"EVALUATE THE EFFICACY OF GARCINIA MANGOSTANA (MANGOSTEEN) INCORPORATED NANO BIO GEL AGAINST PERI-IMPLANTITIS PATHOGENS" – AN VITRO STUDY

BY

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Dissertation

Submitted to
KLE Academy of Higher Education and Research
Belagavi, Karnataka
In partial fulfillment
of the requirements for the degree of

MASTER OF DENTAL SURGERY

In

PROSTHODONTICS AND CROWN & BRIDGE

(BRANCH – I)

Under the guidance of

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2019 – 2022

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ACKNOWLEDGEMENT

"Feeling gratitude and not expressing it is like wrapping a present and not giving it".

A sense of triumph is very much justified at this stage of completion of my dissertation, even more so is a sense of gratitude to all my peers, mentors and well-wishers.

My salutations to ALMIGHTY GURU – A tangent between zero and infinity – for his divine grace bestowed when needed.

In Loving Memory of my Beloved Father Late ***Mr. KANTILAL INDERMAL SHAH.***

I gladly utilize this opportunity to express my deep sense of gratitude and indebtedness to all my TEACHERS.

"The task of the excellent teacher is to stimulate "apparently ordinary" people to unusual effort. The tough problem is not in identifying winners: it is in making winners out of ordinary people."

I feel honored to be a student of my respected teacher and guide ***Dr. PRASHANT A KARNI*** ***MDS,*** *Reader, Department of Prosthodontics and Crown and Bridge, KAHER Vishwanath Katti Institute of Dental Sciences, Belagavi, without whose everlasting inspiration, incessant encouragement, constructive criticism, and with valuable suggestions for improvement, the completion of this study would not have been possible. His unlimited patience, meticulous supervision at every step and everlasting zeal for perfection has not only enabled me to complete the dissertation, but has also helped me tremendously during the postgraduate programme.*

I am grateful to ***Dr. ANANDKUMAR G. PATIL$_{MDS}$*** *and* ***Dr. RAGHUNATH PATIL$_{MDS}$,*** *who have been the pillar of support and were always available to me and have been a source of endless guidance all throughout.*

I would like to thank ***Dr. RAMESH NAYAKAR$_{MDS}$, Dr. MAHANTESH BEMBALGI$_{MDS}$ and Dr. SANTOSH NELOGI$_{MDS}$,*** *for their valuable guidance and keen personal interest in critical analysis of this study material, without which this study would be incomplete.*

I would also like to thank, ***Dr. HEMA$_{MDS}$, Dr. SOUNALAYA$_{MDS}$, Dr. ABHIJIT$_{MDS}$, Dr. SAYED$_{MDS}$, Dr. ADITYA$_{MDS}$, Dr. SWAPNIL$_{MDS}$, Dr. MALLIKARJUN$_{MDS}$, Dr. VEENA$_{MDS}$, Dr. SUVIDHA$_{MDS}$,*** *for their support, their innovative ideas and friendly discussions in the matter of mutual professional interest which were of considerable value & helped me to broaden my source of knowledge.*

I am also very grateful to my co-guide ***Dr. S. C. METGUD $_{MD}$*** *Professor, Dept. of microbiology, JNMC KLE for helping me since the beginning and giving valuable guidance for performing cell culture assays.*

I would like to extend thanks to ***Dr. U. B. BOLMAL****, Dept. of pharmacology, KLE for helping me and giving valuable guidance for performing pharmacological formulation.*

I would like to thank Gogte Institute of Technology, Udyambagh Belagavi for assisting me with surface roughness evaluation.

A word of thanks to ***Prof. Mr. SHIVALINGAPPA JAVALI,*** *Statistician, for providing a scientific meaning to this study by way of its statistical analysis.*

I would like to thank all the technicians and non-teaching staff for their services and help whenever required.

I am grateful to ***Dr. (Mrs.) ALKA KALE*** ***MDS,*** *Principal, KLE V.K Institute of Dental Sciences, Belagavi, for extending her help and cooperation towards the completion of this dissertation.*

I extend my heartfelt gratitude to my dearest friend and senior ***Dr. TEJASHREE CHOUGULE- JAISWAL*** ***MDS*** *for her constant support, encouragement and her tremendously valuable inputs and guidance without whom this study would not be complete.*

My special thanks to ***Dr. VISHAKHA DARE****,* ***Dr. HIMA BINDU*** *and* ***Dr. YAMINI PAGAR*** *who have helped me generously without any hesitation and for their constant support.*

I am thankful to all my colleagues ***Dr. HARPREET, Dr. RUTVI, Dr. RAHUL*** *and* ***Dr. DIVYA*** *for their constant support and cooperation. My sincere thanks to my loving seniors* ***DR. RITWIK, Dr. OVAIS, Dr. DIVYESH, Dr. HARSHALI, Dr. RICHA, Dr. MEEKHA and Dr. SAYALI*** *for generously sharing insight and for their unwavering support. I extend my heartfelt thanks to my dear juniors****, Dr. RAISA, Dr. PALLAVI, Dr. SONALI, Dr. MITALI and Dr. POONAM, Dr. SHREYA, Dr. SWAPNIL, Dr. KARUNA, Dr. NISHA, and Dr. AISHWARYA.*** *Not to forget* ***Dr. NIKHIL*** *who has been generously supportive.*

*I take pleasure in thanking my friends****, Ms. STUTI WADHWA, Mr. YUGANK S. P, Dr. POOJA DIALANI and Dr. NIVEDITA S.*** *for their constant support during the study.*

I would not have completed this dissertation without the unconditional support of my family who has always been there for me whenever I needed them, with encouragement and unconditional love to empower me all the time.

I owe everything of what I am to my mother, ***Mrs. SANGEETA SHAH,*** *my grandparents* ***Late Shri. INDERMAL L***, ***Smt. BADAMIBAI L, Late Shri NEMICHAND M*** *and* ***Late Smt. SUHASBAI M,*** *my uncle and aunty* ***Mr. & Mrs. GULAB PUSPHA JAIN and Mr. & Mrs. VIJAYKUMAR MANJU JAIN,*** *my dearest siblings* ***Mr. SANDEEP JAIN***, ***Ms. RIYASHI SHAH, Dr. SHIRIN JAIN, Mr. ASHWIN JAIN, Mrs. SALONI T, BHAVY JAIN and Mr. NITIN JAIN*** *who gave form to all my dreams and aspirations. I extend my heartfelt thanks to my maternal family* ***Mr. & Mrs. JAYANTI VIMALA JAIN***, ***Mr. & Mrs. SURESH SANCHAL JAIN*** , ***Mr. & Mrs. PRAVEEN MADHU JAIN***, ***Mrs. PUSPHA*** *and* ***Mrs. KANCHAN*** *for their constant support.*

I owe every success to them and I humbly acknowledge that everything I am today is because they loved me.

Thank you, one and all.

Dr. AAYUSHI K SHAH

LIST OF ABBREVIATIONS USED IN THE STUDY

GROUP TE	Titanium experimental group (Nano bio gel)
GROUP TC	Titanium control group (CHX)
GROUP ZE	Zirconia experimental group (Nano bio gel)
GROUP ZC	Zirconia control group (CHX)
PB	Probing depth
S.D.	Standard Deviation
C.V.	Coefficient of Variation
S.E.	Standard of error
ANOVA	Analysis of variance
hrs.	Hours
L	Litre
M	milli
CHX	Chlorhexidine
HA	Hydroalcoholic
PBS	Phosphate Buffer Solution
IOPA	Intra oral periapical
CLSI	Clinical Laboratory Standard Institute Guidelines
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration

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 Garcinia Mangostana (mangosteen) incorporated Nano bio gel against *S. aureus* and *P. gingivalis* peri– implantitis pathogens adhered to Titanium and Zirconia discs.

METHODS

A total of 60 discs of commercially available pure Titanium grade 4 and Zirconia were fabricated of diameter 5 mm and a width of 2 mm (ASTM F67). The discs were subdivided into four groups as control and experimental. The *Garcinia mangostana* fruit was authenticated and the extract was formulated using the pericarp of the fruit. The extract was prepared in hydroalcoholic and phosphate buffer solution. The prepared extract was subjected to antibacterial assay (MIC and MBC) using serum dilution and disk diffusion method. Once the MIC and MBC values were achieved the formulation of the nano bio gel was carried out. The gel formulation was carbopol based and 5 % of the hydroalcoholic extract was used along with other

ingredients. Once the nano bio gel was formulated, the Ttitanium and Zirconia discs were subjected to antibacterial testing in vitro using disk diffusion method.

RESULT

The collected data was subjected to statistical analysis using two way ANOVA and Tukey's multiple post hoc. There was statistically significant difference between the control and experimental group ($P < 0.05$).

CONCLUSION

This present study showed that the G. Mangostana incorporated nano bio gel has greater antibacterial activity against early peri implant pathogens in Titanium implant material. On the other hand when compared to the Chlorhexidine (CHX) gel control group (TC and ZC), the experimental group (TE and ZE) showed higher inhibition of *S.aureus* and *P.gingivalis* peri implant pathogens.

KEYWORDS

garcinia mangostana, Chlorhexidine gel , peri-implantitis, nano bio gel, disc diffusion.

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INTRODUCTION

Prosthodontics is the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of the oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/ or maxillofacial tissues by using biocompatible substitutes. The treatment options for missing single or multiple teeth varies from a removable partial denture, fixed partial denture and dental implants.[1]

Dental implant prosthodontics is the selection, planning, placement, and fabrication of missing teeth and/ or associated structures, and maintenance of restoration(s). Dental Implant is any object or material, such as an alloplastic substance or other tissue, which is partially or completely inserted or grafted into the body for therapeutic, diagnostic, prosthetic, or experimental purposes. By definition of dental implants any artificial, biocompatible material is considered as an implant, here onwards the term dental implant would specifically denote the dental implant used for therapeutic purposes in oral implantology. The treatment of a partially edentulous patient has various phases, thus its objective is complex and the treatment requires intelligent planning and efficient execution.[1] It is therefore obvious that the implants should have certain health promotional and preventive values which are correlated with the dentist's concept of required therapy, as well as certain physical characteristics which yield restorative and remedial values that are in congruence with patient needs and desires.[1]

The goal of modern dentistry is to restore normal contour, function, comfort, esthetics, speech and health regardless of the atrophy, disease or injury of the stomatognathic system. However the more teeth a patient is missing the more arduous

this goal becomes with traditional dentistry. As a result of research advances in implant designs, materials and techniques have led to predictable success in their application and several types of implants are now available for use and rehabilitation of different clinical problems.[2,3]

There are four main types of dental implant designs that have been developed and used in clinical dentistry, including a subperiosteal form, blade form, ramus frame, and endosseous form. However, here we will focus on endosseous implants which are the most frequently used implants in dentistry today.[2,3]

One of the most important developments in dental implantology occurred in 1957, when a Swedish orthopedic surgeon called Per-Ingvar Brånemark started to study bone healing and regeneration and discovered that bone could grow in the vicinity of Titanium (Ti) and that it could be adhered effectively to the metal without being rejected. Brånemark thus named this phenomenon 'osseointegration,' and he performed many more experiments using both animal and human subjects. He defined this process as “Direct structural and functional connection between ordered, living bone and the surface of a load bearing implant”. In simple words it is a direct bone implant interface formed when an implant is allowed to heal in bone in an undisturbed manner; it is analogous to functional ankylosis. [4,5]

The U.S. Food and Drug Administration approved the use of Ti dental implants in 1982, and in 1983 Dr. Matts Andersson developed the Procera (Nobel Biocare, Zurich, Switzerland) computer-aided design and computer-aided manufacturing (CAD / CAM) method of high precision, repeatable dental crown manufacturing. Recent progress in the last century has concentrated on materials and techniques to improve quality and anchorage of implant with the bone. [5]

Implant therapy offers many advantages over conventional fixed or removable treatment options. The survival and success rates reported by many implant investigators often exceed the success rates of traditional dental treatment. The success and predictability of osseointegrated dental implants have forever changed the philosophy and practice of dentistry. Patient's expectations from a treatment plays a potential role to their final satisfaction from the treatment outcomes, the ever increasing awareness among the patients have improved the expectation of the treatment. The dental implant has brought paradigm shift in the conventional treatments, patients have better treatment outcomes and quality of life with dental implants. Although dental implants have greater success rate, it may sometimes lead to implant failure due to various factors. In recent years, use of dental implants has increased significantly as an important treatment modality for the reconstruction of the dental system; however, concomitant with this increase in the use of dental implants, the incidence of peri-implantitis has increased, too.[6]

The term peri-implantitis was introduced in the 1980s to describe a destructive inflammatory process affecting the soft and hard tissues around osseointegrated implants, leading to the formation of a peri-implant pocket and loss of supporting bone. The prevalence of peri-implantitis was reported to be around 20-40%. Clinicians should monitor the surrounding tissues for signs of peri-implant disease by observing changes.[7]

The two principal phenomena in peri implantitis are Mucositis and Peri-implantitis. On the one hand, mucositis is characterized by the presence of an inflammation confined to the peri-implant mucosa and reversible in case of effective treatment. And on the other hand, peri-implantitis is characterized by a loss of

supporting bone, both anatomically and radiographically confirmed, combined with an inflammatory reaction of the surrounding soft tissues.[8]

Bacterial peri-implant pocket colonization is part of the initial peri-implantitis pathways. Micro-organisms most commonly associated with implant failure are aerobic and anaerobic. Gram-negative motile types anaerobes i.e *Porphyromonas gingivali, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Bacteroides forsythus, Treponema denticola, Prevotella nigrescens, Peptostreptococcus micros and Fusobacterium nucleatum* are involved in the pathogenesis of peri implantitis. These species were found significantly at higher levels in peri-implantitis sites. High levels of *Porphyromonas gingivalis* increased the risk for further attachment loss in patients. Within an hour after the installation of dental implants, bacteria can be identified and a complex biofilm is formed within 2 weeks. [9]

Staphylococcus aureus is a Gram-positive aerobic bacteria and it is part of the early colonizing bacteria. *Staphylococcus aureus* has a strong affinity to Titanium surfaces and its presence in infection is important in the development of peri-implantitis. Such forms of peri-implant contamination will be especially rapid in partially dentulous patients with an active periodontal disease: peri-implant sulcus colonization by these microorganisms will be seen from the first month following the implant's contact to its prosthetic part. The bacterial communities present in peri-implant space frequently differ widely from those found in neighbouring teeth's sulcus. [10]

In view of the homogeneity, treatment protocols used to treat gingivitis and periodontitis have been used for management of peri-implant mucositis and peri-implantitis respectively. The management of peri-implantitis can be non-surgical and

surgical. While the primary line of treatment is non- surgical disruption of biofilm and reduction of bacterial loads, additional surgical procedures and adjunctive therapies like use of mechanical debridement and use of topical chlorhexidine (CHX).[11]

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.[12]

The treatment goal in peri-implant disease is to reduce the bacterial load, shift the bacterial composition of the biofilm, and improve cleanability of the affected implants. 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. Among many herbal compounds *Garcinia mangostana (mangosteen)* has antibacterial, antiviral and antifungal properties. An improvised

herbal extract formulated as nano bio gel could be revolutionary adjunctive therapy in early phases of peri-implantitis.

Garcinia mangostana (mangosteen) has a long history of use as a medical plant, mostly in Southeast Asia. The fruits of *G. mangostana* are the most treasured part of this plant and are famous for the remarkably pleasant flavour. Therefore, mangosteen was even named as the 'Queen of Tropical fruits'. The fruit *G. mangostana* has been used for hundreds of years around the world, mostly in Southeast Asia, as a medicine for a great variety of medical conditions. Over the past decades, it was shown that mangosteen contains high amounts of bioactive ingredients. Its pericarp contains a variety of xanthones, such as α-, β-, γ-mangostins which have remarkable biological activities such as antioxidant, antitumoral, antibacterial, antiviral, antifungal, antiallergic, and anti-inflammatory properties. The antibacterial activity against *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Bacillus subtilis, Propionibacterium acnes, Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella typhimurium, Proteus sp, Klebsiella sp, Escherichia coli, Enterococcus spp, and vancomycin resistant Enterococci (VRE)* of the mangosteen has been reported. The major constituents from the xanthone fraction of G. mangostana were found to be *α -mangostin* and *γ -mangostin.* This plant was claimed to have a remarkable antiinflammatory effect and to reduce cell damage. The herbal G. Mangostana has not reported any side effects, hence it could reform the traditional non-surgical management of peri-implant pathology.[13]

Nanogels in medicine applications have a highly promising future. Nanogels could be used to deliver active drug compounds in controlled drug delivery

applications. Nanogels are hydrophilic and amphiphilic polymer chains that are swollen nano-sized networks. These can be configured to accommodate biologically active molecules through physical cross-linking. Nanogels can easily incorporate drugs, bio-macromolecules, and proteins of low molecular mass availability of various polymer systems and ease of alteration of their characteristics have rendered nanogels formulations advantageous. They have high biocompatibility and biodegradability. The biggest advantage of these gels is reduced premature leakage of the drug from the solution. Nanogel appears to be an excellent candidate to improve the design of gels for optimum delivery of drugs to the target cells rather than to the normal cells.[14] Hence Nano gels incorporated with the G. Mangostana herbal extract is a promising and versatile oral drug delivery systems.

In one such effort nano bio gel is combined with Garcinia mangostana to yield clinically acceptable therapeutic results in treatment of peri-implantitis. This present study intended to formulate and evaluate a Garcinia Mangostana (Mangosteen) incorporated nano bio gel for their antibacterial activity against peri-implantitis pathogens.

NEED FOR THE STUDY

Dental implantology has revolutionized dentistry in many ways. Replacement of the lost tissues using the conventional techniques may not be always possible due to complex and challenging clinical conditions. However implant dentistry can overcome this problem even in complex situations like jaw atrophy, compromised health condition etc. Acceptance of osseointegrated implant supported prosthesis has thus increased by the patients. Implant therapy offers many advantages over conventional fixed or removable treatment options and is the treatment of choice. The survival and success rates reported by many implant investigators often exceed the success rates of traditional dental treatment.

The term peri-implantitis was introduced in the 1980s to describe a destructive inflammatory process affecting the soft and hard tissues around Osseointegrated implants, leading to the formation of a peri-implant pocket and loss of supporting bone.

The management of the peri-implantitis is non-surgical and surgical, non-surgical treatment modality is the primary line of treatment. Chlorhexidine gluconate (CHX) is commonly prescribed as non-surgical antimicrobial therapy in treatment 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 form (1%). Chlorhexidine has side effects of discoloration of teeth, tongue and restorative materials, taste alteration, burning of mucous membrane and sometimes allergic reactions.

To overcome these side effects, the customized gel contains herbal *Garcinia mangostana* (Mangosteen) which is locally available in India (southern regions) and has antibacterial properties.

Garcinia Mangostana (Mangosteen) pericarp contains a variety of xanthones, α-, β- and γ-mangostins which have remarkable biological activities such as antioxidant, antitumoral, antibacterial, antiviral, antifungal, antiallergic, and anti-inflammatory properties. The antibacterial activity of Garcinia Mangostana (Mangosteen) against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported.

Bioactive agents present within the prepared Garcinia Mangostana (Mangosteen) extract such as Xanthones are smaller than 10^{-9}μ hence known as Nano Bio particles.

Nanogels are used to deliver all biologically active agents and drugs in a controlled and sustained release manner. Availability of various polymer systems and ease of alteration of their characteristics have rendered nanogel formulations advantageous.

This present study intends to formulate and evaluate a Garcinia Mangostana (Mangosteen) incorporated nano bio gel for their antimicrobial activity against peri-implantitis pathogens.

HYPOTHESIS

NULL HYPOTHESIS:

There is no difference in the antimicrobial effect on the peri-implantitis pathogens when treated with Garcinia Mangostana incorporated nano bio gel and Chlorhexidine.

RESEARCH HYPOTHESIS:

There is a difference in the antimicrobial effect on the peri-implantitis pathogens when treated with Garcinia Mangostana incorporated nano bio gel and Chlorhexidine.

AIM OF THE STUDY

To investigate the efficacy of Garcinia Mangostana (mangosteen) incorporated Nano bio gel against *S. aureus* and *P. gingivalis* peri– implantitis pathogens adhered to Titanium and Zirconia discs.

OBJECTIVES

1. To evaluate the efficacy of Garcinia Mangostana (Mangosteen) incorporated nano bio gel against *P. gingivalis* and *S. aureus* peri– implantitis pathogens adhered to Titanium and Zirconia discs.
2. To evaluate the efficacy of Chlorhexidine gel against *P. gingivalis* and *S. aureus* peri– implantitis pathogens adhered to Titanium and Zirconia discs.
3. To compare the antimicrobial activity of Garcinia Mangostana (mangosteen) incorporated nano bio gel with chlorhexidine gel (CHX) against *P. gingivalis* and *S. aureus* peri– implantitis pathogens adhered to Titanium and Zirconia discs.

REVIEW OF LITERATURE

1. **Sennerby L (1991)** conducted a study 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.[15]
2. **A. Mombelli (1993)** did a study 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 bacteria in the successful implants had very low cultivable numbers, and the majority of them were gram-positive cocci.[16]
3. **Gerald mcdonnell (1999)** studied 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. [17]
4. **Yu-Ling Huang (2001)** did a study evaluate Garcinia mangostana for its contents, and four novel compounds were discovered: three minor xanthones, garcimangosone A (1), garcimangosone B (2), and garcimangosone C (3), and a

benzophenone glucoside, garcimangosone D. (4). Spectral (NMR and MS) and chemical approaches were used to determine the structures of these four compounds. Garcinia mangostana L. (Guttiferae) fruit hulls have been reported to be used as an anti-inflammatory, astringent, and to treat diarrhoea. 1,8-desoxygartanin, R-mangostin, and -mangostin are among the several xanthones found in them. 1-6 From the EtOAc section of this plant's fruit hulls, we isolated four novel compounds: three minor xanthones, garcimangosone A (1), garcimangosone B (2), and garcimangosone C (3), and a benzophenone glucoside, garcimangosone D (4).[18]

5. **Gintaras Juodzbalys (2003)** created an acid-etched implant surface similar to that produced by sandblasting and acid etching, and to compare it to the surfaces of various commercially available screw-type implants. An electron microscope was used to scan all etched surfaces, and digital images were created for visual evaluation and description. A mixture of sulfuric and hydrochloric acids produced the most similar surface to the sandblasted/acid-etched surface.[19]
6. **Wanlop Weecharangsan (2006)** studied the antioxidative and neuroprotective properties of different extracts from mangosteen fruit hulls (Garcinia mangostana Linn., GM). In this study, four extracts were used: water, 50% ethanol, 95% ethanol, and ethyl acetate. The antioxidative activity of the extract was determined using the 2,2-diphenyl-1-picrylhydrazyl free-radical scavenging test at doses of 1, 10, 50, and 100 g/ml. Two extracts (water and 50% ethanol) were chosen for their protective activity in NG108-15 neuroblastoma cells against H_2O_2 induced oxidative stress and cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay based on their free radical scavenging activity. All of the extracts were shown to have antioxidative properties. The free-radical

scavenging activity of the water and 50% ethanol extracts was high, with IC50 values of 34.98, 82.24 and 30.76, 81.66 g/ml, respectively. On NG108-15 cells, both water and 50% ethanol extracts showed neuroprotective action. For both the water and 50% ethanol extracts, the greatest activity was reported at a concentration of 50 g/ml. Except at a high concentration of 100 g/ml, none of the extracts were hazardous to the cells in a cytotoxicity test. The findings suggested that water and 50% ethanol extracts from the GM fruit hull could be effective neuroprotectants. [20]

7. **José Pedraza-Chaverri (2008)** did a review of mangosteen's phytochemistry and pharmacology. Mangosteen has long been used to treat skin infections and wounds due to its anti-inflammatory properties. Dysentery, various urinary problems, cystitis, and gonorrhoea are among the other ailments for which it is used. The evolution of this botanical medication into a widely utilised nutraceutical is highlighted in this review. Mangostana is currently readily available all over the world. As a result, research into mangosteen's phytochemical ingredients and biological activity has increased at the same time. Individual xanthones as well as extracts of G. mangostana were evaluated in depth for their biological activity. [21]
8. **Stefan Renvert (2008)** did a review of the literature to assess non-surgical therapy options for peri-implant mucositis and peri-implantitis. Mechanical non-surgical therapy has been shown to be successful in the treatment of peri-implant mucositis lesions. Furthermore, using antibacterial mouth rinses in addition to mechanical therapy improved the outcome of such mucositis lesions. Nonsurgical treatment for peri-implantitis lesions was found to be ineffective. Clinical and microbiological characteristics were only little influenced by the use of

chlorhexidine. Adjunctive local or systemic antibiotics, on the other hand, have been demonstrated to minimize bleeding during probing and probing depths.[22]

9. **Lindhe J Meyle (2008)** conducted a study to determine the causes of peri-implant mucositis, peri-implantitis and their management. Poor oral hygiene, dental cement, a history of periodontitis, and cigarette smoking are all risk factors. Mechanical debridement is required in the treatment of peri-implant mucositis in order to eliminate the plaque. In the treatment of peri-implant mucositis lesions, mechanical non-surgical treatment is beneficial. Peri-implantitis treatment In the treatment of peri-implantitis, non-surgical mechanical cleaning of the implant surface with or without an adjuvant antibacterial treatment is ineffective.[23]
10. **Sarin Tadtong (2009)** studied the tyrosinase inhibition and antibacterial activity of mangosteen pericarp extract against pathogenic microorganisms in the oral cavity by using the agar dilution method, the researchers discovered that mangosteen pericarp extract had antibacterial activity against pathogenic bacteria in the oral cavity, including Streptococcus mutans (DMST18777), Porphyromonas gingivalis (DMST2136), and Streptococcus pyogenes (DMST17020) at MICs of 0.01 mg/ml, and Staphylococcus aureus (ATCC2). [24]
11. **Arasali Sulaiman Zarena (2009)** did a study to evaluate the antioxidant activities of garcinia mangostana pericarp extract produced using various solvents and polarity mixes. The fine powder (5g) was extracted with several solvents using a Soxtec equipment. Ethyl acetate (EtOAc), hexane (HX), acetone (Ace), and acetone: water (80:20; AW), methanol (MeOH), and ethanol were the solvent systems used (EtOH). The extractions took two hours, including a 30-minute first boil. Following that, the extract was filtered using Whatman No. 1 paper. In this

study it was found that Ethyl acetate and acetone are appropriate solvents for extracting antioxidant components from mangosteen.[25]

12. **Antonio Fernández-Barbero (2009)** studied the internal structure, refractive index, and mechanical properties of the polymer network in this study. They are known as super absorbent materials since they can absorb hundreds of times their own weight in solvent. They react quickly to local environmental changes, which is critical in the miniaturisation of devices and the development of microsensors. Microgels have found use as carriers of therapeutic medications and as diagnostic agents since size changes are followed with changes in interior dimensions.[26]
13. **Krisztina Ungvari (2010)** evaluated three cleaning solutions in a study. 3% H_2O_2 (5 min), saturated citric acid (pH 14 1) (1 min), or chlorhexidine gel were used to cure commercially pure (grade 4) machined Titanium discs (5 min). The dimethylthiazolyl-diphenyltetrazolium bromide (MTT) and bicinchoninic acid (BCA) protein content assays were used to study human epithelial cell attachment (24-h observation) and proliferation (72-h observation). The Titanium surface is not harmed by these chemicals. In contrast to chlorhexidine gel, cleaning with H_2O_2 modestly promotes human epithelial cell development.[27]
14. **Ravichandran Veerasamy (2011)** studied Garcinia mangostana leaf extract as a reducing agent to test a simple and environmentally friendly biosynthesis of silver nanoparticles. When aqueous silver ions were exposed to leaf extract, they were reduced and silver nanoparticles with an average size of 35 nm were formed. UV–Visible, Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy (TEM) methods were used to characterise the silver nanoparticles. These biologically generated nanoparticles were also proven to be highly effective against a variety of multi-drug resistant human diseases.[28]

15. **Irmgard Hauser-Gerspach (2011)** studied the gaseous ozone's antibacterial activity on bacteria adhering to various Titanium and Zirconia surfaces, as well as the attachment of osteoblast-like MG-63 cells to ozone-treated surfaces Streptococcus sanguinis (DSM20068) and Porphyromonas gingivalis (ATCC33277) adhered to Titanium and Zirconia discs as substrates. Gaseous ozone (140 ppm; 33 mL/s) was used to treat the test specimens for 6 and 24 seconds. Gaseous ozone was found to have selective efficiency in reducing adhering bacteria on Titanium and Zirconia without impacting osteoblastic cell adhesion and proliferation.[29]
16. **S. M. V. Palmeira (2012)** investigated antioxidant activity of mango peel extracts. The amounts of polyphenol, anthocyanin, and carotenoid in an acetone extract of peels were measured. Ripe mango peels had more anthocyanins and carotenoids than raw mango peels, but raw mango peels showed a high polyphenol content. The antioxidant activity of ripe and raw mango peels extracted in acetone was assessed utilising a variety of antioxidant methods, including reducing power activity, DPPH free radical scavenging activity, iron-induced lipid peroxidation in liver microsomes, and soybean lipoxygenase inhibition. The IC50 values were found to range between 1.39 and 5.24 g gallic acid equivalents. As a result, mango peel extract showed good antioxidant activity in a variety of systems and could be employed in nutraceuticals and functional foods.[30]
17. **Salah Sakka (2012)** conducted a study to determine the primary causes of early and late implant failure, as a thorough understanding of this unavoidable clinical fact is critical in the field of oral implantology. The most common causes of early implant failure were a lack of primary stability, surgical trauma, and infection.

Because the primary bone healing process is disrupted, early signs of infection may indicate a far more serious outcome than if the same complications occur later. The most common factors associated with late failure appear to be occlusal overload and peri-implantitis.[31]

18. **Binit Shrestha (2012)** investigated the antibacterial properties of grape seed on peri-implantitis microbiota in this study. The grape seed extract was tested using a disc diffusion test against peri-implantitis microflora found in craniofacial implants, including reference strains of Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Candida albicans (C. albicans), and clinical strains of S. aureus, Klebsiella pneumonia (K. pneumonia), and Candida parapsilosis (C. The study's findings revealed that grape seed possesses antibacterial properties that can be investigated further and developed for use in the treatment of infected skin abutment interfaces of craniofacial implants.[32]
19. **Binit Shrestha (2013)** The study determined mangosteen pericarp extract has antibacterial effects on the peri-implantitis microbiota prevalent around craniofacial implants. The disc diffusion test was used to compare the mangosteen pericarp extract to peri-implantitis microflora reference cultures of Staphylococcus aureus (ATCC6538), Candida albicans (ATCC10231), and clinical strains of Staphylococcus aureus and Candida parapsilosis. The modified agar dilution millipore method was used to determine the minimum inhibitory concentrations (MIC) and minimum cidal concentrations (MCC). The extract was then mixed with a 50:50 solution of polyethylene glycol and propylene glycol to make a paste, which was then evaluated for antibacterial activity. It was concluded that mangosteen extract inhibited S. aureus reference strain at MIC and MCC of 1.25 mg/mL and 2.5 mg/mL, respectively, and clinical strain at 2.5 mg/mL and 5

mg/mL. It had little or no activity against Candida albicans and Candida parapsilosis, on the other hand, The extract in combination with polyethylene glycol and propylene glycol inhibited S. aureus in a dose-dependent manner. Mangosteen extract has antibacterial properties against S. aureus, which can be investigated further and developed for application in the treatment of microorganism-induced infection of the skin-abutment interface of craniofacial implants.[33]

20. **Kruti S. Soni (2015)** conducted a study to test a nano-scopic platform for the administration of oral drugs. Swollen nano-sized networks made up of hydrophilic or amphiphilic polymer chains are known as nanogels. They are being developed as drug transport carriers. Low-molecular-mass medicines, bio-macromolecules, and proteins can all be easily incorporated into nanogels. Nanogels appear to have a bright future in medicine, according to recent research. Nanogels are nanoparticles that could be employed in controlled drug delivery applications to distribute active medicinal molecules. This review intends to provide a broad overview of oral nanogels, as well as contemporary fabrication methods and novel applications. [34]
21. **Hyo-Sook Ryu (2015)** study was designed to evaluate after CHX contact, the capacity of modified Titanium surfaces to emit chlorhexidine After preparing four Titanium surfaces, each sample was treated for two hours with either whole saliva or phosphate-buffered saline (PBS). A disc diffusion test utilising Streptococcus gordonii was used to investigate the antibacterial activity of CHX-adsorbed discs. This research demonstrates that Titanium surface changes have a major impact on CHX release, and that SLA and RBM may provide effective CHX absorption capacity in the saliva-filled oral cavity. [35]

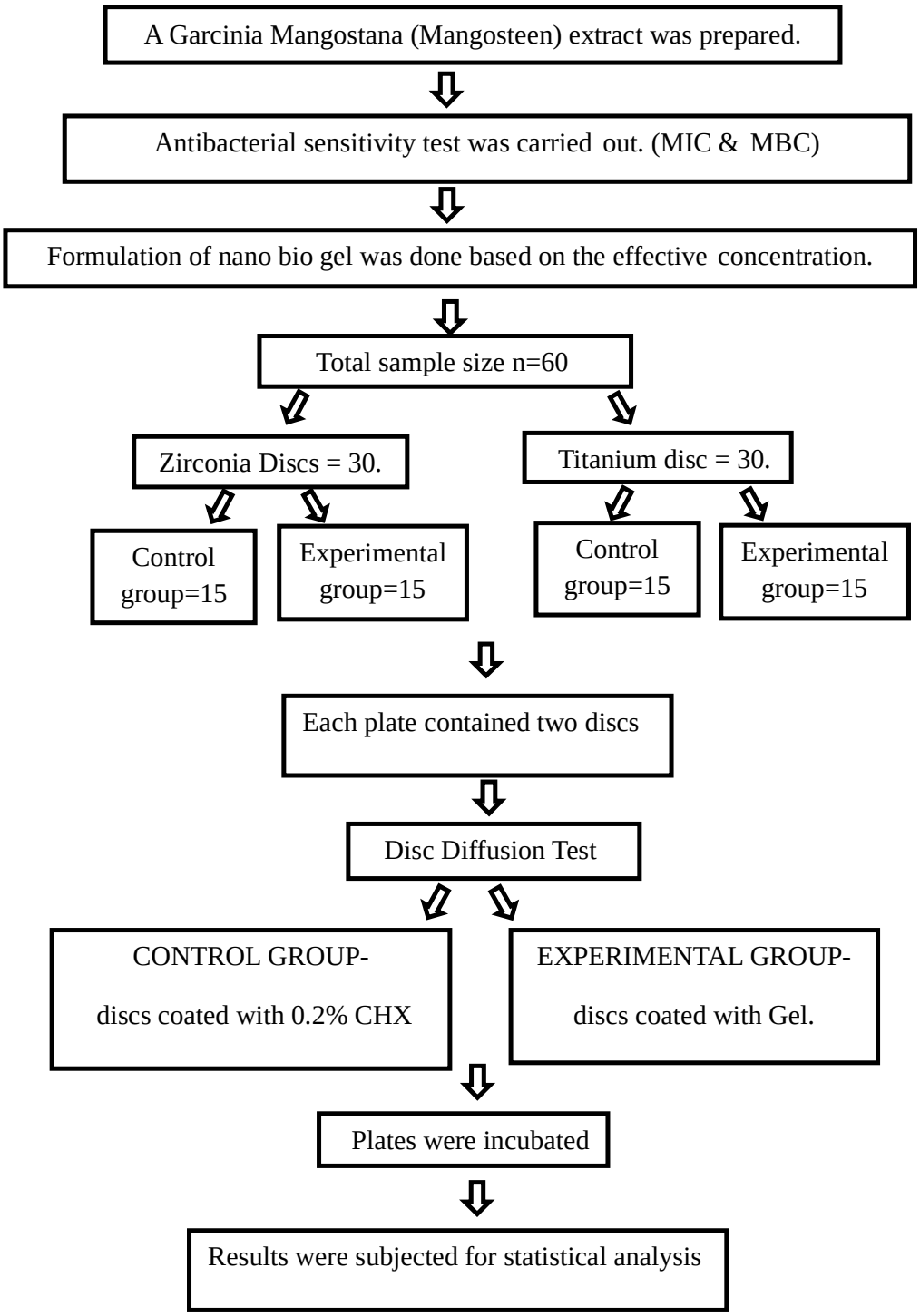
22. **Paula Juliana Pérez-Chaparro (2016)** evaluated the current weight of evidence of the microbiological profile associated with Peri-implantitis in this systematic review. A total of 799 titles were found, with 11 studies being included in this analysis. A predetermined form was used to extract all of the data. According to the findings of this systematic review, "Moderate Evidence" supports the association of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia with the aetiology of peri-implantitis, and "Some Evidence" supports the association of Prevotella intermedia and Campylobacter rectus with the aetiology of peri-implantitis. [36]
23. **Farzane pakdel (2017)** evaluated and compared the antibacterial properties of extracts of Allium sativum and Ziziphora clinopodioides essential oil on S. aureus and P. aeruginosa in this study. Aqueous and methanolic extracts of garlic and ziziphora essential oil were generated in this in vitro study done at Tabriz University of Medical Sciences between March and July 2017. The reference broth macro dilution method and disc diffusion technique were used in the test. Because the essential oil of ziziphora has a positive antibacterial impact on S. aureus, its extract can be utilised as an independent antimicrobial agent or in combination with other antibiotics to treat S. aureus infections.[37]
24. **Priscilla R. Varges (2019)** In this study a Continuous shear rheometry was used to study the effect of physical and chemical variables on the consistency of Carbopol940 and 941 gels. Small strain testing was used to determine the effect of polymer concentration, temperature, neutralisation, and different solvents and neutralizers on the fundamental properties of Carbopol gels. Gels were linear viscoelastic and elastic solids to a first approximation. Creep compliance

increased linearly with concentration and had no link to the type of solvent or neutralizer used.[38]

25. **Yanlong Yin (2020)** carried out a study to assess the specific and distinctive capabilities of nanogels as carrier systems for the delivery of a variety of cargo molecules in comparison to other nanomaterials. These are soft materials having crosslinked networks that can store tiny molecular medicines, biomacromolecules, and inorganic nanoparticles, allowing them to be used for therapy and imaging of a variety of illness conditions. Nanogels' stimuli-responsive behaviour can be easily controlled by selecting constituent polymer and crosslinker components to achieve a desired response at the site of action, giving them the ability to actively participate in the carrier system's intended function rather than being passive cargo carriers.[39]
26. **Woo-Ri Jung (2021)** studied the prevalence and abundance of 9 representative periodontal pathogens Aggregatibacteractinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Tannerellaforsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr), Peptostreptococcus anaerobius (Pa), and Eikenella corrodens (Ec) +in the saliva samples of periodontally healthy subjects (PH) and patients with periodontitis who underwent supportive periodontal therapy (SPT). The prevalence and abundance of periodontal pathogens in the PH group were compared with those in periodontally healthy young subjects (94 subjects; aged <35 years) DNA copy numbers of *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Tannerellaforsythia* (*Tf*), *Treponema denticola* (*Td*), *Prevotella intermedia (Pi), Fusobacterium nucleatum* (*Fn*), *Campylobacter rectus* (*Cr*), *Peptostreptococcus anaerobius (Pa*), and *Eikenella*

corrodens (*Ec*) were analyzed using real-time polymerase chain reaction. The detection frequencies of all pathogens, except *Aa*, were high in the PH and SPT groups. Compared with the PH group, the SPT group exhibited significantly lower total bacteria and *Fn* abundance and higher *Pg* abundance ($P<0.05$). The age-specific pathogen distribution analysis revealed a significantly low *Aa* abundance and high *Tf* and *Cr* abundance in the PH group. The clinical parameters and microbial profiles were similar between the SPT and PH groups. However, patients with periodontitis require supportive care to prevent recurrence. As the abundance of some bacteria varied with age, future studies must elucidate the correlation between age-related physiological changes and periodontal bacterial composition. [40]

METHODOLOGY



MATERIALS AND METHODS

SOURCE OF DATA:

This study was conducted in-

- This study was conducted in- KAHER's KLE VKIDS Department of Prosthodontics and Crown & Bridge
- KAHER's Dr. Prabhakar Kore's Basic science research center (BSRC)
- Department of Microbiology, JNMC, Belagavi.
- Department of Pharmaceutics, KLE College of Pharmacy, Belagavi.
- Authentication of *Garcinia Mangostana (Mangosteen)* - Agharkar Research Institute, Pune.

METHOD OF COLLECTION OF DATA:

INCLUSION CRITERIA:

- Even disks with a diameter 5 mm and a width of 2 mm (ASTM F67).
- Optimal Surface Roughness of 0 - 5 μm was included in the study

EXCLUSION CRITERIA:

- Discs with surface irregularities

  Optimal Surface Roughness above 5 μm was excluded from the study.
- Discs with casting defect.

MATERIALS USED IN THE STUDY:

Material	Description	Manufacturer
Hydroalcoholic solvent (175ml/75ml)	Ethanol	Fisher Scientific
Mueller hinton agar	Culture media	Hi-media
Brucella blood agar	Culture media	Hi-media
Phosphate buffer solvent (6.8 ph)	Sodium chloride, sodium dihydrogen orthophosphate dihydrate, potassium chloride, distilled water.	Fisher Scientific
Carbopol gel base	Carbopol 940	OEM manufacturers
Distilled water	-	-
Garcinia Mangostana (Mangosteen) incorporated Nano Bio Gel	Pericarp of Garcinia Mangostana	Novel
Chlorhexidine Gel	Hexi gel 1%	ICPA Health products

ARMAMENTARIUM USED IN THE STUDY:

ARMAMENTARIUM	DESCRIPTION	MANUFACTURER
Incubator	Sr.no: ZBCI-08444	Remi elektrotechnik ltd India
Weighing machine	Unibloc weighing balance, ELB series	Shimadzu Balances
Profilometer	Surfcom Flex 50 A	Zeiss India
Water bath	Rectangular water bath	Labec
Anaerobic Jar	Noair	Tecno source

METHODOLOGY:

DETAILS OF THE PROCEDURES CONDUCTED DURING THE RESEARCH:

MATERIAL PREPARATION -

A total of 60 discs of commercially available pure Titanium grade 4 and Zirconia were fabricated of diameter 5 mm and a width of 2 mm (ASTM F67). The discs were subdivided into four groups as control and experimental.

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

SURFACE ANALYSIS -

The surface roughness was examined using Profilometer. The optimal surface roughness calculated from 0 - 5 μm was used in the study.

The Radiographs evaluation was done using Intraoral periapical (IOPA) X-ray of titanium and zirconia discs to assess the casting defect.

METHOD OF EXTRACTION –

The fruits are cleaned and peeled, and then the peels are dried and grinded into powder using an electric blender. The coarse powder is passed through a sieve to form a fine powder. The total weight of the powder was 50gms**.** The fine powder sample of 25gms was extracted in Hydroalcoholic solution (175ml ethanol + 75 ml of PBS at pH6.8) and another 25gms was extracted in Phosphate buffer solution (6.8 pH) for 3 days using a shaker. Then the extract was filtered through blotting paper and Whatman no.1 paper and the samples were stored at 4°C until use.

The filtered extract is placed in rotary evaporater at 50^{o} C until the reduction of extract. Afterwards the reduced extract was transferred into a china dish and placed in water bath at 50^{o} C temperature for 3 days. A total of 10 grams of hydroalcoholic extract and 10 grams of PBS extract was produced**.**

TO CHECK FOR THE ANTIBACTERIAL PROPERTY OF THE PBS AND HYDROALCOHOLIC EXTRACTS:

To assess antimicrobial properties of the Mangosteen extract and 1% Chlorhexidine gel an in vitro pilot study was carried out using standard strain of S. aureus (ATCC 25923) and P. gingivalis (ATCC 33277). These strains were maintained on nutrient agar slope and thioglycolate medium respectively at 4^{0} C. These strains were sub cultured onto agar plates at 37^{0} C for typical colony morphology and used for the study. Anti-microbial activity was tested as per CLSI guidelines (Clinical Laboratory Standard Institute Guidelines) by using Mueller Hinton Agar and Brucella Blood Agar on 90mm diameter petri dishes.

The inoculum was standardized by diluting in sterile saline to adjust the density of 0.5 Mc Farland opacity equivalent to 1×10^6 to 5×10^6 colony forming units (cfu/ml). The antimicrobial activity was assessed using sequential dilution method confirmed with disc diffusion method. This procedure was carried out for mangosteen extract and chlorhexidine gel.

The 100mg of extract was dissolved in 10ml of distilled water and further dilutions were made to achieve the final concentrations of 100mg, 10mg, 5mg, 2.5mg, 1.25mg, 0.675mg in BHI broth (Mueller Hinton Broth) for S. aureus and Thioglycolate Broth for P. gingivalis. The disc diffusion method was carried out using Mueller Hinton Agar and Brucella Blood Agar.

The MIC was considered as the lowest concentration resulting in negative subculture. The experiment was carried out in 8 replicates for each extract.

Minimum inhibitory and bactericidal concentrations and Zone of inhibition of extract against S. aureus and P. gingivalis was established.

The reported results revealed higher potential of the hydroalcoholic extract to prevent S. aureus and P. gingivalis proliferation compared to PBS extract. The values of MIC, MBC were recorded at 5 % for hydroalcoholic extract**.** The 5% hydroalcoholic extract was incorporated in the Nano bio gel.

FORMULATION OF NANO BIO GEL –

- The weighed quantity of Carbopol 940 (3gms) was distributed in 100ml of distilled water with a help of magnetic stirrer for 2 hrs. The soaked polymer solution was kept for 24hrs for complete hydration.
- Weighed quantity of extract 5gms (5%) was dispersed in the above polymer gel with constant stirring at high-speed propeller for half hour.
- To above mixture 5% Glycerin, 0.5% Sodium benzoate, and 0.01% Methyl paraben were added as preservatives.
- The ph was adjusted to 7.0 with Triethanolamine QS.
- Final product was stored in air tight container at Room temperature.

DISK-DIFFUSION TEST-

- The disk-diffusion test was performed to assess the effectiveness of 5% Mangosteen incorporated nano bio gel against *S.aureus* and *P.gingivalis* standard bacterial strains.

PREPARATION OF STANDARD INOCULUM -

- The standard strain of P. gingivalis (ATCC 33277) was inoculated on enriched brucella blood agar supplemented with haemin and vitamin K at 37°C for 72 - 96 hours.
- The standard microbial strain of S. aureus (ATCC 25923) inoculated on Mueller hinton agar at 37°C for 18 - 24 hours.

- Fresh isolates were transferred to sterile saline and a microbial suspension was prepared and the turbidity adjusted to 0.5 McFarland standard to yield approximately 10^6 CFU/mL. The inoculum was evenly spread on brucella blood agar and Mueller hinton agar.

METHOD OF INOCULATION – Lawn cell culture

- The agar plate was streaked with standard bacterial strains.
- After the inoculum dries, an 6mm diameter and 3mm depth well were punched with a sterile cork borer.
- The sterile pure Titanium grade 4 and Zirconia were fabricated of diameter 5 mm and a width of 2 mm (ASTM F67).
- Each plate contained two discs:

1. *Experimental group-* The disc was coated with Nano bio gel.

2. *Control group-* The disc was coated with 1% Chlorhexidine (CHX) gel.

- The plates were incubated at 37°C for -

  Aerobic – 18 to 24 hours for S. aureus

  Anerobic – 72 to 96 hours for P. gingivalis

SETTING UP THE ANAEROBIC JAR –

A catalyst and chemicals such as Sodium hydrogen carbonate, Citric acid and Sodium borohydrate were used to create anaerobiasis**.** The culture media was placed inside the jar, the air inside was pumped out and replaced with either unmixed Hydrogen or as a 10%CO2+90%H2 mixture. The catalyst acts and the oxygen was used up in forming water with the hydrogen. The manometer registers this as a fall in the internal pressure of the jar. Hydrogen is pumped in to fill up the jar so that the pressure inside equals atmospheric pressure. The jar was incubated at desired temperature settings**.**

ZONE OF INHIBITION –

There was absence of bacterial colonies around the antibacterial agent forming a zone of inhibition.

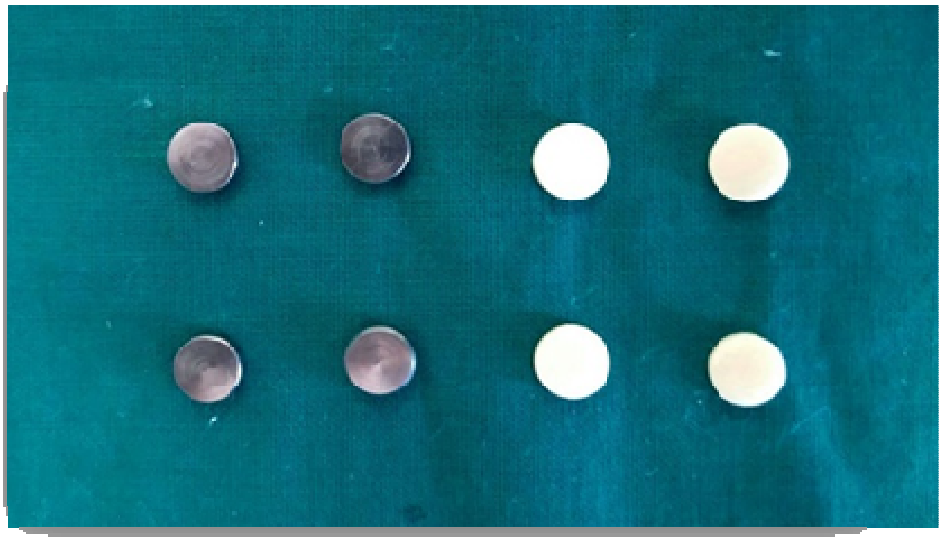


Fig 1: Zirconium and Titanium discs.

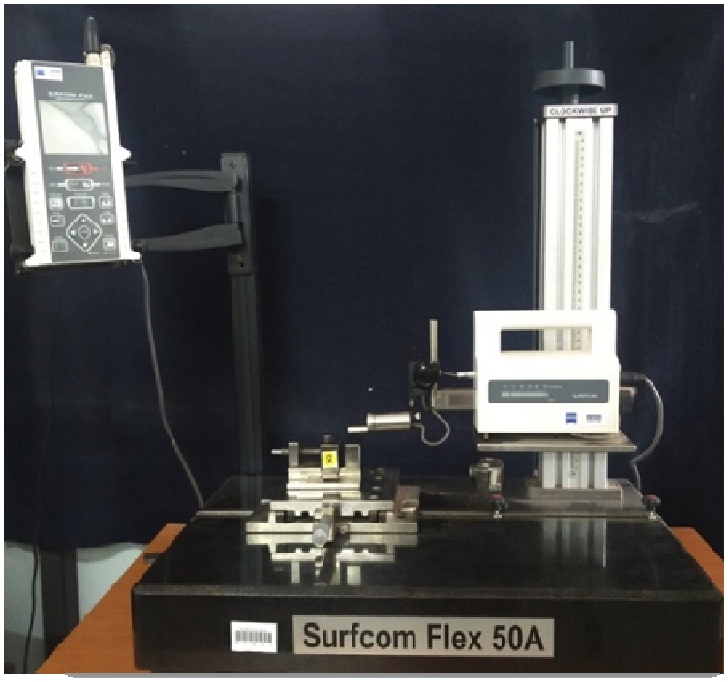


Fig 2: Profilometer.

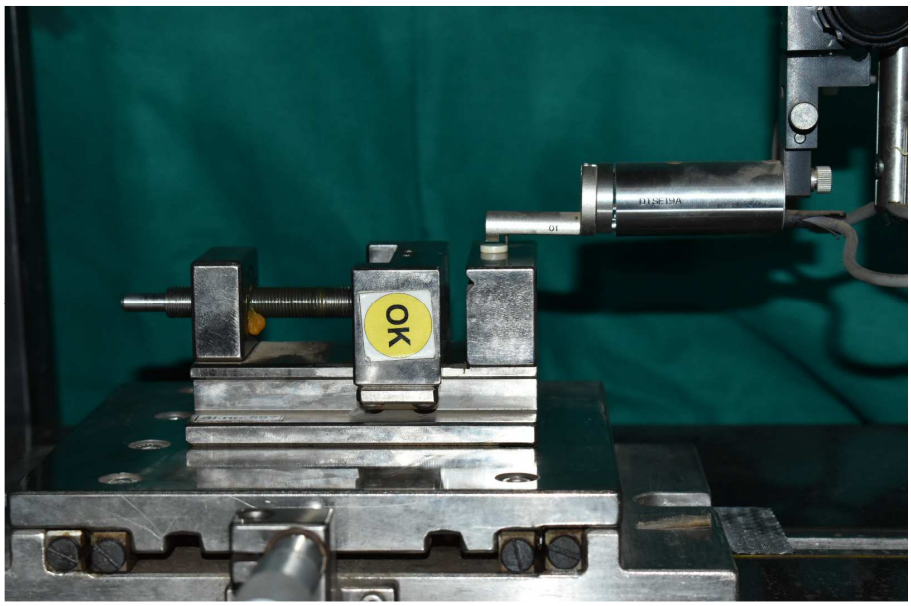


Fig 3: Testing the surface roughness of Zirconia disc.

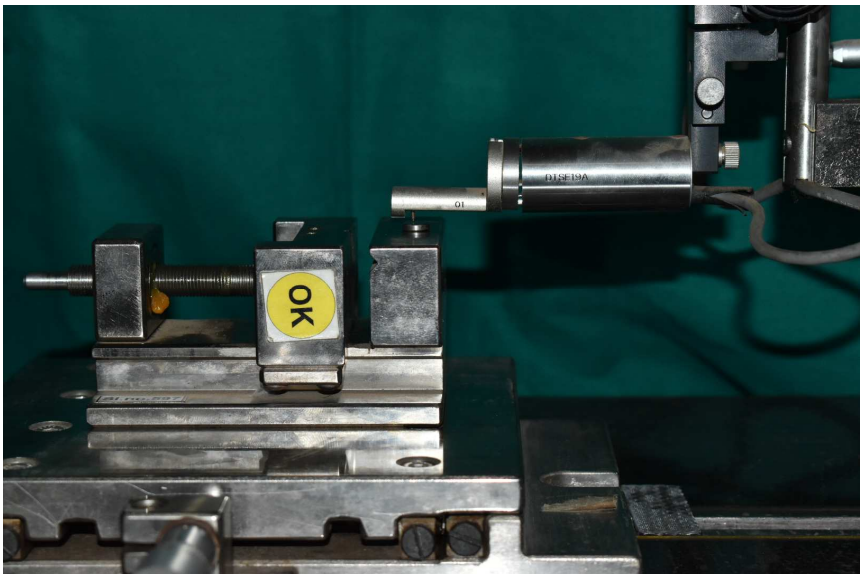


Fig 4: Testing the surface roughness of Titanium disc.

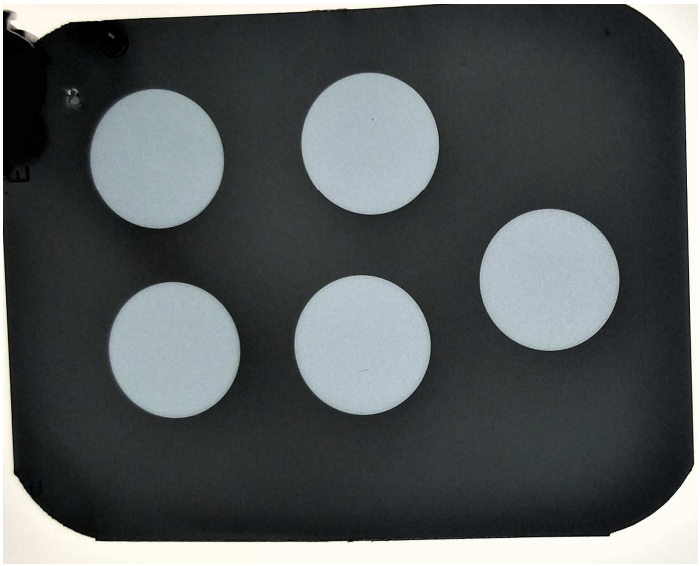


Fig 5: Evaluation of surface defects using IOPA.

Fig 6: G. Mangostana (Mangosteen) fruit powder.

Fig 7: Hydroalcoholic solution and PBS solution on mechanical shaker.

Fig 8: Optimisation of extract in evaporator to separate ethanol from herbal extract.

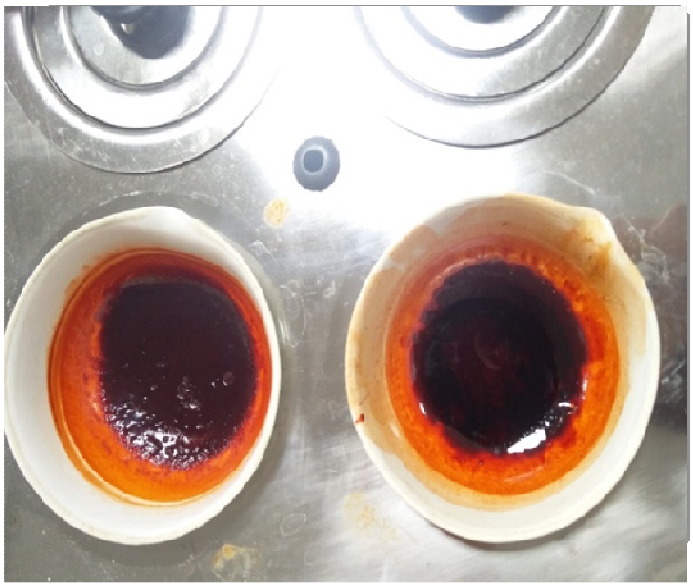


Fig 9: Herbal extract placed in controlled water bath in China dish to facilitate evaporation to obtain semi solid mixture.

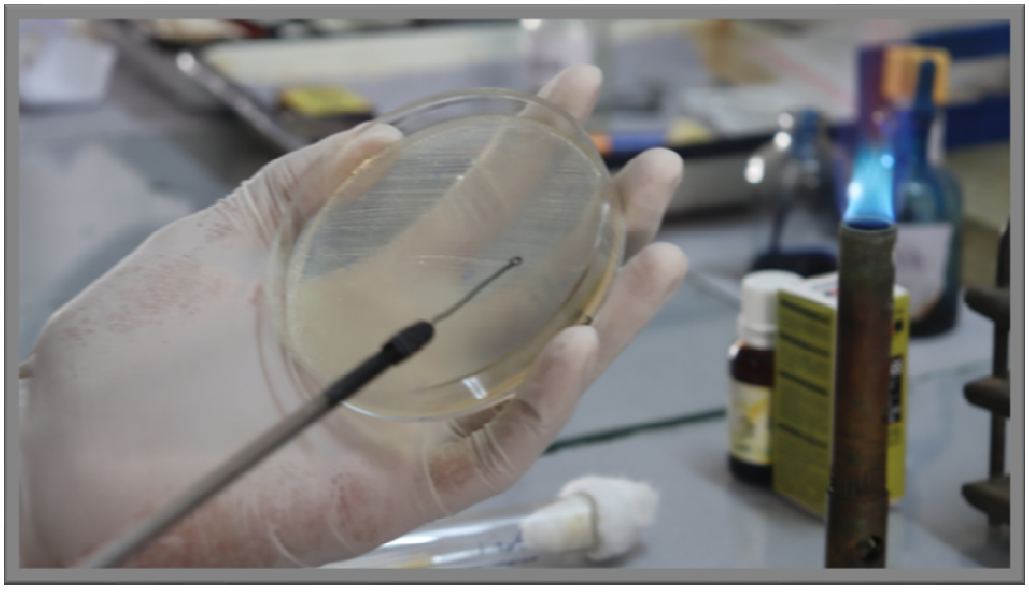


Fig 10: Inoculating loop used for streaking for inoculation.

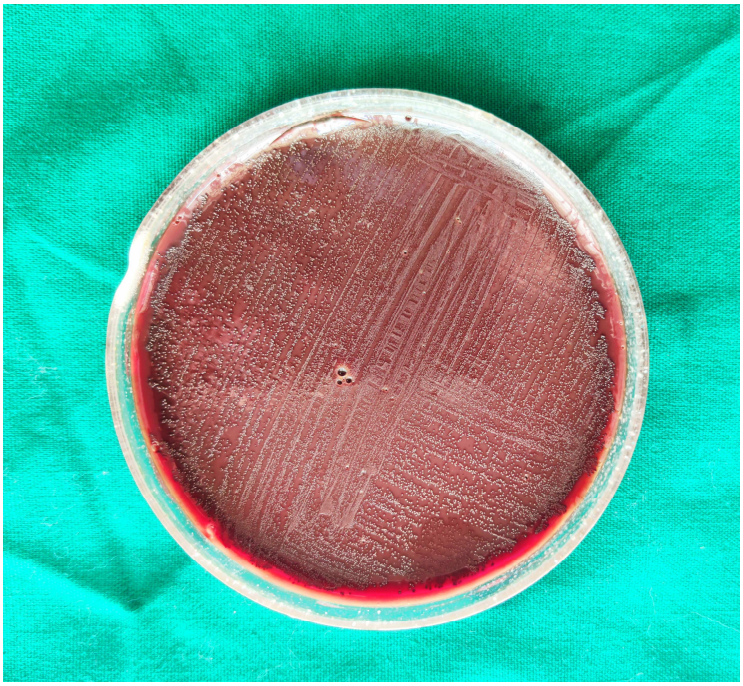


Fig 11: P. gingivalis strain on Brucella Blood Agar.

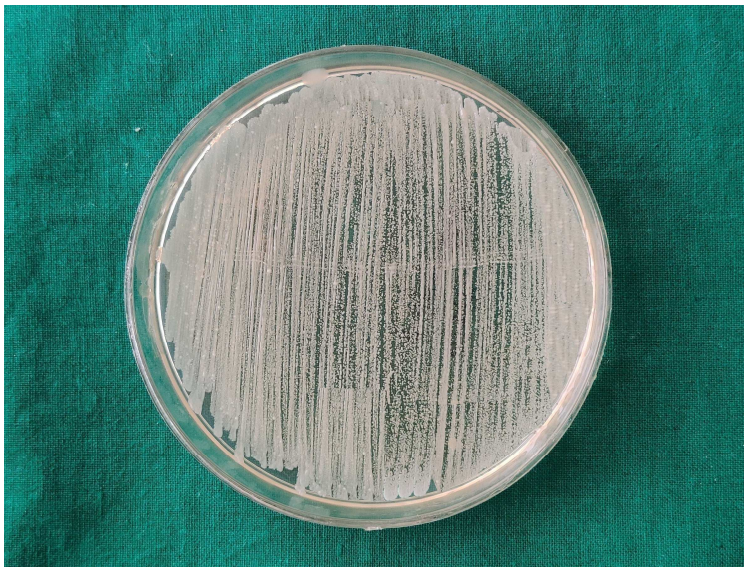


Fig 12: S. aureus strain on Mueller hinton agar.

Fig 13: Materials: Anaerobic jar, Sodium hydrogen carbonate, Citric acid, Sodium borohydrate.

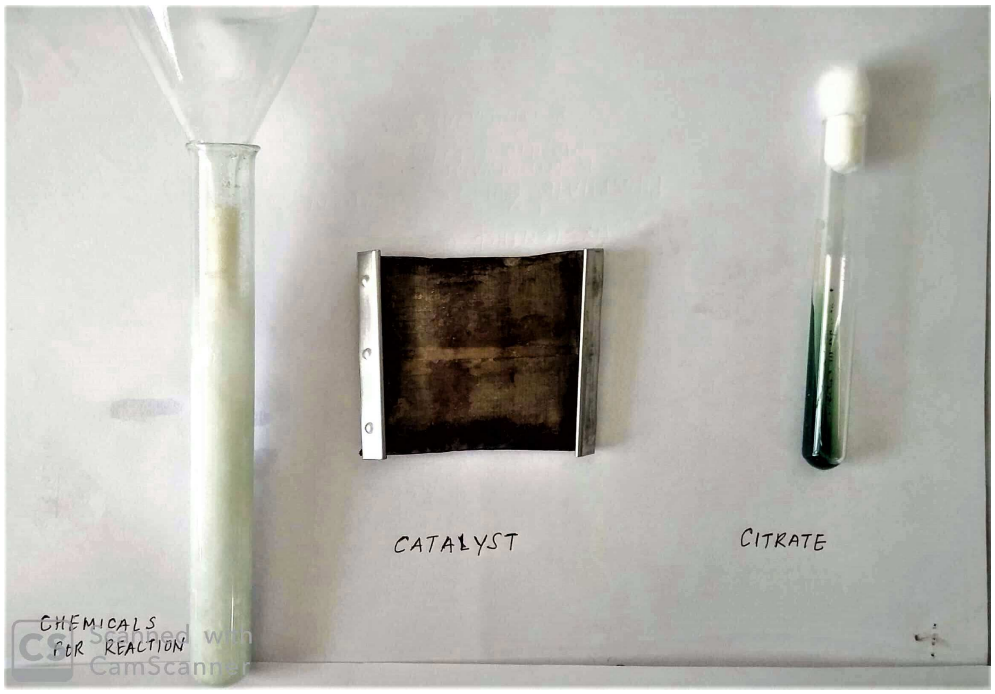


Fig 14: Setting up of anaerobic jar.

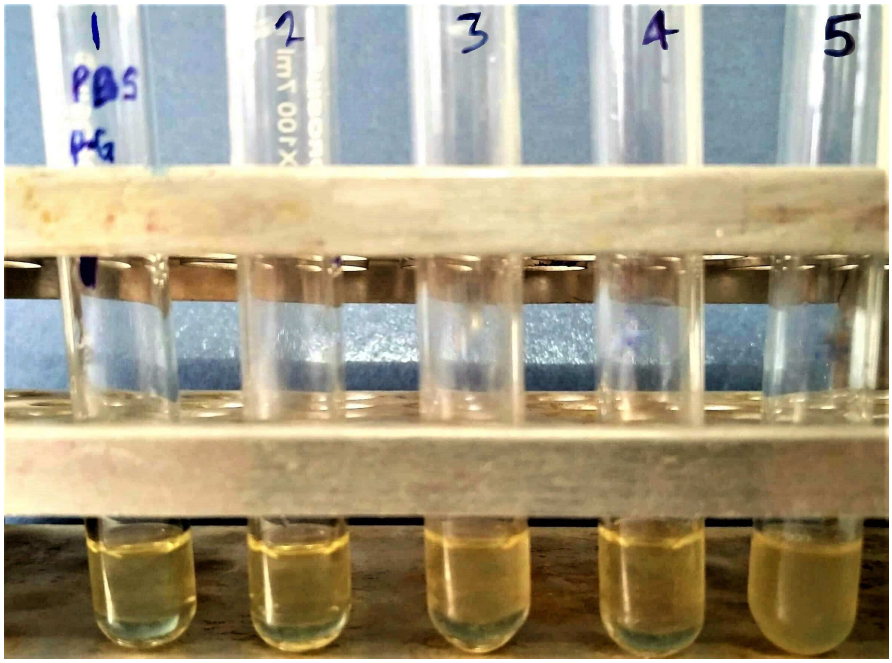


Fig 15 : Antibacterial activity of PBS Mangosteen extract against P.gingivalis assessed by sequential Microdilution.

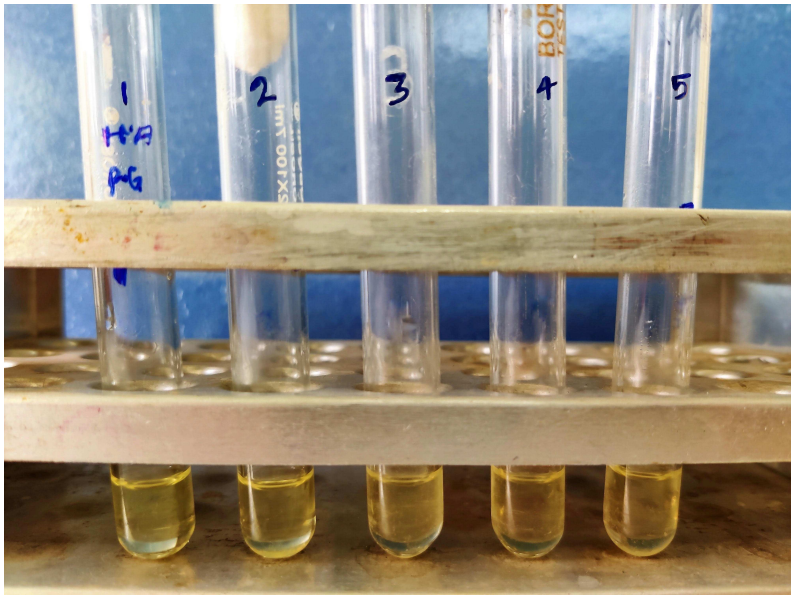


Fig 16 : Antibacterial activity of HA Mangosteen extract against P.gingivalis assessed by sequential Microdilution.

Fig 17: MIC of Mangosteen extract against S.aureus assessed by Disc diffusion method. (Zone 1 – 0.675mg, Zone 2 – 1.25mg , Zone 3 – 2.5mg, Zone 4 – 5mg, Zone 5 – 10mg).

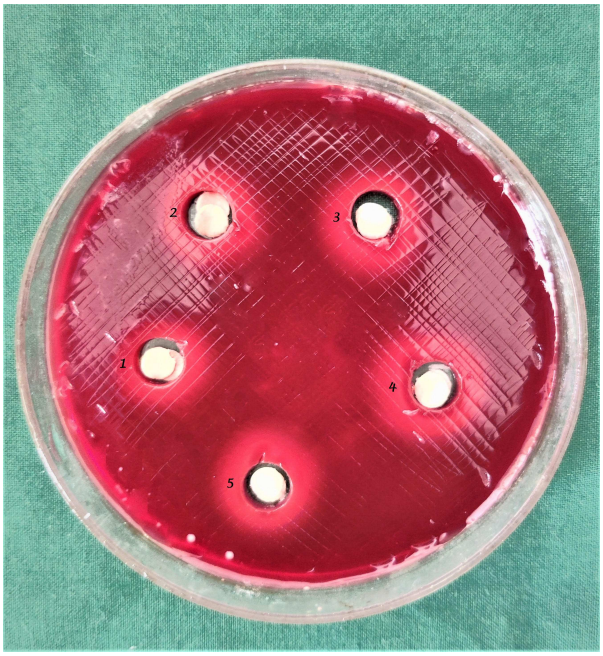


Fig 18: MIC of Mangosteen extract against P.gingivalis assessed by Disc diffusion method. (Zone 1 – 0.675mg , Zone 2 – 1.25mg , Zone 3 – 2.5mg, Zone 4 – 5mg, Zone 5 – 10mg).

FORMULATION NANO BIO GEL

Fig 19 : Materials: HA extract, Glycerine, Sodium benzoate, Carbopol.

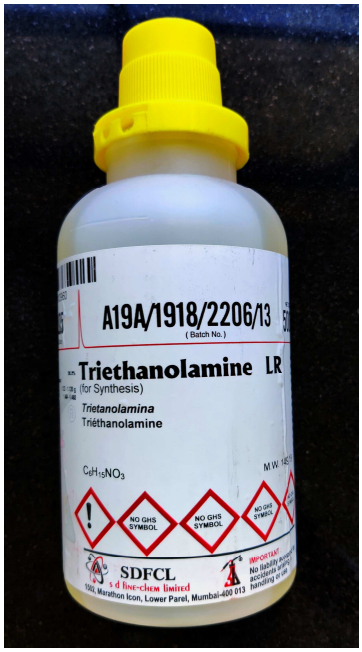


Fig 20 :Materials: Triethanolamine QS for PH adjustment.

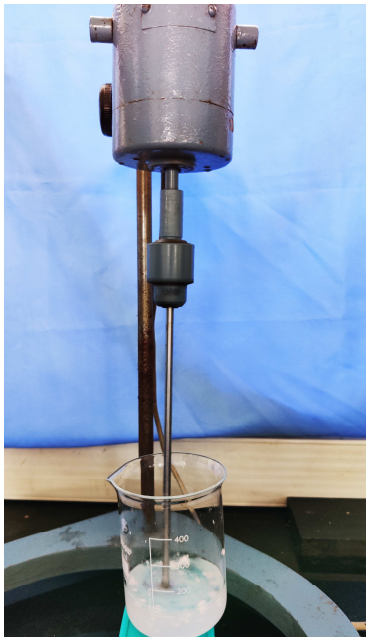


Fig 21: Magnetic stirrer – Carbopol with Distilled water.

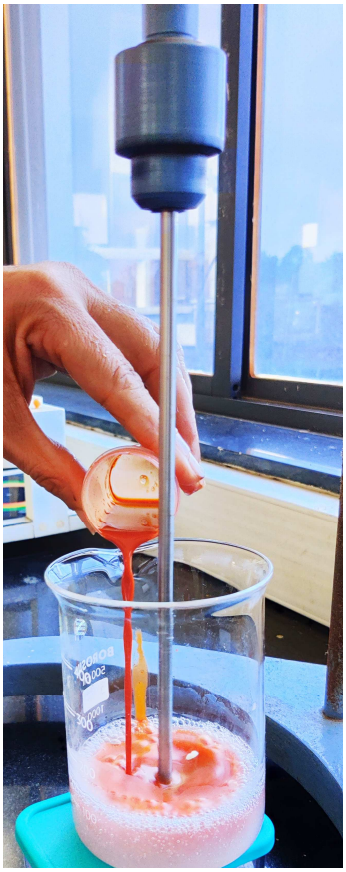


Fig 22: Magnetic stirrer –5% extract added.

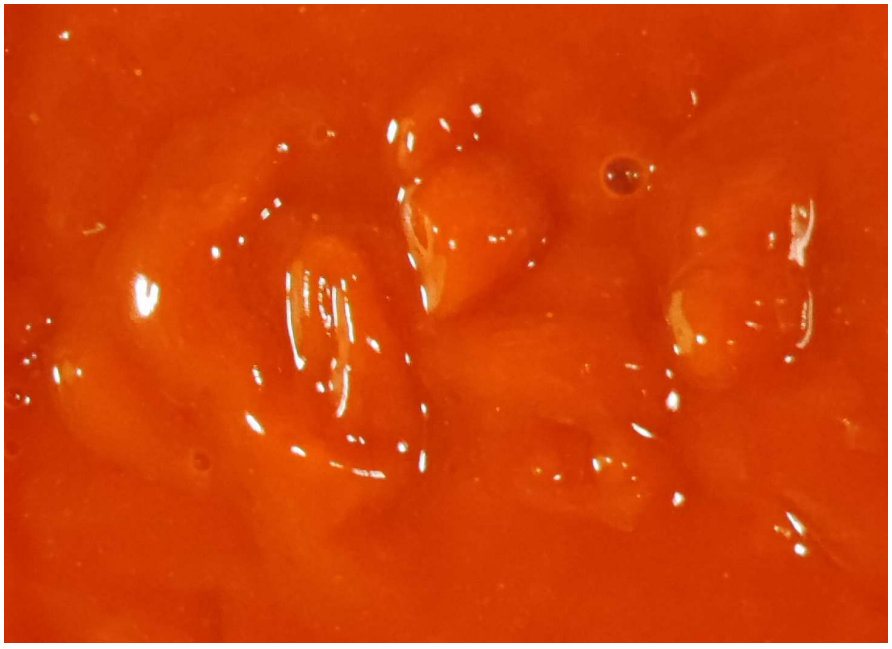


Fig 23: G. Mangostana incorporated Nano Bio Gel.

DISC-DIFFUSION METHOD

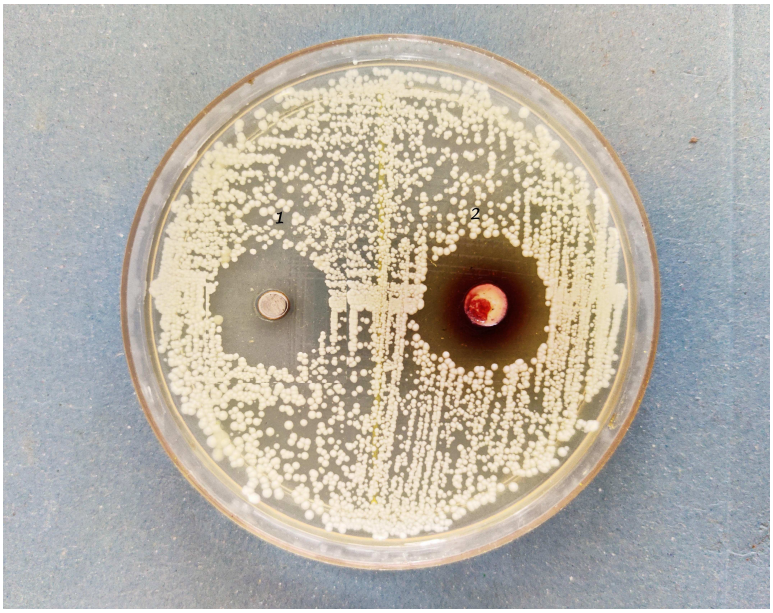


Fig 24: Two discs placed in the Mueller hinton agar plate, Zone one coated with 1% CHX gel and Zone two coated with 5% Nano bio gel.

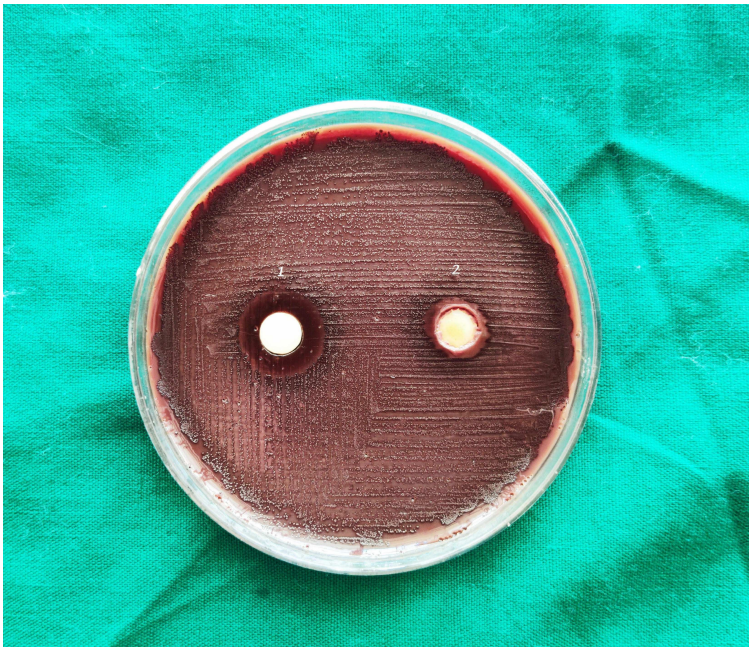


Fig 25: Two discs placed in the Brucella Blood Agar plate, Zone one coated with 5% Nano bio gel and Zone two coated with 1% CHX gel.

RESULTS

The values of microbial count which revealed the effect of the 5% Garcinia Mangostana (mangosteen) incorporated Nano bio gel and 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 Experimental group which was the Garcinia Mangostana (mangosteen) incorporated Nano bio gel and the Control group in which a commercially available Chlorhexidine gel.

Comparison between two gels (G. Mangostana nano bio gel and Chlorhexidine) against two microorganism (S.aureus and P. gingivalis) using two different implant materials (Titanium and zirconia) 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 tests:-

- Two way ANOVA
- TUKEY'S MULTIPLE POSTHOC.

DESCRIPTIVE STATISTICS

A. Summary of number of zones in S.aureus organism in two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia)

Gels and materials	n	Mean	SD	SE
Nano gel with Titanium	15	24.60	2.44	0.63
Nano gel with Zirconia	15	25.67	2.44	0.63
Chlorhexidine gel with Titanium	15	22.53	0.92	0.24
Chlorhexidine gel with Zirconia	15	18.80	0.68	0.17

B. Summary of number of zones in P. Gingivalis organism in two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia)

Gels and materials	n	Mean	SD	SE
Nano gel with Titanium	15	23.47	0.74	0.19
Nano gel with Zirconia	15	20.40	0.91	0.24
Chlorhexidine gel with Titanium	15	21.33	0.82	0.21
Chlorhexidine gel with Zirconia	15	17.07	0.88	0.23

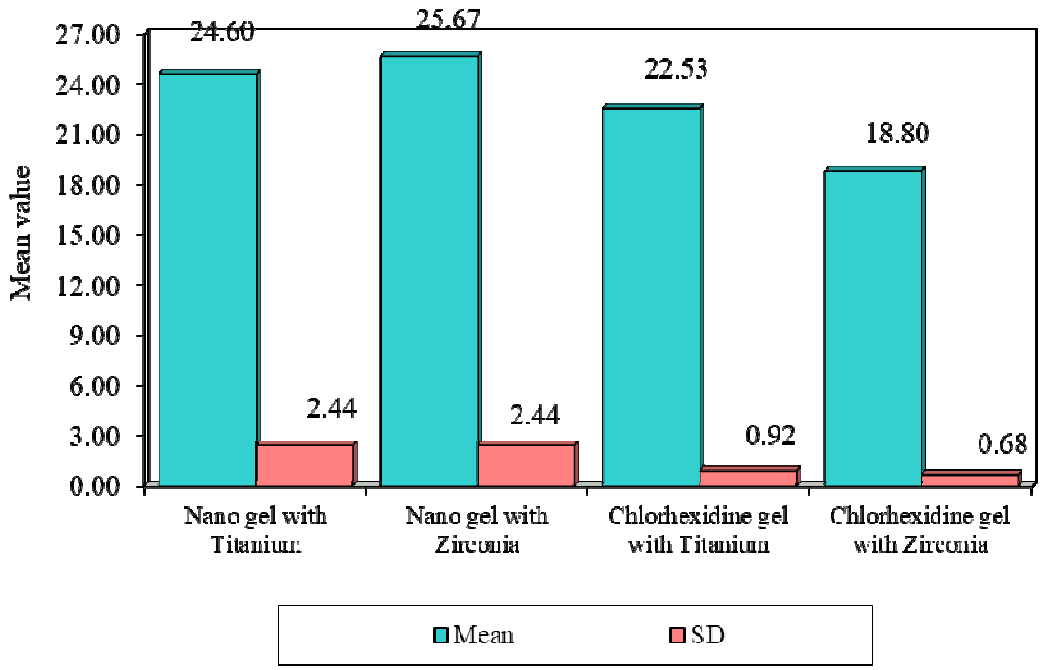
1. S.AUREUS

Comparison of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in S.aureus organism by two way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Main effects					
Gels	1	299.27	299.27	90.5562	0.0001,S
Materials	1	26.67	26.67	8.0692	0.0063,S
2-way intersection effects					
Gels x Materials	1	86.40	86.40	26.1441	0.0001,S
Error	56	185.07	3.30		
Total	59	597.40			

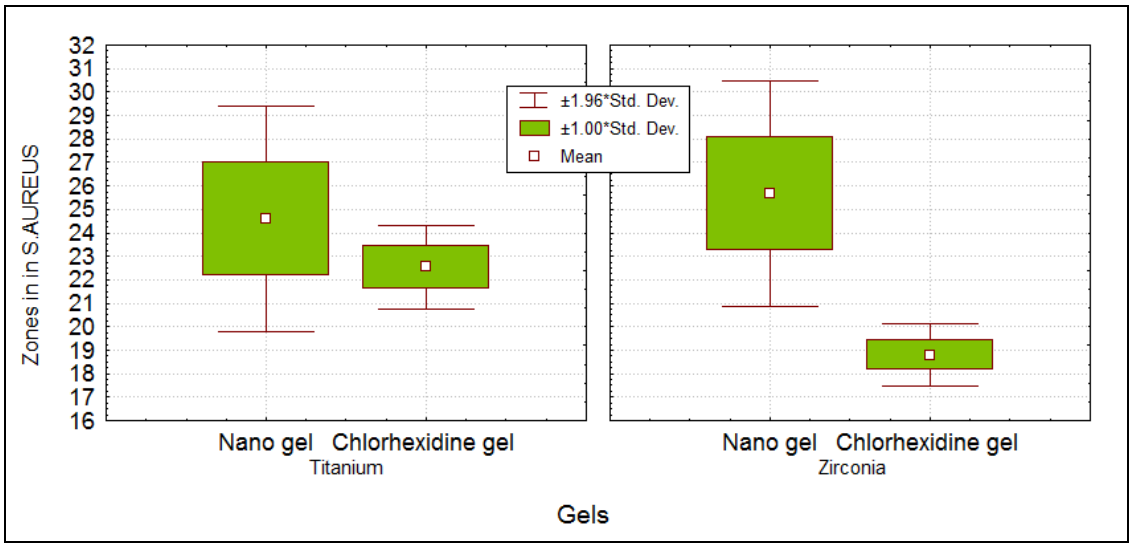
When intergroup comparison was done for the percentage for antibacterial activity against peri-implant pathogen using two-way ANOVA, statistically significant results were observed with p-value (<0.05). **(Graph 1)**

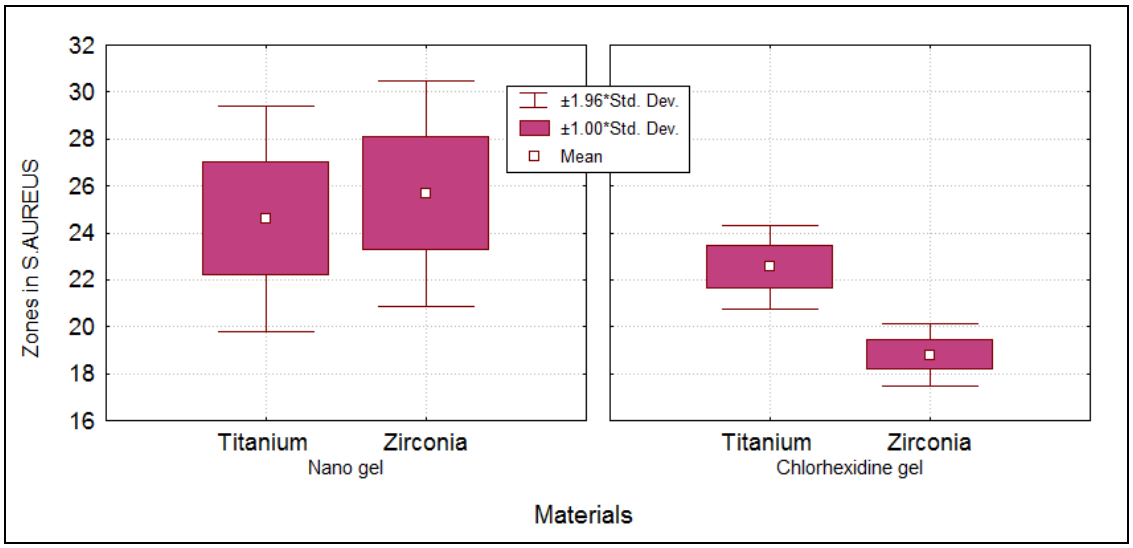
Graph 1: Comaprison of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in S.AUREUS organism



Pair wise comparison of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in S.aureus organism

Gels and materials	Nano gel with Titanium	Nano gel with Zirconia	Chlorhexidine gel with Titanium	Chlorhexidine gel with Zirconia
Mean	24.60	25.67	22.53	18.80
SD	2.44	2.44	0.92	0.68
Nano gel with Titanium	-			
Nano gel with Zirconia	P=0.3832, NS	-		
Chlorhexidine gel with Titanium	P=0.0151,S	P=0.0002,S	-	
Chlorhexidine gel with Zirconia	P=0.0002,S	P=0.0002,S	P=0.0002,S	-





Intergroup comparison by tukey's multiple posthoc procedures was done and statistically significant results were observed in few groups. There was statistically significant result for the groups TE (Titanium experimental group), group TC (Titanium control group) and Group ZC (zirconia control group). The comparison between the group TE and group ZE was not statistically significant as there was difference found between the activity. The experimental group had an higher activity. The titanium implant material had more antibacterial activity when compared to zirconia against S.aureus peri-implant pathogen. **(Graph 2 & 3)**

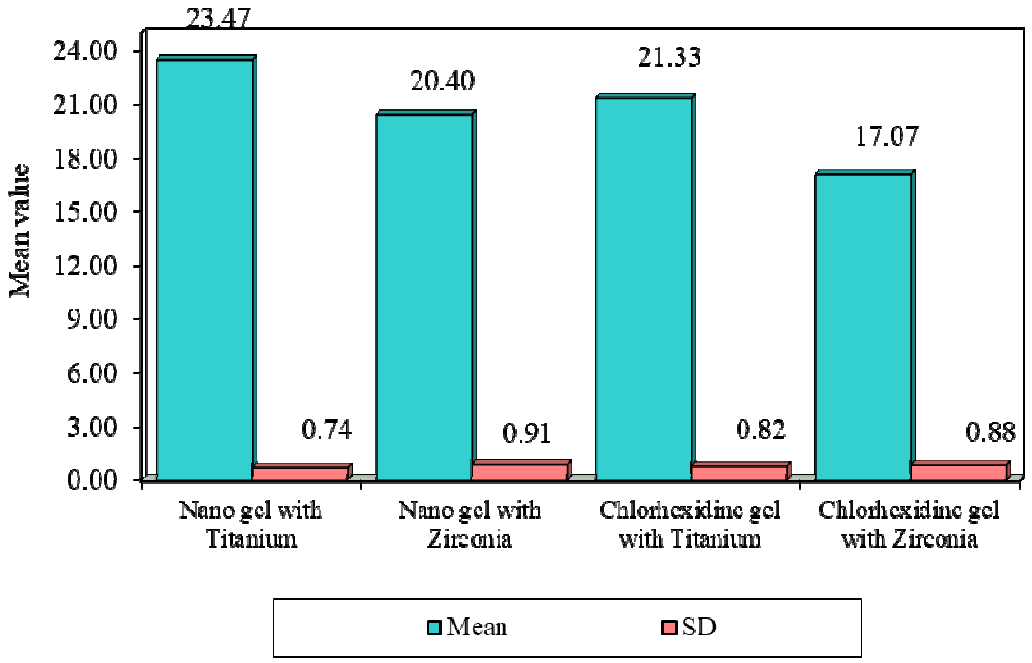
2. P. GINGIVALIS

Comaprison of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in P. Gingivalis organism by two way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Main effects					
Gels	1	112.07	112.07	158.4781	0.00001,S
Materials	1	201.67	201.67	285.1852	0.00001,S
2-way intersection effects					
Gels x Materials	1	5.40	5.40	7.6364	0.0077,S
Error	56	39.60	0.71		
Total	59	358.73			

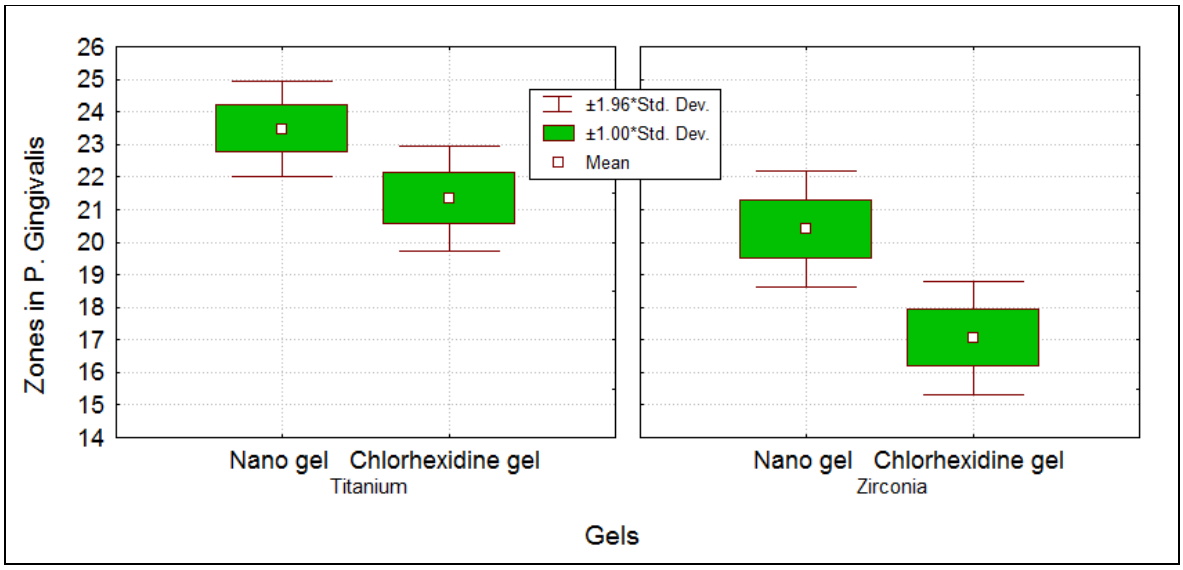
When intergroup comparison was done for the percentage for antibacterial activity against peri-implant pathogen using two-way ANOVA, statistically significant results were observed with p-value (<0.05). **(Graph 4)**

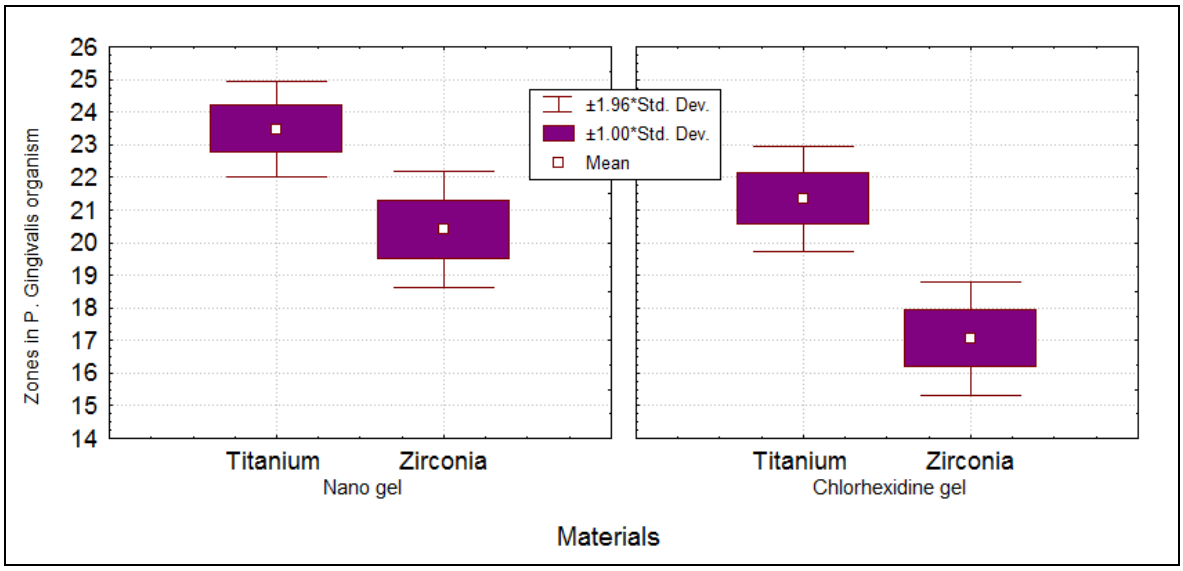
Graph 4: Comaprison of of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in P. Gingivalis organism



Comparison of two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia) with number of zones in P. Gingivalis organism

Gels and materials	Nano gel with Titanium	Nano gel with Zirconia	Chlorhexidine gel with Titanium	Chlorhexidine gel with Zirconia
Mean	23.47	20.40	21.33	17.07
SD	0.74	0.91	0.82	0.88
Nano gel with Titanium	-			
Nano gel with Zirconia	P=0.0002,S	-		
Chlorhexidine gel with Titanium	P=0.0002,S	P=0.0184,S	-	
Chlorhexidine gel with Zirconia	P=0.0002,S	P=0.0002,S	P=0.0002,S	-





Intergroup comparison by tukey's multiple posthoc procedures was done and statistically significant results were observed in few groups. There was statistically significant result for the groups TE (Titanium experimental group), group TC (Titanium control group), Group ZC (zirconia control group) and Group ZE (zirconia experimental group). The experimental group had an higher activity against P.Gingivalis peri-implant pathogen. **(Graph 5 & 6)**

When comparing the implant material, according to previous studies titanium has more affinity towards S.aureus as an early colonizer. This study showed that the G. Mangostana incorporated nano bio gel has greater antibacterial activity against early peri implant pathogen in titanium implant material. On the other hand when compared to the CHX control group (TC AND ZC), the experimental group (TE AND ZE) had a better activity against S.aureus and P.gingivalis peri implant pathogens.

DISCUSSION

Peri-implantitis is a multifactorial condition which is associated with inflammation of surrounding structure and loss of supporting bone around the implant. Failure of dental implants may occur by peri-implantitis and/or lack of osseointegration. Since bacterial infection is one of the main causes of peri-implantitis, both reduced bacterial colonization on and elimination of bacteria from the surface of dental implants are required to treat and even to prevent peri-implantitis. Two of these bacterial species are S. aureus and P. gingivalis. [40]

Although antibiotics are routinely used and are effective against these bacterial infection, due to the resistance of human pathogenic micro-organisms to these antibacterial agents and their side effects, use of alternate herbal medicines for the treatment of these conditions. In addition, herbal medicines are usually more compatible with the body, and usually have no side effects; therefore, in chronic diseases when such medications are used for a long time, they are non-toxic. In addition, these medications are economical and are readily available. [43]

Therefore, the goal was to assess antibacterial efficacy of herbal G. Mangostana incorporated nano bio gel against peri-implantitis pathogens to various Titanium and Zirconia discs. It could be demonstrated that the G. Mangostana incorporated nano bio gel disclosed a significant activity to control adherent bacteria on both materials without causing any surface damages. Adhesion of bacteria on Titanium implants has already been described both in vivo and in vitro. Dominant factors influencing bacterial adhesion are surface free energy and chemical composition, and particularly surface roughness of the material.

In several different test systems, rough surfaces promoted bacterial adhesion whereas smooth surfaces minimized it. **Irmgard Hauser-Gerspach et al** found a lower bacterial adhesion on Titanium-polished surface (Sa, 0.012 μm) in comparison to SLA surface (Sa, 1.554 μm) in the presence of absorbed proteins which is in line with the few previous reports. Comparative studies for Zirconia are scarce. **Hisbergues et al**. concluded that Zirconia might show a lower bacterial colonization but a need for further studies, particularly in the presence of absorbed proteins of a saliva serum pellicle.[27],[29]

Irmgard Hauser-Gerspach et al provides data for Zirconia. It confirms that the lower surface roughness of the zirconia material, showed lower bacterial adhesion when compared to Titanium. The results demonstrate that Zirconia may be a suitable material for implant abutments with a lower colonization potential. The fact that S. sanguinis adhered to a higher extent to both materials than P. gingivalis could be related to its nature as a first colonizer. Staphylococcus aureus has been identified in peri-implantitis lesions by **Leonhardt et al and Renvert et al**. According to **Harris & Richards et al** this is interesting in the sense that foreign bodies are often colonized by S. aureus and it has been reported that Titanium favors colonization by S. aureus.[29]

Relatively, in our study the customized nano bio gel showed a drastic reduction of adhered early colonizer S.aureus on Titanium Disc (implant surface). The results of the formulated nano bio gel was superior to CHX gel.

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. As reported in several studies, CHX has many side effects and cannot be safely used for more than 3 weeks.[35]

The main aspiration of the present study was to develop a safe non-surgical adjunctive topical treatment option without side effects to improvise the treatment standards for the patient. Several studies investigated the phytochemical activity of the Garcinia Mangostana pericarp and have reported no long term side effects.

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 research on different parts of plants with medicinal uses in order to discover new drugs with herbal origins.[37]

In a study by **Binit Shrestha et al** the antimicrobial effects of mangosteen pericarp extract on craniofacial implants were assessed against peri-implantitis microflora strains of Staphylococcus aureus, Candida albicans and Candida parapsilosis by disk diffusion test. Mangosteen extract had potential antimicrobial effects against S. aureus, which can be further studied and developed, to be used in

the treatment of microorganism induced infection of skin-abutment interface of craniofacial implants. This study is one of the initial researches, which focuses on the potential use of G. mangostana as an alternative antimicrobial therapy to reduce peri-implantitis arising from microbial infection around craniofacial implants, especially that arising from the proliferation S. aureus. The study concluded that it was active against S.arues which is in accordance with our study results.[33]

In this study **Ruchadaporn et al** examined the activity of alpha-mangostin against Candida albicans, the most important microorganism implicated in oral candidiasis. Its activity was compared to Clotrimazole and Nystatin. Results showed that alpha-mangostin was effective against C. albicans, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were 1,000 and 2,000 microg/ml, respectively. The C. albicans killing activity of alpha-mangostin was more effective than Clotrimazole and Nystatin. The cytotoxicity of alpha-mangostin was determined and it was found that alpha-mangostin at 4,000 microg/ml was not toxic to human gingival fibroblast for 480 min. The strong antifungal activity and low toxicity of alpha-mangostin make it a promising agent for treatment of oral candidiasis.[51]

Hence, in the present study the novel formulation of G. Manogstana (mangosteen) incorporated nano bio gel showed significant antibacterial activity at 5% against aerobic S.aureus as well as anaerobic P.Gingivalis when compared to CHX gel (control).

This prompts further probing and research into the various theraputic and pharmacologic applications of Garcinia mangostana (Mangosteen). In one such attempt the bioactive ingredients from the pericarp of garcinia mangostana were

incorporated into carbopol based nanobiogel. This in vitro study gave a statistically significant result against nidus formation of S. aureus and p gingivalis strains. During the study, no apparent undesirable effects were appreciated against the integrity of Titanium and Zirconia implant materials, also no changes were observed following usage of the Nano bio gel.

Hence, Garcinia Mangostana (mangosteen) incorporated Nano bio gel is a lucrative, promising and reliable 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 as it is indicative of the therapeutic aid of Garcinia mangostana (Mangosteen) in Peri-implantitis management.

SCOPE OF THE STUDY

In accordance with the results of the present study, a significant reduction of microbial colony forming unit was observed after the application of the novel G. mangostana (mangosteen) incorporated Nano bio gel against the selected strains. It is necessary to establish the effects of this herbal Mangosteen incorporated nano bio gel on different strains of microorganism in the oral cavity.

Further research could be carried out to include the antimicrobial assessment of extended time period since microbial growth could vary with different duration of time. Extended long term effects of the Novel Gracinia Mangostana incorporated Nano bio gel could be investigated.

This present study could also act as a basis to further research the antimicrobial efficacy of various permutation and combination of phytopharmaceutical extracts from different parts of the plants as well as pharmaceuticals composition and optimization of desired antimicrobial properties over a period of time

Further studies could also asses the effect of long term use of the herbal preparation, evaluate the osteogenic property of the nano bio gel and as the research was performed in vitro, additional in vivo parameters with varying circumstances and long-term monitoring can be included.

LIMITATIONS OF THE STUDY

There is scope for further research involving a wider area of interest as follows:

1. As peri-implantitis is caused by multiple complex of microorganism, the effect of the G. mangostana gel on varying microbial strains was not carried out.
2. It is only possible to carry out quantitative analyses, qualitative analyses should also be done and must be correlated with quantitative data.
3. A further topographic assessement can be evaluated for different implant materials.

CLINICAL IMPLICATIONS

In this present study the use of herbal phytopharmaceutical nano bio gel against peri-implantitis pathogens showed reduced microbial count. Hence the nano bio gel containing Garcinia Mangostana (Mangosteen) pericarp extract could be recommended as non-surgical aid in early peri-implantitis.

The use of the herbal Nano bio gel containing Garcinia Mangostana (Mangosteen) can be preferred over commercially available gel as it was found to be more effective than commercially available gel, as it overcomes the anticipated ill effects of long term usage of chemical gels such as chlorhexidine (CHX).

The clinical use of this herbal Nano bio gel as an adjunctive therapy has a great future prospective even as an preventive measure for biofilm formation.

This gel could revolutionize herbal use without reported side effects as an standard and effective non-surgical adjunctive treatment modality.

CONCLUSION

This study concluded the use of herbal Garcinia Mangostana (mangosteen) Nano bio gel significantly reduced the complex strain P.ginigvalis and S.aureus which leads to early peri-implant pathology in comparsion with commercially available topical CHX gel. As established, S.aureus is an early colonizer in peri implantitis and has an affinity towards Titianium implant material, the present study showed significant reduction in the bacterial count in titanium implant material. The formulated herbal Nano bio gel reported no long term side effects when compared to commercially available CHX. This opens the door to further investigations. Even more widely, as a standard clinical treatment modality.

SUMMARY

The present study was conducted with the aim of evaluating and comparing the effect of the two gel namely Garcinia Mangostana (Mangosteen) incorporated Nano bio gel and commercially available Chlorhexidine (CHX) gel.

A total of 60 discs of commercially available pure Titanium grade 4 and Zirconia were fabricated of diameter 5 mm and a width of 2 mm (ASTM F67). The discs were subdivided into four groups as control and experimental. The Garcinina mangostana fruit was authenticated and the extract was formulated using the pericarp of the fruit. The extract was prepared in hydroalcoholic and phosphate buffer solution. The prepared extract was subjected to antibacterial assay (MIC and MBC) using serum dilution and disk diffusion method. Once the MIC and MBC values were achieved the formulation of the Nano bio gel was initiated. The gel formulation was carbopol based and 5 % of the hydroalcoholic extract was used along with other ingredients. Once the final Nano bio gel was prepared, the Titanium and Zirconia discs were subjected to antibacterial testing in vitro using disk diffusion method. The disk-diffusion agar method tested the effectiveness of Mangosteen incorporated Nano bio gel against *S.aureus* and *P.gingivalis.* An agar plate was first streaked with bacteria, After the inoculum dried, a 6 mm diameter and 3mm depth well was punched with a sterile cork borer.

The sterile Titanium and Zirconia discs were used.

Each plate contained two discs:

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

The plates were then incubated for 24 hours (aerobic) 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 Garcinia Mangostana (mangosteen) incorporated Nano bio gel and commercially available chlorhexidine gel were subjected to statistical analysis to draw a conclusion from experimental data.

The novel G. Mangostana incorporated Nano bio gel had a better antibacterial activity when compared to the commercially available CHX (control group).

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ANNEXURE – I- ETHICAL CLEARANCE LETTER

Research and Ethics Committee
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CERTIFICATE

This is to Certify that the synopsis titled

EVALUATE THE EFFICACY OF GARCINIA MANGOSTANA [MANGOSTEEN] INCORPORATED NANO BIO GEL AGAINST PERI - IMPLANTITIS PATHOGENS - AN IN- VITRO STUDY *Submitted by*

Dr. AAYUSHI SHAH *P. G. Student / Staff, Guided by* DR. PRASHANT KARNI *from Department of* PROSTHODONTICS & CROWN & BRIDGE *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

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

महाराष्ट्र असोसिएशन फॉर द कल्टिव्हेशन ऑफ सायन्स
आघारकर अनुसंधान संस्थान
(विज्ञान और प्रौद्योगिकी विभाग, भारत सरकार के अधिन स्वायत्त संस्थान)
गो. ग. आगरकर पथ, पुणे - ४११ ००४.

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दिनांक / Date: 21/08/19

प्रमाणीकरण पत्र / AUTHENTICATION CERTIFICATE

नाम / Name	: -	Dr. Aayushi Kantilal Shah
पता / Address	: -	KLE Vishwanath Katti Institute of Dental Sciences KLE University (Deemed), Belgaum- 590010 Karnataka, India
संदर्भ/ Reference	: -	None
नमूने का नाम / Name of the sample	: -	*Garcinia mangostana* Fruit
नमूने की मात्रा / Amount of sample	: -	Fresh plant material around 100 gm
प्राप्ति की तिथि / Date of the receipt	: -	02/08/2019

Report:

प्राप्त नमूने का टैक्सोनोमिक चरित्रों की मदद से सावधानीपूर्वक अध्ययन किया गया है। हम एतद्वारा प्रमाणित करते हैं कि दिया गया फल का नमूना *Garcinia mangostana* L. [Family: Clusiaceae] का है।

The sample has been critically studied with the help of taxonomic characters. We hereby authenticate that the given fruit sample belongs to *Garcinia mangostana* L. [Family: Clusiaceae].

रितेश कुमार चौधरी

डॉ. आर. के. चौधरी/ Dr. R. K. Choudhary
(वैज्ञानिक/ Scientist)

AUTH 19-171

Scientist
Plant Drug Authentication
Biodiversity and Palaeobiology Group

ANNEXURE – III –SURFACE ROUGHNESS VALUES (RA) USING

PROFILOMETER – 0 to 5 microns

GROUP TITANIUM	GROUP ZIRCONIA
0.253	0.319
0.249	0.149
0.229	0.299
0.228	0.246
0.258	0.358
0.273	0.152
0.323	0.233
0.252	0.309
0.341	0.208
0.172	0.189
0.145	0.256
0.266	0.321
0.111	0.356
0.278	0.234
0.212	0.244

Summary of number of zones in S.AUREUS organism in two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia).

Gels and materials	n	Mean	SD	SE
Nano gel with Titanium	15	24.60	2.44	0.63
Nano gel with Zirconia	15	25.67	2.44	0.63
Chlorhexidine gel with Titanium	15	22.53	0.92	0.24
Chlorhexidine gel with Zirconia	15	18.80	0.68	0.17

Summary of number of zones in P. Gingivalis organism in two gels (Nano and Chlorhexidine) and two materials (Titanium and Zirconia)

Gels and materials	n	Mean	SD	SE
Nano gel with Titanium	15	23.47	0.74	0.19
Nano gel with Zirconia	15	20.40	0.91	0.24
Chlorhexidine gel with Titanium	15	21.33	0.82	0.21
Chlorhexidine gel with Zirconia	15	17.07	0.88	0.23