

Comparative Evaluation of Osteogenic Differentiation Capability and Antimicrobial Effect of Cissus Quadrangularis and Laccifer Lacca Extract Coated Dental Implants against Non-Coated Dental Implants- An In Vitro Study

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Study protocol

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Title

Comparative Evaluation of Osteogenic Differentiation Capability and Antimicrobial Effect of Cissus Quadrangularis and Laccifer Lacca Extract Coated Dental Implants against Non-Coated Dental Implants- An In Vitro Study

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Abstract

Background: Over the past few decades, implantology has achieved numerous advancements. The fundamentals of integrated osteo-implantology have changed significantly, which is altering the therapeutic approaches that the field uses, achieving outstanding outcomes in oral rehabilitation and resolving functional, psychological, and aesthetic issues in cases of partial and complete edentulism. Alternative materials have been studied to enhance implants and bone interference, including bone-conductive and bone-inducing materials. By enhancing mineralization, bone matrix synthesis, and bone cell

adhesion, osseointegration can be enhanced through nanoscale alteration of implant surface coatings. The goal of the implant surface treatments is to create a surface that is biologically active, enhancing the osseointegration process between the implant and bone tissue. For accelerating bone fractures, two well-known ayurvedic formulations are frequently utilized Cissus quadrangularis and Laccifer lacca. Additionally, these formulations include analgesic and anti-inflammatory qualities that lessen the severity of disorders associated to bones and joints. The study focuses on assessing a modification of surface coated implants with herbal extract of Cissus quadrangularis and Laccifer lacca for osteogenic potential differentiation and antimicrobial properties.

Methods:

1. Titanium discs of diameter 10mm and thickness 2mm will be used.
 2. Hydroalcoholic extract of Cissus quadrangularis and aqueous extract of Laccifer lacca will be obtained. Prepared extract will be coated on the titanium discs
 3. Four groups will be prepared
 - Group A: Discs coated with Cissus quadrangularis
 - Group B: Disc coated with Laccifer lacca
 - Group C: Disc coated with combination of C. quadrangularis and L. lacca in 1:1 ratio w/w%.
 - Group D: Non coated discs
 4. Each group will be further divided to perform two tests i.e. Osteoblastic Proliferation test and antimicrobial test.
- **Discussion:** Studies have been carried out with Cissus quadrangularis and Laccifer lacca that studies suggest that they promote bone healing. Srinidhi et al in year 2020 carried out a study with C quadrangularis the study included spectrophotometric analysis to measure the activities of alkaline phosphatase and lactate dehydrogenase, as well as to ascertain their kinetics. Based on the findings, they came to the conclusion that extracts from C. quadrangularis and Lakshadi guggulu exhibit strong osteogenic activity and can be effectively utilized as surface modification materials in titanium implants and dental bone mending. But there is a lack of research that combines the properties of both C. quadrangularis and Laccifer lacca as a surface coating material and hence the study was needed to assess the same.
 - **Clinical Trial registration Number (CTRI): REF/2024/05/084317**

Keywords

- Dental Implants, Cissus quadrangularis, Laccifer lacca, Osteogenic differentiation*

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see <http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/>).

Title {1}	Comparative Evaluation of Osteogenic Differentiation Capability and Antimicrobial Effect of Cissus Quadrangularis and Laccifer Lacca Extract Coated Dental Implants against Non-Coated Dental Implants- An In Vitro Study.
Trial registration {2a and 2b}.	REF/2024/05/084317
Protocol version {3}	1
Funding {4}	No funding received.
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Name and contact information for the trial sponsor {5b}	Not Applicable
Role of sponsor {5c}	There are no sponsors for the study as of now.

Introduction

Background and rationale {6a}

Over the past few decades, implantology has achieved numerous advancements. The fundamentals of integrated osteo-implantology have changed significantly, which is altering the therapeutic approaches that the field uses, achieving outstanding outcomes in oral rehabilitation and resolving functional, psychological, and aesthetic issues in cases of partial and complete edentulism [1]. Alternative materials have been studied to enhance implants and bone interference, including bone-conductive and bone-inducing materials. By enhancing mineralization, bone matrix synthesis, and bone cell adhesion, osseointegration can be enhanced through nanoscale alteration of implant surface coatings [2]. The goal of the implant surface treatments is to create a surface that is biologically active, enhancing the osseointegration process between the implant and bone tissue osseointegration-enhancing capabilities [3].

With the aid of Indian herbs, Ayurveda, a holistic ancient medical system, treats human

diseases and promotes health throughout the Indian subcontinent. For accelerating bone fractures, two well-known ayurvedic formulations are frequently utilized: *Cissus quadrangularis* and *Laccifer lacca*. Additionally, these formulations include analgesic and anti-inflammatory qualities that lessen the severity of disorders associated to bones and joints [4]. This study is to modify dental implant with *Cissus quadrangularis* and *Laccifer lacca* as an alternate surface modification material.

Cissus quadrangularis, also referred to as Veldt Grape or Winged Treebine, is a vine that is extensively utilized for medicinal purposes in India. It is a member of the Vitaceae family. This plant, which grows in warmer climates in Malaysia, Sri Lanka, and India, is edible. Because of its capacity to fuse bones, the plant, also known as a "bone setter," is called *Asthishrinkala* in Sanskrit and *Hodjod* in Hindi. Ascorbic acid, carotene, calcium, anabolic steroids, and phytoestrogenic steroids have all been found in plant extracts [5].

Laccifer lacca another herbal substance that is secreted by the female lac bug is a reddish-brown resin. It is used to strengthen bones and promote the healing of bone fractures. It is thermoplastic, soluble in ethanol and mild alkalis, and has strong adhesion, high scratch hardness, and resilience in lac films [6].

In a review published in 2020, Drs. Keerthi GK, M.V. Saiteja, et al. noted that osteoporosis, one of the risk factors that impedes osseointegration following implant placement, can be appropriately managed with *Laccifer lacca*, a well-known ayurvedic medication that promotes calcium deposition and has effects on bone healing while taking into account implant factors and the importance of a multidisciplinary approach [7].

In 2009, Bhagath Kumar Potu and colleagues carried out research to ascertain the synergistic impact of osteogenic medium and *Cissus quadrangularis* on the processes of proliferation, differentiation, and calcification. The outcome demonstrates that *Cissus quadrangularis* petroleum ether extract also increased the marrow mesenchymal stem cells' rate of proliferation. Compared to control cells, cells cultured in osteogenic media containing *Cissus quadrangularis* showed increased rates of differentiation, calcification, and proliferation [8].

In 2021, Srinidhi Bhat et al carried out the study with *Cissus quadrangularis* and *Lakshadi Guggulu*. The study included spectrophotometric analysis to measure the activities of alkaline phosphatase and lactate dehydrogenase, as well as to ascertain their kinetics. Based on the findings, they came to the conclusion that extracts from *C. quadrangularis* and *Lakshadi guggulu* exhibit strong osteogenic activity and can be effectively utilized as surface modification materials in orthopedic titanium implants and dental bone mending [9].

In order to assess the osteogenic potential of *Cissus quadrangularis* (CQ) hydrogel in improving the osseointegration of titanium to the bone in an experimental rabbit model, Srinidhi

Bhat et al. carried out an in vivo study in 2022. According to the study's findings, C. quadrangularis hydrogel is a strong osteogenic substance that can speed up osseointegration and improve patient adherence to implant therapy [10].

Hence, the study evaluates the osteogenic potential differentiation and antibacterial capabilities of modified surface coated implants with non-coated implants.

Rationale

To ascertain the effectiveness of Laccifer lacca and Cissus quadrangularis in terms of their capacity for osteogenic differentiation capability which in turn promote bone healing and also in terms of antibacterial properties, also to ascertain a more economical and biocompatible surface modification material that will enhance biocompatibility, reducing the risk of adverse reactions or inflammation in surrounding tissues. Biocompatible coatings help minimize the body's immune response to the implant, promoting a more harmonious integration with the surrounding bone and soft tissues.

Objectives {7}

1. To evaluate the osteogenic differentiation capability of non-coated discs.
2. To evaluate the antimicrobial effect of non-coated discs.
3. To evaluate the osteogenic differentiation capability of Cissus quadrangularis and Laccifer lacca extract coated discs.
4. To evaluate the antimicrobial effect of Cissus quadrangularis and Laccifer lacca extract coated discs.
5. To comparatively evaluate the Osteogenic Differentiation capability and antimicrobial effect of Cissus quadrangularis and Laccifer lacca extract coated implants against non-coated discs.

Trial design {8}

The experimental study will be carried out in a solitary setting at the Sharad Pawar Dental College's prosthodontic department.

Methods: Participants, interventions and outcomes

Study setting {9}

The study will be performed at the Department of Microbiology DMIHER Sawangi Meghe, Wardha

Eligibility criteria {10}

Not applicable. As it is a preclinical study and no patients are involved in the trial.

Who will take informed consent? {26a}

Not Applicable. For this type of study, formal consent is not required as no patients are involved in the trial.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Not Applicable. As this is preclinical study no patients are involved and the specimen used will be artificial saliva and no other biologic specimens will be used.

Interventions**Explanation for the choice of comparators {6b}**

Cissus quadrangularis and Laccifer lacca extract coated titanium disks will be compared as against non-coated titanium disks.

Intervention description {11a}

The extract will be coated on the titanium discs by spray coating technique.

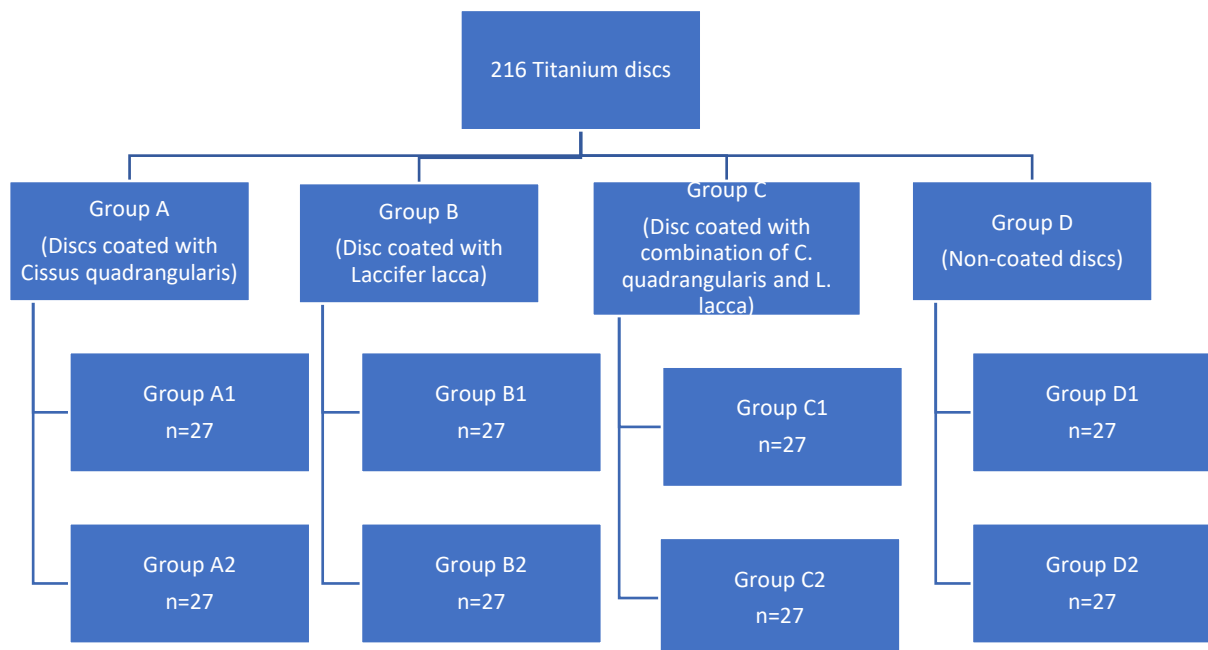
Four groups will be prepared for the tests:

Group A: Discs coated with Cissus quadrangularis extract

Group B: Discs coated with Laccifer lacca extract

Group C: Discs coated with combination of C. quadrangularis and L. lacca extract in 1:2 ratio w/w%.

Group D: Non coated discs



Each group will be further divided into two to perform two tests i.e. Osteoblastic Proliferation test and antimicrobial test.

- 1) Procedure for Osteoblastic proliferation test: MC3T3 cells will be cultured on the discs. After fixing and drying, the discs will be observed under Scanning Electron Microscope for Osteoblastic Proliferation this will be done on 7th, 12th and 21st day after incubation.

Group A1	Group B1	Group C1	Group D1
n=27	n=27	n=27	n=27

- 2) Procedure for Antimicrobial test: The discs will be treated with artificial saliva containing 1% mucin. *S. mutans*, *S. aureus*, *E. coli* will be cultured on the discs. Incubation of the

inoculated discs at a suitable temperature (usually 37°C) for a period of 24-48 hours will be done to promote bacterial growth and biofilm formation, after which the cultured bacteria will be stained using green and red fluorescent nucleic acid. The unattached microbes will be removed and the samples will be washed and fixed. ELISA will be performed for anti-biofilm formation test. The test will confirm the antimicrobial effect of the surface coated discs.

Group A2	Group B2	Group C2	Group D2
n=12	n=12	n=12	n=12

Criteria for discontinuing or modifying allocated interventions {11b}

There won't be any unique requirements for stopping or changing allotted interventions.

Strategies to improve adherence to interventions {11c}

Not Applicable. Because it is preclinical trial and no patients are involved.

Relevant concomitant care permitted or prohibited during the trial {11d}

Not Applicable. Because it is preclinical trial and no patients are involved in this.

Provisions for post-trial care {30}

Not Applicable. Because it is preclinical trial and no patients are involved

Outcomes {12}

At the end of the study, we may expect that the properties such as osteogenic differential potential and antimicrobial properties of herbal extract coated discs will be superior to that of non-coated discs which would lead to a better implant surface coating material in terms of its biocompatibility, antimicrobial properties and osseointegration. Also, will be able to identify which surface coating shows better properties among *Cissus quadrangularis* and *Laccifer lacca* or the combination of both.

For osteogenic potential test:

	STUDY PERIOD						
	Enrolment	Allocation	Post- allocation				Close-out
TIMEPOINT	-t ₁	0	t ₁ (day 0)	t ₂ (day 7)	t ₃ (day 12)	t ₄ (day 21)	t _x
ENROLLMENT:							
Eligibility screen	X						
Informed consent							
(list other procedure)	X						
Allocation		X					
INTERVENTIONS:							
Intervention A (Discs coated with Cissus quadrangularis extract)							
Intervention B (Disc coated with Laccifer lacca extract)							
Intervention C (Disc coated with combination of C. quadrangularis and L. lacca extract)							
Intervention D (Non coated discs)							
ASSESSMENTS:							
List of baseline variables	X	X					
List outcome variables				X		X	
List other data variables			X	X	X	X	

For Antimicrobial Test:

	STUDY PERIOD				
	Enrolment	Allocation	Post-allocation		Close-out
TIMEPOINT	-t ₁	0	t ₁ (day 0)	t ₂ (day2)	t _x
ENROLLMENT:					
Eligibility screen	X				
Informed consent					
(list other procedure)	X				
Allocation		X			
INTERVENTIONS:					
Intervention A (Discs coated with Cissus quadrangularis extract)					
Intervention B (Disc coated with Laccifer lacca extract)					
Intervention C (Disc coated with combination of C. quadrangularis and L. lacca extract)					
Intervention D (Non coated discs)					
ASSESSMENTS:					
List of baseline variables	X	X			
List outcome variables				X	
List other data variables			X	X	

Sample size {14}

The sample size formula used are as follows:

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/\kappa)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

The notation for the formulae are:

n_1 - sample size of Group 1

n_2 - sample size of Group 2

σ_1^2 - standard deviation of Group 1

σ_2^2 - standard deviation of Group 2

Δ = difference in group means

κ = ratio = n_2/n_1

$z_{1-\alpha/2}$ - two-sided Z value (eg. Z-1.96 for 95% confidence interval).

$z_{1-\beta}$ - power

Mean BIC value in group I = 48.08

Mean BIC value in group II = 60.29

σ_1 = SD of BIC value in group I = 18.62

σ_2 = SD of BIC value in group II = 14.74

For detecting mean difference of 12.82 i.e. $\Delta = 60.29 - 48.08 = 12.82$

$\kappa = 1$

$N = \frac{(18.62^2 + 14.74^2)(1.96 + 0.84)^2}{12.82^2}$

= 26.90 = 27 samples needed in each group

Reference: Srinidhi Bhat et al

Power of the Test: 80%

Level of significance: 5% (95% confidence interval)

Recruitment {15}

Not Applicable. As the study will be done on the coated and non-coated titanium implants and no patients are involved in the study.

Assignment of interventions: allocation

Sequence generation {16a}

Not Applicable. Because it is preclinical trial and no patients are involved

Concealment mechanism {16b}

Not Applicable. Because it is preclinical trial and no patients are involved

Implementation {16c}

Not Applicable. Because it is preclinical trial and no patients are involved

Assignment of interventions: Blinding

Who will be blinded {17a}

There will be blinding of Outcome assessors, Data analysts.

Procedure for unblinding if needed {17b}

There is no human involvement in the assignment process; it will be determined by data analysis using spreadsheets containing random numbers. The procedure will be kept completely secret from both research investigators and potential volunteers until the study arm is allocated.

Data collection and management

Plans for assessment and collection of outcomes {18a}

The antimicrobial test for coated and non-coated titanium discs will be assessed after 24-48 hours of inoculation.

Also osteogenic potential will be assessed by MC3T3 cells culture on the coated and non-coated discs. Discs will be observed under Scanning Electron Microscope for Osteoblastic Proliferation this will be done on 7th, 12th and 21st day after incubation.

After this coated and non-coated disc will we compared for both antimicrobial properties and osteogenic potential.

Plans to promote participant retention and complete follow-up {18b}

Not applicable. Because there are no follow ups involved in the study as no patients are involved in the study.

Data management {19}

The principal investigator will handle electronic data management, gather data, and enter it into the database for screening.

Confidentiality {27}

Not applicable Since no patients are participating in the study, no personal information about prospective or enrolled participants will be gathered.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

- 1) MC3T3 cells will be cultured on the coated and non-coated titanium discs. After fixing and drying, the discs will be observed under Scanning Electron Microscope for Osteoblastic Proliferation this will be done on 7th, 12th and 21st day after incubation.
- 2) For antimicrobial test the coated and non-coated discs will be treated with artificial saliva containing 1% mucin. *S. mutans*, *S. aureus*, *E. coli* will be cultured on the discs. Incubation of the inoculated discs at a suitable temperature (usually 37°C) for a period of 24-48 hours will be done to promote bacterial growth and biofilm formation, after which the cultured bacteria will be stained using green and red fluorescent nucleic acid. After that ELISA will be performed for anti-biofilm formation test.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The study will employ both descriptive and inferential statistics, utilizing Chi square test, unpaired t test, and one-way ANOVA. The analysis will be conducted using SPSS 27.0 and GraphPad Prism 7.0 software, with a significance threshold of $p < 0.05$.

Interim analyses {21b}

Interim analyses will be done by comparing data collected from days 7, 12 and 21st. For this, osteogenic potential will be assessed on days 7, 12 and 21.

Antimicrobial test will be done after 48 hours of inoculation to check for bacterial growth and biofilm formation.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Not Applicable. As no subgroups are involved in the study there are no methods for additional analyses.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Not Applicable. As no patients are involved in study and the study will be done the titanium discs.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

Upon reasonable request, the corresponding author will provide the statistical code and datasets used in the current work, together with the complete procedure.

Oversight and monitoring**Composition of the coordinating centre and trial steering committee {5d}**

The committee consist of

1. The head of the department of prosthodontics
2. P.G. guide
3. Research scientist
4. Principal investigator
5. Statistician
6. Data manager

Composition of the data monitoring committee, its role and reporting structure {21a}

Members of the data monitoring committee (DMC) include the research incharges of the department and the institute.

Adverse event reporting and harms {22}

When adverse events and other unanticipated consequences of trial interventions or trial conduct occur, data will be gathered, evaluated, and voluntarily reported.

Frequency and plans for auditing trial conduct {23}

Every month, the Project Management Group will examine the trial behaviour. The independent Data Monitoring and Ethics Committee meets with the Trial Steering Group to examine conduct from the start of the trial until its conclusion.

Ethics and dissemination Research ethics approval {24}

Ethics grant and consent of participants {24}

Name of ethics committee: Datta meghe institute of higher education and research Institutional Ethics Committee.

The Institutional Ethics Committee grants the study ethical permission.

[ref no: DMIHER(DU)/IEC/2024/236]

Consent of participants

Not Applicable. As this is an in vitro study no human participants or patients are involved neither the organs/tissues are involved in the study.

Protocol amendments {25}

Strategies for informing pertinent parties—such as trial participants and ethical committees—of significant protocol modifications {25}

The primary investigator will inform the centers, and they will also receive a copy of the updated protocol if this study get funding primary investigator will notify the sponsor and funder.

Dissemination plans {31a}

Publication

Discussion

As it is preclinical study and to be done in laboratory set up some technical or lab related issues can be faced during the course of the study.

Trial status

[Ref No: DMIHER(DU)/IEC/2024/236] protocol version number 1; begins July 2024, and approximately will be completed by October 2025.

Clinical Trial Number: REF/2024/05/084317

Abbreviations

No abbreviations are used.

Declarations

Acknowledgements

NA

Authors' contributions {31b}

Shruti Deshmukh (Corresponding Author), Sweta Kale Pisulkar, Anita Wanjari, Akansha Bansod, Arushi Beri, Ritul Jain

Funding {4}

No funding received.⁸⁷

Availability of data and materials {29}

The datasets generated and/or analysed during the current study are not publicly available because the study is in progress.

Ethics approval and consent to participate {24}

Ethical approval for the study is obtained from the Datta Meghe Institute of Higher Education and Research Institutional ethics committee.

[ref no: DMIHER(DU)/IEC/2024/236]

Consent for publication {32}

Not Applicable as there are no identifiable images.

Competing interests {28}

The authors declare that they have no competing interests.

Authors' information (optional)

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