

# A Comparative Evaluation of the Antimicrobial Efficacy of Flavonoid of Withania Somnifera Gel with Quercetin and Propolis Gels Against Red Complex Organisms in Chronic Periodontitis Patients: Study Protocol for a Randomised, Double-blind, Parallel-group Controlled Trial

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**Trial registration:** Clinical Trials Registry – India (CTRI): CTRI/2025/06/089506

**Ethics approval:** Institutional Ethics Committee, Datta Meghe Institute of Higher Education and Research (DU): DMIHER(DU)/IEC/2024/17

**Protocol version:** 1.0

**Study status:** Not yet recruiting

## Abstract

**Introduction:** Subgingival red complex organisms — Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola induced chronic inflammation leads to destruction of the tooth-supporting periodontal structures. Scaling and root planning (SRP), the standard mechanical therapy, cannot reliably eliminate these pathogens from inaccessible sites such as furcations and root concavities. Chlorhexidine, the adjunctive chemical agent of choice, is limited by side effects on long-term use, prompting interest in natural phytochemicals such as quercetin, propolis and Withania somnifera as adjuncts to mechanical therapy recommended due to their distinct properties.

**Methods and analysis:** Present randomized, double-blind, parallel-group, controlled clinical trial will be conducted in the Department of Periodontology and Implantology, Sharad Pawar Dental College and Hospital, Wardha. Forty-five patients (Both males and females) with Stage III Grade B or Grade C periodontitis will be enrolled and randomly allocated equally by SNOSE (sequentially numbered, opaque, sealed envelopes). All the patients will receive SRP in addition to quercetin gel (Group A), Withania somnifera gel (Group B), and propolis gel (Group C), twice application daily for 7 days. Probing pocket depth, clinical attachment level, plaque index and bleeding index will be recorded at baseline and 6 weeks by a single calibrated examiner. Gingival crevicular fluid levels of P. gingivalis, T. forsythia and T. denticola will be quantified at baseline and 6 weeks using Multiplex Polymerase chain reaction. The primary outcome: reduction in red complex organism levels; secondary outcomes: alteration in clinical periodontal parameters will be recorded. Data will be analysed using SPSS v27.0 and GraphPad Prism v7.0, with chi-square, paired/unpaired t-tests, one-way and two-way ANOVA with Tukey post-hoc test;  $p < 0.05$  will be considered significant.

**Ethics and dissemination:** The protocol has been approved by the Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research (DMIHER(DU)/IEC/2024/17). Written informed consent will be obtained from all participants. Findings will be disseminated through peer-reviewed publication and scientific presentation.

**Trial registration number:** CTRI/2025/06/089506 (Clinical Trials Registry – India).

## Keywords

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### Power and Limitations of The Study

- To the best of our knowledge this will be the first randomized controlled trials to directly compare the antimicrobial efficacy of three natural phytochemical gels — Quercetin, *Withania somnifera* and Propolis — against red complex organisms .
- Red complex organism levels in gingival crevicular fluid will be quantified objectively by Multiplex Polymerase Chain Reaction (MT-PCR) which will more authenticate and justified in comparison to relying solely on clinical parameters alone.
- The randomised, double-blind, parallel-group design with a single calibrated examiner minimises performance and assessment bias.
- The 6-week follow-up period, while adequate to demonstrate short-term antimicrobial effect, may not represent the long-term durability of the clinical and microbiological benefits.
- As a single-centre trial, will be conducting on small sample size may be the limitations of study.

### Introduction

#### Background and Rationale

Tenacious inflammatory immunopathology is one of the important component that may contribute to the destruction of the supportive tissues of the teeth, resulting in apical shift of junctional epithelium associated absolute pocket formation and its sequelae.<sup>1</sup> The prevalence of mild-to-moderate and severe periodontitis is about 50 and 11% worldwide in addition , it will act as a risk factor for wide varieties of systemic disorders and adverse pregnancy outcomes.<sup>2,3</sup>

Host microbial interaction and dysbiotic subgingival microbiota especially — *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* — are grouped as the "red complex" on the basis of their extracellular proteolytic activity, anaerobic amino acid fermentation and toxic metabolite production, and are regarded as the key pathogens driving disease progression.<sup>4</sup>

Management of Periodontitis via Scaling and root planning (SRP) still remains the gold-standard therapy, but it may not eliminate pathogenic bacteria's persist within soft tissue or in inaccessible sites such as furcation/root concavities to instrumentation.<sup>5-6</sup> Chlorhexidine gluconate has therefore been widely used as an adjunctive to SRP but its long-term use is limited by tooth discolouration, taste alteration and mucosal irritation.<sup>7</sup> Such limitations might guided the researchers to look for safe profile containing natural

phytochemicals with distinctive antimicrobial &/ anti-inflammatory properties.

Quercetin, the most abundant dietary flavonoid, effective against Gram-positive and Gram-negative pathogens<sup>8</sup> well as it inhibit virulence factors of *P. gingivalis*, including gingipain proteases and haemagglutinin at sub minimally inhibitory concentration.<sup>9</sup> Propolis, a resinous bee product with complex composition having broad spectrum of activities,<sup>10</sup> has proven its antimicrobial efficacy against pigmented anaerobic periodontal pathogens.<sup>1</sup>

<sup>11, 1</sup>*Withania somnifera* (Ashwagandha), widely used in Indian medicine, contains withanolides and sitoindosides with distinctive properties such as anti-inflammatory, immunomodulatory and antimicrobial activity, and investigated antimicrobial efficacy only against *P. Gingivalis* and *P. Intermedia* in preliminary studies.<sup>12</sup> whereas other two red complex organisms were not evaluated yet.

Despite the individual promise of these three phytochemicals, few clinical studies have directly compared their antimicrobial efficacy against red complex organisms in patients with chronic periodontitis individually but no study has evaluated dose-dependent antimicrobial activity of *Withania somnifera* against these pathogens in addition to Quercetin & Propolis gel all under single study protocol to the best of our knowledge. This trial has therefore been designed to generate comparative clinical evidence to address this gap.

#### Objectives

The primary aim is to evaluate and compare the antimicrobial efficacy of flavonoid of *Withania somnifera* gel, quercetin gel and propolis gel, used as adjuncts to SRP, against red complex organisms in patients with chronic periodontitis. The specific objectives are to:

1. To evaluate gingival crevicular fluid (GCF) levels of *P. gingivalis*, *T. forsythia* and *T. denticola* in the Quercetin (grape seed extract) gel group;
2. To evaluate GCF levels of *P. gingivalis*, *T. forsythia* and *T. denticola* in the *Withania somnifera* gel group;
3. To evaluate GCF levels of *P. gingivalis*, *T. forsythia* and *T. denticola* in the Propolis gel group; and
4. To compare GCF levels of *P. gingivalis*, *T. forsythia* and *T. denticola* between the three groups.

## Methods and Analysis

### Trial Design

This will be a randomized, controlled, parallel-group, double-blind clinical trial with three arms allocated in a 1:1:1 ratio, conducted in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) guidance.<sup>13</sup>

### Study Setting

The trial will be conducted in the Department of Periodontology and Implantology, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Higher Education and Research (Deemed to be University), Sawangi (Meghe), Wardha, Maharashtra, India. The parent institution has the clinical and laboratory infrastructure required for periodontal examination, preparation of phytochemical extract facility, microbial sample storage facility whereas microbiological analysis will be performed at Maratha Mandel Clinical Research Laboratory, Belgaum, Karnataka.

### Eligibility Criterion

#### Inclusion criteria

- Age 20–65 years
- Diagnosis of Stage III Grade B or Grade C periodontitis<sup>14</sup>
- Deep periodontal pocket with clinical attachment level  $\geq 5$  mm and/or probing pocket depth  $\geq 4$  mm
- Willingness to provide informed consent and comply with the study protocol

#### Exclusion criterion

- Systemic disease (e.g., diabetes) that could alter periodontal disease progression or treatment outcome
- Periodontal therapy within the preceding 6 months
- Systemic antibiotic or anti-inflammatory medication within the preceding 3 months
- Use of any other topical gels storage
- Known allergy to Withania somnifera or related herbal compounds
- Pregnant or lactating women

### Interventions

#### Phase 1 — Preparation of herbal gels.

Withania somnifera roots will be procured, washed, air-dried and powdered before Soxhlet extraction with 80% hot methanol. The filtrate will be sequentially re-extracted with petroleum ether, ethyl ether and ethyl acetate; the ethyl acetate fraction (containing bound flavonoids) will be hydrolysed with 7% H<sub>2</sub>SO<sub>4</sub> to remove bound sugars, and the resulting flavonoid extract formulated into a topical gel.<sup>15,16</sup> Quercetin<sup>15</sup> and propolis gels<sup>16</sup> will be formulated using standardised extraction and formulation procedures. Preparation of all extract and gel will be performed by principle investigator whereas packing will be

performed by trained investigator who will not be the part of study protocol and will done the blinding of respective gel formulation.

#### Phase 2 — Clinical intervention.

After SRP under standardised conditions by a trained clinician, participants will receive one of the adjunctive gels applied using 5 ml syringe twice daily at 1 hour interval for 7 days.

- Group A (n=15): SRP + Quercetin (grape seed) gel
- Group B (n=15): SRP + Withania somnifera gel
- Group C (n=15): SRP + Propolis gel are the just proposed outline group allotted as per blinded protocol will be considered final.

No concomitant periodontal therapy, systemic antibiotics or other topical agents will be permitted during the study period. Any protocol deviation if observed so will not be considered for study protocol.

### Outcomes

**Primary outcome:** reduction in gingival crevicular fluid levels of *P. gingivalis*, *T. forsythia* and *T. denticola* will be measured by Multiplex- PCR system at baseline and 6 weeks post treatment.

**Secondary outcomes:** change from baseline to 6 weeks in probing pocket depth, clinical attachment level, full-mouth plaque index<sup>17</sup> and full-mouth bleeding index<sup>18</sup> recorded by a single calibrated examiner using a William's graduated periodontal probe.

### Participant Timeline

Eligible participants will be screened, consented and examined at baseline, will undergo SRP with allocated blinded adjunctive gel application over 7 days, and will be re-evaluated for clinical and microbiological parameters at 6 weeks post-intervention.

### Study Flow

#### Chart

##### Sample Size

Sample size will be calculated using mean probing depth values from prior investigations (mean probing depth at week 4: Group A = 5.1 mm, SD 0.5; Group B = 4.5 mm, SD 0.91; mean difference 0.8 mm). At 80% power and 5% significance, 13 patients were required per group; allowing for attrition/ withdrawal if any 15 patients per group (45 patients total) will be recruited minimally.

#### Recruitment

Patients attending the outpatient Department of Periodontology and Implantology and who meet the eligibility criterion will be identified by the principle clinicians and invited to participate honorarary. Recruitment will continue until the target sample size is achieved.

#### Assignment of Interventions

#### Sequence generation and allocation concealment.

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Eligible participants will be randomly allocated in a 1:1:1 ratio to Group A, B or C using SNOSE (sequentially numbered, opaque, sealed envelopes) until the point of intervention.

## Implementation.

A Trained investigator who will not be the part of study protocol will generate the allocation using SNOSE and blinding and unblinding (demasking) the testing gel till the time Principle investigator complied all the recorded blinded data & will submit to the trained investigator who will done the unmasking of the blinding and unmasked data of each group will be submitted to the Principle investigator for statistical analysis utilizing mean, standard deviation, paired 't' test, One way ANOVA & Chi square test will be done and final interpretation will be drawn.

## Blinding

This is a double-blind trial: participants and the outcome assessor (examiner recording clinical parameters and interpreting laboratory results) will be blinded to group allocation. The gels will be identically packaged and coded by an independent investigator. Un-blinding will be done only if serious adverse event occur.

## Data Collection Method:

### Clinical parameters.

Probing pocket depth, clinical attachment level, plaque index and bleeding index will be recorded at baseline and at 6 weeks by a single calibrated examiner using a William's graduated periodontal probe.

### Microbiological sampling.

Supragingival and subgingival biofilm will be removed with an ultrasonic scaler before sampling. Gingival crevicular fluid will be collected using absorbent endo-paper points placed at the deepest isolated periodontal pocket in the maxillary /mandibular arch until a consistent volume is obtained, transferred to Eppendorf tubes and stored at  $-70^{\circ}\text{C}$  until analysis. Samples will be analysed for P. gingivalis, T. forsythia and T. denticola using a commercially available Multiplex-PCR system as per the manufacturer's protocol.

### Data Management

Data will be recorded systematically twice at an interval of 1 hour and average will be considered as final to reduce the intra examiner error for all baseline and post operative evaluation. Arbitrary imputation will not be undertaken without statistical justification, and any such method will be fully documented. Participant records will be stored securely with access restricted to authorized personnel.

### Statistical Methods

Data will be analysed using descriptive and inferential statistics in SPSS version 27.0 and GraphPad Prism version 7.0. Categorical variables will be compared

using the chi-square test. Intra-group comparisons (baseline vs 6 weeks) will be made using the paired t-test, and inter-group comparisons using the unpaired t-test. Comparisons across the three groups will be made using ANOVA, with pairwise comparisons by Kruskal Wallis test. A p-value  $<0.05$  will be considered statistically significant.

## Sample size calculation

The sample size formulae 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

$\sigma_1$  = standard deviation of Group 1

$\sigma_2$  = standard deviation of Group 2

$\Delta$  = difference in group means

$\kappa$  = 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 probing depth in group A at week 4=5.1

Mean probing depth in group B at week 4=4.5

$\sigma_1$  = SD of probing depth in group A at week 4=0.5

$\sigma_2$  = SD of probing depth in group B at week 4=0.91

For detecting mean difference of 0.8 i.e.  $\Delta = 5.1 - 4.5 = 0.8$

K=1

$$N = \frac{(0.5*0.5 + 0.91*0.91)(1.96+0.84)^2}{0.8*0.8}$$

$$= 13.20 = 13$$

= 13 patients required in each group (13\*3=39 total patients)

**Reference :** Dr Sabgreetga Subramanian

**Power of the Test:** 80%

**Level of significance:** 5%, 95% confidence interval

## Data Monitoring and Auditing

Periodic review and auditing of study records will be undertaken to verify consistency and adherence to protocol. Study records will be made available to authorized personnel for verification. The principal investigator and supervisor will jointly oversee trial conduct and safety.

## Harms

Adverse events, including local mucosal irritation, allergic reaction or taste alteration attributable to the study gels, will be recorded at each visit and reported to the Institutional Ethics Committee as per institutional policy such patients data will not be recorded for final assessments / participants will be withdraw from the study.

## Ethics and Dissemination

### Research ethics approval.

The study protocol has been reviewed and approved by the Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research (DU), Wardha (Ethics approval number: DMIHER(DU)/IEC/2024/17). The trial is registered with the Clinical Trials Registry – India (CTRI Registration No.: CTRI/2025/06/089506).

### Protocol amendments.

Any amendment to the study protocol if so will be documented in writing and submitted to the Institutional Ethics Committee for approval. Changes affecting study design, methodology or participant safety will be communicated to the relevant authorities and to participants; fresh informed consent will be obtained before continued participation where a major amendment is made.

### Consent.

The nature, purpose and procedures of the study will be explained to all potential participants in a language they understand, and written informed consent will be obtained before enrolment.

### Confidentiality.

Participant data confidentiality will be maintained throughout the study; records will be de-identified for analysis and stored securely with access limited to the research team.

### Declaration of interests.

The authors declare no competing interests.

### Access to data.

The principal investigator and supervisor will have full access to the final dataset. De-identified data will be made available upon reasonable request to the corresponding author following publication.

### Dissémination policy.

Findings will be disseminated through peer-reviewed publication, scientific conferences and the institutional thesis repository, regardless of outcome direction. Authorship will follow ICMJE criteria.

### Trial Status

The study is at the protocol/planning stage; participant recruitment has not yet commenced.

### Data Availability Statement

No data are currently associated with this protocol. Data generated during the trial will be made available upon reasonable request to the corresponding author, subject to institutional data-sharing policy.

### Discussion

Non-surgical periodontal therapy alone is often insufficient to eradicate periodontal pathogens: Octavia (2018)<sup>19</sup> observed only a modest reduction in *T. forsythia* three months after SRP, underscoring the need for adjunctive therapy. Dave RD et al. (2016)<sup>20</sup> reported munaka Honey & pomegranate mouthwash is the most effective against A.

*Actinomycetemcomitans* followed by *F. Nucleatum*, *P. Gingivalis*, *P. Intermedia* as compare to 0.2% chlorhexidine when tested on red and green complex periodontal pathogens.

Interest in natural phytochemicals as periodontal adjuncts has grown because of their antimicrobial and anti-inflammatory properties. He Z (2020)<sup>9</sup> reported that quercetin inhibited virulence factors of *P. gingivalis* without affecting bacterial growth, suggesting a role in controlling pathogenicity rather than mere bacteriostasis. Shawky and Lotfy (2021)<sup>12</sup> found propolis to have greater antibacterial activity against *P. gingivalis* and *Prevotella intermedia* than ginseng extract, although this evidence remains largely in-vitro. The antimicrobial potential of *Withania somnifera* against periodontal pathogens has also been demonstrated,<sup>21,22</sup> but its efficacy against red complex organisms specifically has not been examined in vivo.

This comparative gap and the absence of head-to-head clinical evidence for these three phytochemicals against red complex organisms in the same trial population will be rationale for the present study. By combining validated clinical periodontal parameters with objective microbiological quantification of red complex organisms in gingival crevicular fluid, this trial will be designed to generate evidence to guide the selection of an effective, natural adjunctive agent for chronic periodontitis and to reduce reliance on chlorhexidine and its associated side effects.

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