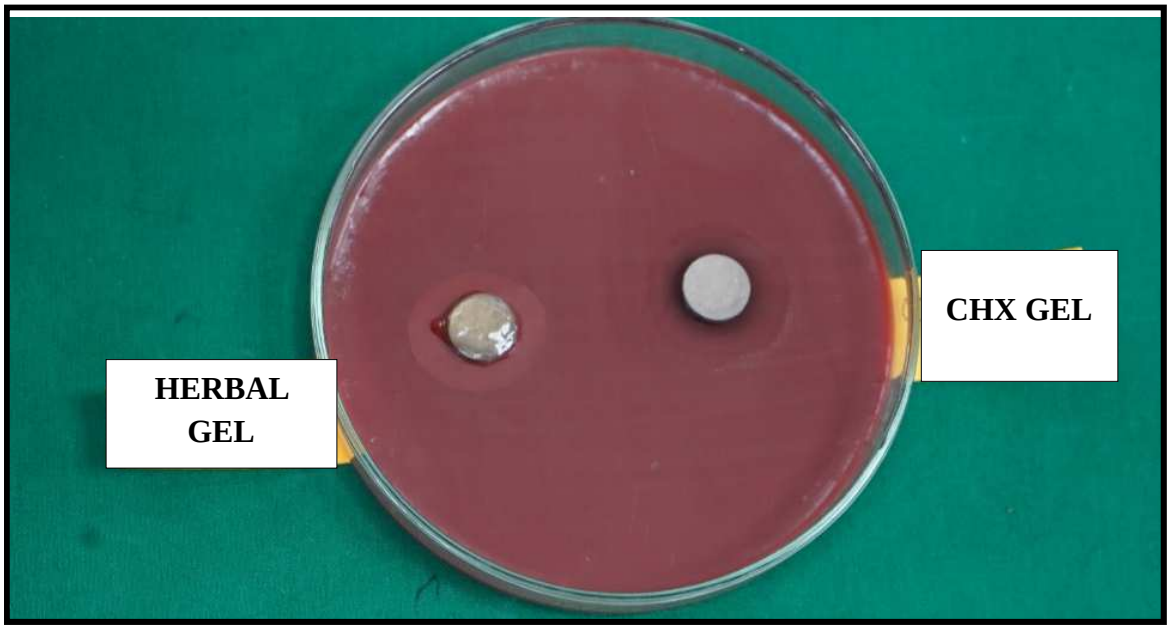


Figure 12: (C) Zone of inhibition on P.intermedia and chlorhexidine on day 10 by disc diffusion method

RESULTS

The values of microbial count which revealed the effect of the novel herbal gel containing aqueous orange peel extract and alcoholic ginger extract with 1% commercially available Chlorhexidine gel were subjected to statistical analysis to draw a conclusion from experimental data.

Descriptive statistical measures such as Mean, Standard deviation were calculated for all the study groups in order to collectively compare the two groups i.e the Control group which was the herbal gel and the Experimental group in which a commercially available Chlorhexidine gel.

Comparison between two gels (Herbal gel and Chlorhexidine) against two microorganism (P. gingivalis and P.intermedia) using Titanium implant material was assessed using statistical tests which determined the mean zone formation for the Control group and Experimental group was found to be statistically significant ($p = <0.05$).

Statistical Analysis was done by using the following test:-

- Kolmogorov Smirnov test
- Parametric tests

Table 4: Normality of Zone of inhibition (mm) scores in P.Gingivalis and P intermedia at different treatment time points in two groups by Kolmogorov Smirnov test

Organisms	Treatment times	Herbal gel group		Chlorhexidine group	
		Z-value	p-value	Z-value	p-value
P.Gingivalis	Day 4	1.0400	0.2300	1.2050	0.1100
	Day 7	1.1770	0.1250	1.1770	0.1250
	Day 10	1.3050	0.0660	1.2050	0.1100
	Day 4 to Day 7	0.7930	0.5550	0.9940	0.2770
	Day 4 to Day 10	1.0510	0.2190	0.8200	0.5120
	Day 7 to Day 10	1.1040	0.1740	1.1540	0.1390
P. intermedia	Day 4	1.3190	0.0620	1.1560	0.1380
	Day 7	1.1560	0.1380	1.2090	0.1325
	Day 10	1.2050	0.1100	1.1960	0.1140
	Day 4 to Day 7	1.2030	0.1110	1.2060	0.1090
	Day 4 to Day 10	0.7550	0.6180	0.9110	0.3780
	Day 7 to Day 10	0.7830	0.5710	1.2210	0.1020

The Zone of inhibition (mm) scores in P.Gingivalis and P.intermedia at different treatment time points in two groups follow normal distribution. Therefore, the parametric tests were applied.

DESCRIPTIVE STATISTICS

PORPHYROMONAS GINGIVALIS

Table 5: **Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores in P.Gingivalis at different treatment time points by Independent t test**

Treatment times	Herbal gel group		Chlorhexidine group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Day 4	25.59	1.54	27.35	0.70	-4.2912	0.0002*
Day 7	24.24	0.66	27.24	0.66	-13.1681	0.0001*
Day 10	20.41	0.94	25.65	0.70	-18.4083	0.0001*
Day 4 to Day 7	1.35	1.54	0.12	0.93	2.8349	0.0079*
Day 7 to Day 10	3.82	1.13	1.59	0.87	6.4579	0.0001*
Day 4 to Day 10	**5.18**	1.91	**1.71**	1.05	6.5657	0.0001*

*p<0.05 indicates significant

Independent t test was done to compare two independent groups in order to determine whether they are statistically significant.

Above table indicates that, when herbal gel was compared with chlorhexidine gel on day 4, chlorhexidine gel showed zone of inhibition with the mean of 27.35±0.70 when compared with herbal gel that is 25.59 ±1.54

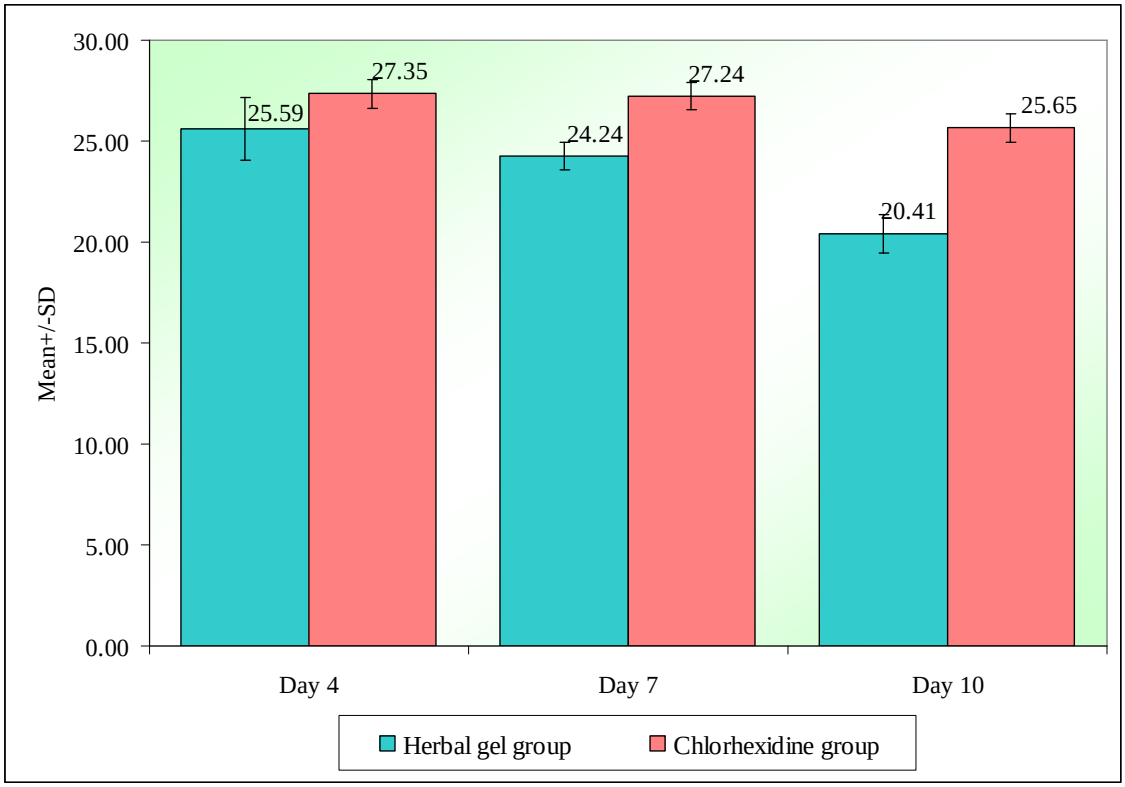
On day 7, chlorhexidine gel showed 27.24±0.66 and herbal gel showed 24.24±0.66, similarly on day 10, chlorhexidine gel showed increased zone of inhibition than herbal gel that is 25.65±0.70 and 20.41±0.94 respectively.

When intergroup comparsion was done of herbal gel (day 4^{th} to day 7^{th}) was done , mean was 1.35±1.54 when compared with chlorhexidine gel which was 0.12±0.93.

From day 7^{th} to day 10^{th} mean of zone of inhibition of herbal gel was 3.82±1.13 and in chlorhexidine gel was 1.59±0.87.Similarly from day 4^{th} to day 10^{th}, mean of zone of inhibition in herbal gel increases when compared with chlorhexidine gel that is 5.18±1.91 and 1.71±1.05 respectively .This increase in mean difference values was due to the result of decreased antimicrobial efficiency .

Microbial efficiency decreased from day 4 to day 10 in herbal gel. But in chlorhexidine group from 4^{th} to 10^{th} day the mean difference is significantly less and there is no much difference from day 4 to day 10 .Thus there is a significant difference in between herbal gel group and chlorhexidine group.

Graph 1: Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores in P.Gingivalis at different treatment time points



Graphical representation:

Above graph indicates graphical representation of the comparison between herbal gel and chlorhexidine gel group on 4^{th},7^{th}, 10^{th} day on Porphyromonas gingivalis. It shows that zone of inhibition of herbal gel gradually reduced as the day increases when compared to chlorhexidine gel. Herbal gel showed comparable results with chlorhexidine on Day 4^{th}.

Table 6: Comparison of different treatment time points with Zone of inhibition (mm) scores in P.Gingivalis in Herbal gel group and Chlorhexidine group by Dependent t test

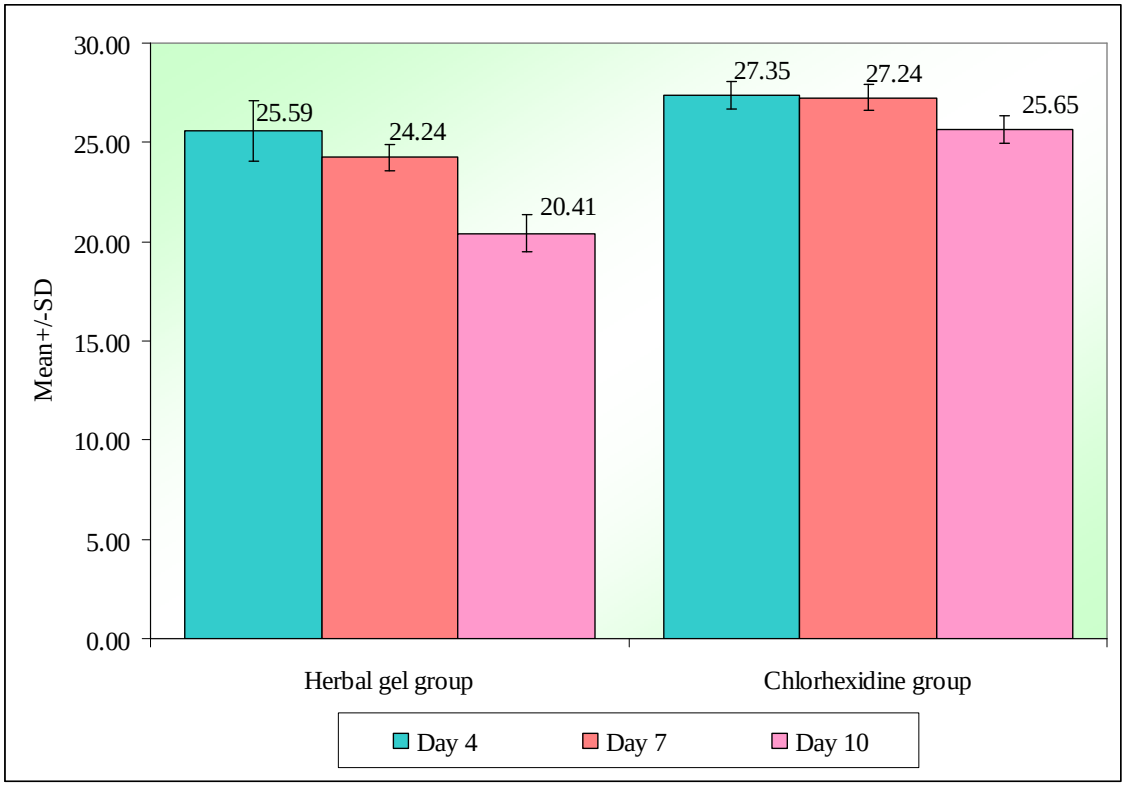
Groups	Changes from	Mean Diff.	SD Diff.	% of change	t-value	p-value	Effect size
Herbal gel group	Day 4 to Day 7	1.35	1.54	5.29	3.6253	0.0023*	0.8630
	Day 7 to Day 10	3.82	1.13	15.78	13.9375	0.0001*	
	Day 4 to Day 10	5.18	1.91	**20.23**	11.1648	0.0001*	
Chlorhexidine group	Day 4 to Day 7	0.12	0.93	0.43	0.5230	0.6082	0.6810
	Day 7 to Day 10	1.59	0.87	5.83	7.5247	0.0001*	
	Day 4 to Day 10	1.71	1.05	**6.24**	6.7197	0.0001*	

*$p<0.05$ indicates significant

Dependent test was used to compare the sample means from two related groups. It helps to determine the % of change from one group to other.

As the zone of inhibition decreased at different time intervals, the % of change in herbal gel was more when compared with chlorhexidine gel that is 20.23% and 6.24% respectively.

Graph 2: Comparison of different treatment time points with Zone of inhibition (mm) scores in P.Gingivalis in Herbal gel group and Chlorhexidine group.



Graphical representation:

The above graph indicates that, on intergroup comparison of herbal gel and chlorhexidine gel the effect or the zone of inhibition of herbal gel was reducing when compared with the chlorhexidine gel .

PREVOTELLA INTERMEDIA

Table 7: Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores P. intermedia at different treatment time points by independent t test

Treatment times	Herbal gel group		Chlorhexidine group		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Day 4	15.53	1.62	21.76	1.25	-12.5358	0.0001*
Day 7	14.76	1.25	21.24	1.39	-14.2455	0.0001*
Day 10	11.71	0.85	21.35	1.22	-26.7366	0.0001*
Day 4 to Day 7	0.76	0.83	0.53	1.42	0.5898	0.5595
Day 7 to Day 10	3.06	1.48	-0.12	1.50	6.2302	0.0001*
Day 4 to Day 10	**3.82**	1.94	**0.41**	1.58	5.6103	0.0001*

*p<0.05 indicates significant

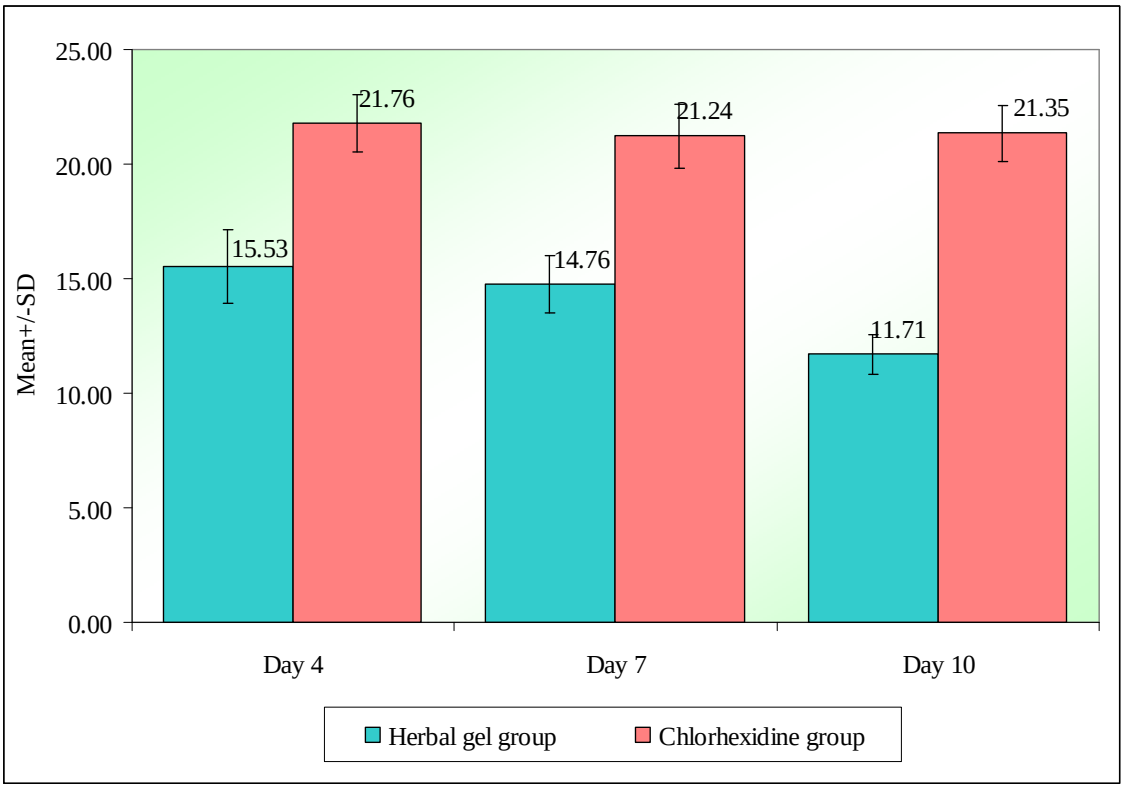
The above group indicates the comparison of herbal gel and chlorhexidine gel on Prevotella intermedia pathogen. It was done on the bases of different time zones that was on 4^{th} 7^{th} and 10^{th} day. It showed that the mean gradually decreased from 15.53±1.62 to 11.71±0.85 that is from 4^{th} day to 10^{th} day respectively, when compared to chlorhexidine gel that is 21.76±1.25 to 21.35±1.22 from 4^{th} day to 10^{th} day respectively.

On intergroup comparison from day 4 to day 7, mean of zone of inhibition in herbal gel was 0.76±0.83 and in chlorhexidine gel was 0.53 ±1.42. Similarly, when compared from day 7 to day 10 mean of zone of inhibition increased in herbal gel

than chlorhexidine gel group that is 3.06±1.48 and -0.12 ±1.50 respectively. Whereas from day 4 to day 10 , mean of zone of inhibition showed significant increase in the mean values on comparsion with chlorhexidine gel that is 3.82±1.94 and 0.14±1.58 respectively. This increase in the mean difference was due to reduced antimicrobial efficiency as the day increases.

It indicates that microbial efficiency reduced from day 4 to day 10. In chlorhexidine group from day 4 to day 10 mean difference is significantly less.

Graph 3: Comparison of Herbal gel group and Chlorhexidine group with Zone of inhibition (mm) scores P. intermedia at different treatment time pointsGraphical representation:



The above graph represents zone of inhibition of herbal gel and chlorhexidine gel from day 4th to day 10th. It shows that effect of herbal gel on each day was less as compared with chlorhexidine gel on Prevotella intermedia pathogen.

Table 7: Comparison of different treatment time points with Zone of inhibition (mm) scores in P. intermedia in Herbal gel group and Chlorhexidine group by dependent t test

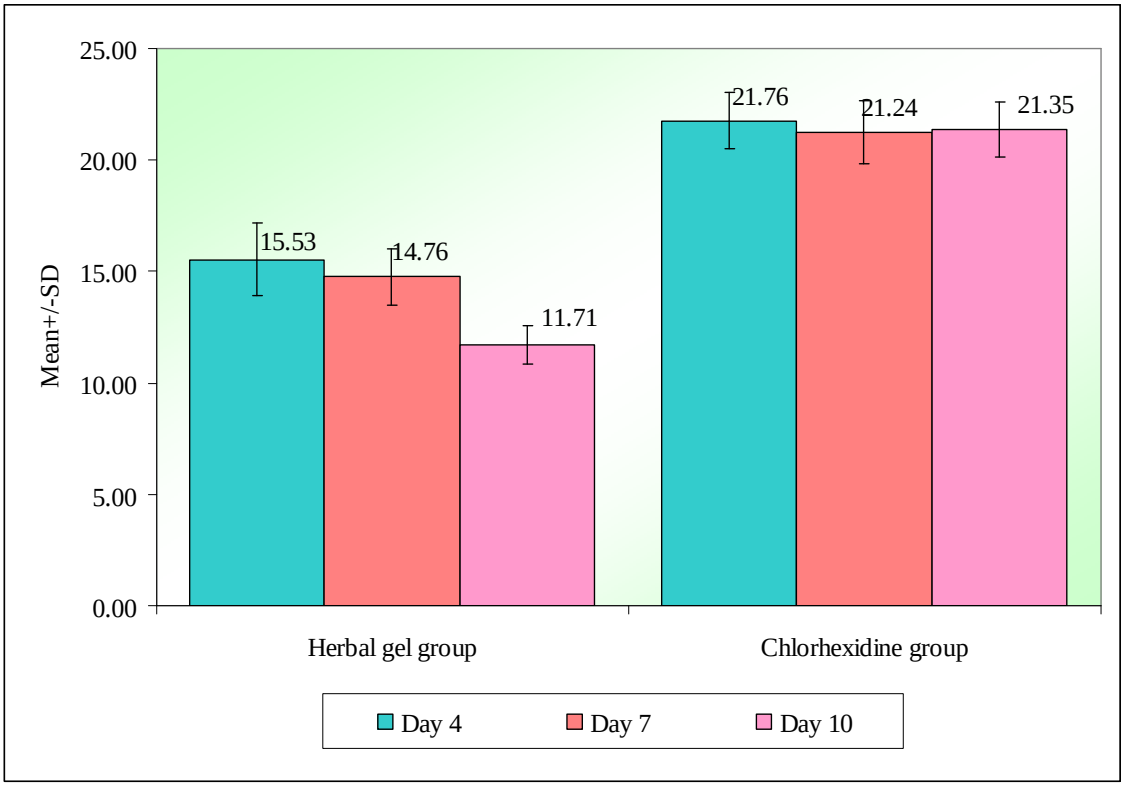
Groups	Changes from	Mean Diff.	SD Diff.	% of change	t-value	p-value	Effect size
Herbal gel group	Day 4 to Day 7	0.76	0.83	4.92	3.7925	0.0016	0.7970
	Day 7 to Day 10	3.06	1.48	20.72	8.5343	0.0000	
	Day 4 to Day 10	**3.82**	**1.94**	**24.62**	8.1092	0.0000	
Chlorhexidine group	Day 4 to Day 7	0.53	1.42	2.43	1.5378	0.1436	0.0680
	Day 7 to Day 10	-0.12	1.50	-0.55	-0.3244	0.7498	
	Day 4 to Day 10	0.41	1.58	1.89	1.0722	0.2995	

*$p<0.05$ indicates significant

Dependent test was used to compare the sample means from two related groups. It helps to determine the % of change from one group to other.

As the zone of inhibition decreased at different time intervals, the % of change in herbal gel was more when compared with chlorhexidine gel that is 24.62% and 1.89% respectively from 4^{th} to 10^{th} day.

Graph 4: Comparison of different treatment time points with Zone of inhibition (mm) scores in P. intermedia in Herbal gel group and Chlorhexidine group



Graphical representation:

As shown on the graph, effect of herbal gel gradually decreases with increased in time when compared to chlorhexidine gel.

Cytotoxicity of the gel:

After checking the antimicrobial property of the herbal gel, cytotoxicity of the herbal gel was done. Cytotoxicity of the gel was checked on L929 mice fibroblasts. It showed that there was total 37% cell proliferation and 63% cell death.

DISCUSSION

Dental implants are one of the most popular and common treatment plan for replacing missing tooth or teeth in completely or partially edentulous patients with high success rate (95-98%) and patient acceptability. Among various dental implant materials, titanium implants have been more popular as a way of restoring missing teeth. Due to the fact that they inherit exceptional biocompatible qualities, these materials have gained increased significance and are now acceptable for usage in the biomedical industry. In the year 1969, Branemark and his colleagues discovered that titanium and bone may come into close touch with one another. The term "osseointegration" was used to describe this process. It was contingent on the choice of implant design, processing, material employed, as well as the kind of bone that was used to accept the implant. Titanium grades IV and V are utilised for dental applications because to their biological features, which promote osseointegration and biocompatibility. These qualities make titanium an ideal material for dental applications.[4-5] Hence, in our study we have used titanium grade V material.

People who have few missing teeth are currently the most common candidates for oral implant treatment, and numerous reports have been published demonstrating successful long-term outcomes with a variety of oral implant systems. Because the majority of partially edentulous patients who are treated seem to be middle-aged people, that is, people who are between the ages of 40 and 50 when they are provided with oral implants, it is reasonable to anticipate that the patients will be able to function with their implant-supported constructions for a significant amount of time. It is generally accepted that periodontitis is caused when teeth are exposed to plaque for an extended period of time. It should not come as a surprise that the soft tissues

surrounding the implants are reported to respond to the presence of bacteria in a manner that is comparable to how the gingiva does because it was discovered that the microflora at implants in partially edentulous patients is comparable to that at teeth. In addition, patients who have lost teeth as a result of periodontitis prior to the placement of an implant show a higher degree of marginal bone loss, and even loss of the implant, during the follow-up process.[4-5]

As a consequence of this, it appears that the responses of teeth and implants to the microbiological environment of the mouth are comparable, provided that the teeth and implants are subjected to the same agents for the same amount of time.[40]

Nevertheless, in spite of their enormous success, the number of complications and rates of failure have been steadily increasing. According to **Salah et al**, keys reasons for early and late implant failure in majority of the cases are lack of primary stability surgical stress and infection. One of the most prevalent biological complications that may impact functioning implants is an infection called peri-implantitis. It is a destructive inflammatory illness that is related with the development of pockets and the loss of bone around the implants. Variations in the bone's marginal level following early remodelling, together with bleeding on peri-implant probing (BOP), are considered as diagnostic indicators for this condition. Around 13% of implants and 18.5% of patients are affected by periimplantitis, and the frequency of the condition has increased from 0.4 to 43.9% in the last 3–5 years. However, the condition manifests itself in varying degrees and intensities across a variety of patients and implant types. Even though bacteria are the primary cause of peri-implantitis, the likelihood of acquiring the condition may be increased by a number of other variables. The identification of these determinants, regardless of

whether they are innate or can be changed, is essential not only for the prevention but also the treatment of the illness. Periodontal disease, a lack of maintenance, using cigarettes or smokeless tobacco, hyperglycemia, and obesity are among the risk factors that have been identified for this condition. Regionally specific risk factors such as insufficient management of plaque, mucositis, malposition of the implant, and inadequately constructed prosthesis or presence because of the extra cement, certain hereditary variables, cardiovascular and autoimmune illnesses, high-dose blood pressure medication, and hormone replacement therapy may enhance the susceptibility to peri-implantitis. Vaping and smoking water pipes have a considerable negative impact on periodontal tissues, and as a result, they should be regarded to be possible risk factors for peri-implantitis. It's also possible that occlusal loading and the presence of titanium particles contributed to the beginning of the illness as well as its development.[41]

According to research conducted by Zitzmann et al, the incidence of the development of peri-implantitis is approximately six times greater in patients who have a history of periodontitis than it is in individuals who do not have a history of periodontal inflammation. After ten years, 10 to 50 percent of the dental implants exhibited symptoms of peri-implantitis.[7]

There is a wide variety of microorganisms that might cause problems for the periodontium that surrounds an implant. The majority of these species belong to the red complex or the orange complex of microorganisms. Gram negative organisms such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola are all members of the red complex seen in a periodontitis cases and Prevotella intermedia and fusobacterium nucleatum are members of the orange complex which are seen in

peri implantitis cases as per the research study don by gloria et al. Other members of the oral microbiome include Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Parvimonas micra, and Camylobacter rectus. Gram-positive aerobic bacteria, such Staphylococcus aureus, are also included in the group of early colonising bacterias.[8]

Porphyromonas gingivalis is one of the primary periodontal pathogens, and it is characterised by high levels of virulence and the ability to destroy periodontal tissue. Another organism that is present in peri implantitis that is observed rather often is Prevotella intermedia, which has an effect on the immunological response of the host and may cause periodontal damage.[8-9] Considering the frequency of these pathogens in both peri implantitis and periodontitis from metagenomic studies, theses two pathogens were selected to check the anti microbial efficacy of formulated herbal gel.

The management of gingivitis and periodontitis have been utilised in the management of peri-implant mucositis and peri-implantitis respectively. Non-surgical treatment for peri-implantitis focuses on preventing infections by cleaning the surface of the implant. The goal is to remove the stuck biofilm and lower the number of bacteria that can cause disease. Curettes, air abrasive devices, ultrasonic devices and lasers are some of the mechanical debridement for implant surface treatments that have been evaluated for the treatment of peri-implantitis. Primarily they are more aimed at subgingivally to decontaminate the exposed implant surfaces.[12]

The air-spray of sodium bicarbonate is the foundation of most powered air-abrasive systems that are now in use. Because of their high abrasiveness, they have the potential to cause harm to both hard and soft tissue. Recently, a powered air abrasive system that is based on a low-abrasive amino-acid glycine powder has been

demonstrated as an effective method of removing biofilm from the root surface. This was accomplished without causing any damage to either hard or soft tissues, and it has been recommended for use in debriding implant surfaces. Similarly ultrasonic devices and lasers have been suggested due to its anti-infective, physical, and ablative capabilities. Because of its ability to remove subgingival plaque and calculus effectively without significantly damaging the implant, the erbium-doped yttrium aluminium garnet laser is the laser that has shown the highest potential for use in the treatment of peri-implantitis.[21]

Inspite of all these available methods ,it is difficult to reduce bacterial loads to levels that are compatible with the health of tissue using only mechanical means, so adjunctive therapies, such as antiseptics and antibiotics, have been proposed as a way to improve the results of nonsurgical debridement. Chlorhexidine-based products, such as gels, irrigation solutions, and shampoos, have also been suggested as a way to improve the outcomes of nonsurgical debridement and/or rinses, as well as in a variety of formulated combinations and application schedules, such as repeated irrigation of the peri-implant pocket with 0.2% chlorhexidine in one session, single application of 1% chlorhexidine gel with a disposable syringe, applied subgingivally in each implant. So, in the present study chlorhexidine was used to check the antimicrobial property on P.gingivalis and P.intermedia. Results showed that there was increased zone of inhibition of chlorhexidine on P.gingivalis with mean of 1.71±1.05 and on P.intermedia it was 0.14±1.58.

Several antibiotic delivery strategies, either locally or systemically, have been put through their paces and evaluated: a single-unit dose of 1 mg of minocycline and 3 mg of poly(glycolide-co-dl-lactide) placed submucosally at each treatment site[21]

Systemic antibiotics were used; however, there are no controlled clinical studies that have been conducted to evaluate their effects. Depending on the ultimate goal of the surgical intervention, a number of different surgical procedures have been suggested, including the following: access for cleaning and decontaminating the implant surface (access flaps); access for washing and decontaminating the implant itself plus the exposure of the damaged areas for the purpose of cleaning (apically repositioned flaps); and access for cleaning as well as the goal of bone regeneration and re-osseointegration (regenerative techniques).[21]

Chlorhexidine gluconate (CHX) is commonly prescribed as non-surgical antimicrobial agent in management of peri-implantitis. CHX works against Gram-positive and Gram-negative anaerobic and aerobic bacteria, fungi, yeasts as well as several viruses. It is prescribed as a rinsing solution (0.2%) or in gel topical form (1%). Although, chlorhexidine has side effects such as discoloration of teeth, tongue and restorative materials, dysgeusia, desquamative gingivitis, burning of mucous membrane and sometimes allergic reactions. Contact with conjunctiva can cause permanent damage and accidental contact with the tympanum can cause ototoxicity. Contact sensitivity to CHX was first reported by Calnan (1962). CHX is known to elicit allergic contact dermatitis, including connubial contact dermatitis, generally after prolonged and repeated application (Krautheim et al. 2004). It can also cause contact urticaria, photosensitivity, fixed drug eruption and occupational asthma. On the whole, although sensitivity to CHX is rare, this complication should be kept in mind during its application. Therefore, its use should be limited to a maximum of 3 weeks.[13]

According to **Hyo-Sook Ryu et al** , combination of mechanical and chemical therapy may efficiently control peri-implant infection rather than mechanical debridement alone. This is because unlike natural teeth, rough implant surfaces fabricated for good osseointegration attract oral bacteria and develop a biofilm, making effective debridement difficult. Limited evidence also suggests that rough surfaces show greater risk of peri-implantitis than smooth surfaces. Therefore, further decontamination by adjunctive antiseptic agents is recommended to effectively eliminate pathogenic bacteria and improve debridement outcomes. Several anti-plaque chemical agents are commercially available, including citric acid, H_2O_2, and chlorhexidine (CHX). CHX has some side effects, including bitter taste and the formation of extrinsic stains on the teeth and tongue as well as cell cytotoxicity against a human fibroblast.[25]

According to a study by **Farzane Pakdel et al** antibiotics are invaluable medications for the treatment of many human diseases; however, excessive use of these medications results in microbial resistance. Therefore, researchers have prioritized their study on different parts of plants with medicinal uses in order to discover new drugs with herbal origins.[42]

To overcome the side effects and toxicity of the commercially available CHX gel, alternate methods to control the infection are the herbal compounds, which are non-toxic and safe to use. Phytotherapy, also known as phytochemistry or phytoecology, is the study of the use of plant extracts as medications. It is possible that phytotherapeutic agents may prove to be an easily accessible, highly useful, and cost-efficient option that can be used as a supplement to the therapy of periodontitis. These natural products have an excellent acceptance rate among patients and a high

tolerance level. Utilization of these drugs does not contribute to any antibiotic resistance. Neem, tulsi, aloe vera, ginger, turmeric, chamomile, tea tree, eucalyptus, orange peel extract showed antibacterial activity against various organisms such as Porphyromonas gingivalis, Provotella intermedia, Staphylococcus aureus etc.[14]

Ginger contains gingerols, in its rhizomes and has beneficial health activities including antioxidant, anti-inflammatory, anti-microbial and antifungal properties.[14] Similarly Orange peel (citrus reticulata) contains high levels of phenolic compounds including several flavonoids. It has been used in the treatment of various skin and stomach ailments and as a immune-enhancing agent. It also shows anti-bacterial activity against many organisms such as Pseudomonas aeruginosa, Staphyloloccus aureus, Prevotella intermedia, Porphyromonas gingavalis etc.[15]

Hence, this study was conducted to check antibacterial property of the ginger extract and orange peel extract on Porphyromonas gingivalis and Prevotella intermedia.

In a study done by **Mankar et al**, evaluated the antimicrobial property of orange peel extract, 0.2% chlorhexidine and saline against dental biofilm -forming bacteria such as Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans. Results showed that Chlorhexidine and orange peel showed anti microbial activity against these organisms at different concentrations. Mean zone of inhibition increased with increased concentration.[15]

Sana et al, checked the antimicrobial property of aqueous and alcoholic ginger extract against porphyromonas gingivalis .It showed that both the extracts had

bacteriostatic and bactericidal actions whereas alcoholic ginger extract was more active in inhibiting the organism with MIC(Minimum inhibitory concentration) of 30%(0.3g/ml) concentration and MBC(Minimum bactericidal concentration) of 60%(0.6g/ml) concentration when compared to aqueous ginger extract with MIC(Minimum inhibitory concentration) of 50% (0.5g/ml) and MBC(Minimum bactericidal concentration) of 80% (0.8g/ml).[24]

Ferdinan Pasaribu et al did research in which citrus peel extract of ethanol at a concentration of 25% has the strongest antibacterial effectiveness against the three bacteria when compared to solvents. The diameter of the inhibition zone against A. actinomycetemcomintans is 16.05 mm, while the diameter of the inhibition zone against P. gingivalis and F. nucleatum is 12.75 mm and 11.67 mm, respectively. There are statistically significant differences between the citrus peel extract with concentrations of 25%, 12%, 6,25%, and 3,125% (p 0.05). Citrus peel extract has an antibacterial effect, as shown by its ability to prevent the development of bacteria such as A. actinomycetemcomintans, P. gingivalis, and F. nucleatum.[34]

In a study done by **Dr. Aritra Mandal et al,** discovered that a mouthwash containing 4% citrus sinesis was just as effective as 0.2% chlorhexidine in lowering the Plaque Index, and it was much more successful in lowering the gingival inflammation and gingival bleeding index. As an alternative to 0.2%, citrus sinesis mouthwash with a concentration of 4% may be used for a limited period of time without the risk of experiencing any adverse effects. Use of mouthwash containing chlorhexidine may help reduce plaque and gingival irritation.[37]

So, in the present study, a herbal gel was formulated using aqueous orange peel extract and alcoholic ginger extract at 0.4% concentration, to check the anti-microbial

property against P.gingivalis and P intermedia . It was found that there was significant increase in mean difference values of zone of inhibition from day 4 to day 10 in herbal gel against Porphyromonas gingivalis as compared to chlorhexidine gel which was 5.18±1.91 and 1.71±1.05 respectively. But on day 4, the mean of zone of inhibition of herbal was comparable with chlorhexidine group that is 25.59 ±1.54 and 27.35±0.70 respectively. This indicates that zone of inhibition decreased with increased time. Similarly on Prevotella intermedia, day 4 to day 10, mean of zone of inhibition showed significant increase in the mean values on comparsion with chlorhexidine gel that is 3.82±1.94 and 0.14±1.58 respectively. This increase in the mean difference was due to reduced antimicrobial efficiency as the day increases.

In this present study, chlorhexidine was used as positive control and showed better anti-microbial activity against P.gingivalis and P.intermedia in terms of MIC and a greater zone of inhibition, establishing itself as gold standard .

A study done by **A. NALBANTSOY et al** to check antimicrobial and cytotoxicity of ginger. The ethanol extract on the L929 cells showed a cytotoxic impact on both human cervical cancer (HeLa) cell-lines and mouse fibroblast (L929) cell-lines. The amount of cytotoxicity is proportional to the amount of ethanol extract present. At a concentration of 80 micrograms per millilitre, the cytotoxicity was measured, and it steadily rises at greater doses.. The calculated IC50 value was 101.0 µg/ml.[38]

KEERTHANA T et al evaluated the cytotoxicity of citrus sinensis against fibroblasts. On comparison with CHX on these cells, the results reveal that CSE has a nontoxic impact up to a level of 200 g/ml and does not have a detrimental effect on

the fibroblasts, even at concentrations as high as 50%. The peel extract from Citrus sinensis showed much reduced cytotoxic activity.[39]

When cytotoxicity of herbal gel was checked on L929 mice fibroblasts, cell viability was found to be less. This may be due to the higher concentration which was used to make the gel or due to the interaction of the components present in the extracts which were used. The present study only evaluated the cytotoxicity of the acquired concentration, so cytotoxic effect can be reduced by further alterations of the concentrations.

Although chlorhexidine remains to be the gold standard, the formulated herbal gel showed comparable antimicrobial activity against peri implantitis pathogens and it can be used as an alternative for maintenance of early peri-implantitis. The outcome of this in vitro study can be of further interest in the field of clinical application in dentistry.

SCOPE OF THE STUDY

- This present study was done to check the antimicrobial property of the herbal gel on the two main predominant organisms (porphyromonas gingivalis and prevotella intermedia) , it is necessary to check the effect of the herbal gel on the different strains of the micro organisms which are present in peri-implantitis .Also antimicrobial property and cytotoxicity can be checked using different concentrations .
- On the basis of this study, different types of herbal extracts from different parts of the plants in combination with pharmaceuticals compostion can be used to check the antimicrobial properties against peri implantitis organisms.
- Further research can also assess the long term effect of the herbal gel and can also evaluate the osteogenic potential of the same. As the study was performed in vitro , in vivo parameters should also be included .

LIMITATIONS OF THE STUDY

Limitations of the study are as follows:

- Peri-implantitis is caused by various organisms, but the effect of the herbal gel was only evaluated on Porphyromonas Gingivalis and Prevotella Intermedia.
- It is an in vitro study, so there maybe few parameters that may affect the long term effect of the herbal gel.
- Topographic changes should be evaluated for different implant materials.
- Anti-microbial property was checked using only one concentration, so zone of inhibition on varying concentrations can be evaluated

CLINICAL IMPLICATIONS

- In this present study the use of herbal gel against peri-implantitis pathogens showed comparable results with chlorhexidine gel could be recommended as non-surgical aid in early peri-implantitis.
- The use of the herbal gel containing aqueous orange peel extract and alcoholic ginger extract can be preferred over commercially available gel as it was found to be effective and can be used to overcomes the anticipated ill effects of long term usage of chemical gels such as chlorhexidine (CHX).
- The clinical use of this herbal gel as an adjunctive therapy has a great future prospective even as an preventive measure for biofilm formation.
- This gel with further modification can revolutionize herbal use as an standard and effective non-surgical adjunctive treatment modality

CONCLUSION

This study concluded the anti-bacterial property of herbal gel inhibited the complex strain P.ginigvalis and Prevotella Intermedia which lead. to early peri-implantitis when compared with commercially available topical CHX gel. The formulated herbal gel reported toxicity on the acquired concentration so, further research can be done by reducing the concentrations. This opens the door to further investigations.

SUMMARY

The present study was conducted with the aim of evaluating and comparing the effect of the two gel namely herbal gel containing aqueous orange peel extract and alcoholic ginger extract and commercially available Chlorhexidine (CHX) gel.

A total of 68 discs of commercially available pure Titanium grade 5 was fabricated of diameter 10 mm and a width of 2 mm. The discs were subdivided into two groups as control and experimental. The orange peel and ginger was authenticated and the extract was formulated. The prepared extract was subjected to antibacterial assay (MIC and MBC) using serum dilution method for individual extract . Combination MIC and MBC was evaluated. Once the MIC and MBC values were achieved the formulation of the herbal gel was initiated. The gel formulation was carbopol based and extracts was used along with other ingredients. Once the final herbal gel was prepared, the Titanium discs were subjected to antibacterial testing in vitro using disk diffusion method. The disk-diffusion agar method tested the effectiveness of herbal gel against *P.gingivalis and P.intermedia*. An agar plate was first streaked with bacteria, After the inoculum dried, well was punched with a sterile cork borer.

The sterile Titanium discs was used.

Each plate contained two discs:

1. *Experimental group-* disc coated with herbal gel.
2. *Control group-* disc coated with 1% Chlorhexidine gel.

The plates were then incubated for and 48 hours (anerobic) at 37 ℃.

When the antibacterial activity was present, no colonies grew where the concentration in the agar is greater than or equal to the effective concentration. This is called the zone of inhibition.

The values of microbial count which revealed the effect of the herbal gel and commercially available chlorhexidine gel were subjected to statistical analysis to draw a conclusion from experimental data.

The novel herbal gel had a comparable antibacterial activity when compared to the commercially available CHX (control group).

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

Sl. No. : 1458

CERTIFICATE

This is to Certify that the synopsis titled

comparative evaluation of anti microbial efficacy of a novel herbal gel containing orange peel extract and ginger extract with chlorhexidine gel on peri implantitis Pathogens- An invitro study *Submitted by*

Dr. _____ *P. G. Student / Staff, Guided by* _____ *from Department of* Prosthodontics & Crown & Bridge *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 – AUTHENTICATION LETTER

Herbo Nutra
Natural Ingredients Solutions Provider

Company Address:
A-2/66, UPSIDC SITE 5,
Kasna Surajpur Industrial Area,
Gautam Budh Nagar, Greater Noida
Uttar Pradesh – 201308, India
Phone: +91-9911016820
Email: info@herbonutra.com
Web: www.herbonutra.com

Certificate of Analysis

Product Name:	Orange Peel Extract
Batch Number:	HN / OPE /1100992
Quantity:	25 kg
Manufacture Date:	Aug 20
Exp Date:	July 23

Analysis	Specification	Results
Appearance	Brown Fine Powder	Complied
Odor	Characteristic	Complied
Taste	Characteristic	Complied
Assay / Extract Ratio	10:1	Complied
Loss on Drying	≤5.0%	3.34%
Sieve Analysis	Pass 60 mesh	Complied
Bulk Density	45-55g/100mL	Complied
Heavy Metal	NMT 20ppm	Complied
As	NMT 2ppm	Complied
Microbiology		
Total Plate Count	NMT 500cfu/g	Complied
Yeast & Mold	NMT 100cfu/g	Complied
E.Coli	Negative	Complied
Salmonella	Negative	Complied

Conclusion	Conform with specification
Storage	Store in cool & dry place. Keep away from strong light and heat.
Shelf life	3 years when properly stored

The information herein is correct based on our knowledge; please evaluate the raw material prior to use in a finished product

Computer generated certificate, valid without signature.

UKASCB®
UNITED KINGDOM APPROVAL
ISO 9001:2015 Certified
★ Optical Emission Spectrometry
★ PMI
★ Hardness Testing
★ Ultrasonic Flaw Detection
★ Ultrasonic Thickness Gauging
★ Dye Penetrant Testing

MTL

METAL TEST LAB

(Recognised By Government Deptts & Undertakings)

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Phone : 6743 7546 • Mobile : 9224778882 / 9223371637 • E-mail : metaltestlab2016@gmail.com

TEST REPORT

T/C No : G/ 40321 DATE 04/03/2021

PARTY NAME : SPECIAL METALS

REFERENCE : -

MATERIAL DESCRIPTION: TITANIUM DISC

GRADE : TI GR.5

%	C %	Si %	Mn %	P %	S %	Cr %	Mo %	Ni %	Al %
COMP	0.0580	--	--	--	--	--	--	--	6.00
REQD	--	--	--	--	--	--	--	--	5.5000
	0.0800	--	--	--	--	--	--	--	6.7500

%	Co %	Cu %	Nb %	Ti %	V %	W %	Pb %	Fe %	N %
COMP	--	--		87.88	5.00 --	--	--	0.069	--
REQD	--	--	--	--	3.5000	--	--	--	--
	--	--	--	--	4.5000	--	--	0.40 00	--

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.

QUALITY IS OUR MOTTO

ANNEXURE – III

Summary of number of zones on Porphyromonas gingivalis organism on herbal gel and chlorhexidine gel

	4th Day	**7th Day**	**10th Day**
Herbal gel	27mm	24mm	20mm
	24mm	24mm	22mm
	23mm	23mm	22mm
	27mm	25mm	22mm
	25mm	25mm	21mm
	27mm	24mm	20mm
	26mm	25mm	20mm
	25mm	24mm	21mm
	24mm	25mm	20mm
	26mm	23mm	20mm
	22mm	24mm	19mm
	27mm	24mm	20mm
	27mm	25mm	21mm
	26mm	24mm	20mm
	26mm	24mm	20mm
	26mm	25mm	20mm
	27mm	24mm	19mm

Chlorhexidine gel	28mm	27mm	25mm
	27mm	26mm	26mm
	28mm	27mm	26mm
	28mm	27mm	26mm
	26mm	28mm	27mm
	28mm	27mm	26mm
	28mm	28mm	25mm
	27mm	27mm	26mm
	28mm	28mm	25mm
	27mm	28mm	26mm
	26mm	27mm	25mm
	27mm	26mm	25mm
	27mm	27mm	26mm
	28mm	28mm	27mm
	27mm	27mm	25mm
	27mm	28mm	25mm
	28mm	27mm	25mm

Summary of number of zones in Prevotella intermedia organism on herbal gel and chlorhexidine gel

	4th Day	**7th Day**	**10th Day**
Herbal gel	16mm	15mm	12mm
	17mm	15mm	12mm
	16mm	16mm	13mm
	16mm	16mm	11mm
	13mm	13mm	12mm
	13mm	13mm	12mm
	16mm	15mm	12mm
	17mm	15mm	10mm
	17mm	15mm	10mm
	16mm	16mm	12mm
	17mm	16mm	13mm
	13mm	13mm	12mm
	13mm	13mm	11mm
	14mm	13mm	12mm
	17mm	16mm	11mm
	16mm	16mm	12mm
	17mm	15mm	12mm

Chlorhexidine gel	20mm	20mm	20mm
	23mm	23mm	22mm
	23mm	20mm	20mm
	20mm	20mm	23mm
	22mm	23mm	22mm
	22mm	20mm	22mm
	20mm	20mm	20mm
	23mm	20mm	20mm
	23mm	22mm	23mm
	20mm	20mm	20mm
	20mm	22mm	22mm
	22mm	20mm	20mm
	22mm	22mm	22mm
	23mm	22mm	22mm
	22mm	23mm	20mm
	23mm	23mm	22mm
	22mm	20mm	23mm