

Evaluation Of Antimicrobial Efficacy Of Novel Dental Varnish Containing Grape Seed Extract And Nanohydroxyapatite Against Streptococcus Mutans – An In-Vitro Study

Syamantika Ray¹, Lumbini Pathivada², Karthik M. Krishna³, Archana Singh⁴, Sakshi Sharan⁵, Niraj Singh⁶

^{1,5}Post Graduate, Department of Pediatric and Preventive Dentistry, Rungta College of Dental Sciences

²Professor & Head, Department of Pediatric and Preventive Dentistry, Rungta College of Dental Sciences

³ Professor and Dean, Department of Periodontics and Oral Implantology, Rungta College of Dental Sciences

⁴Reader, Department of Pediatric and Preventive Dentistry, Rungta College of Dental Sciences

⁶Post Graduate, Department of Conservative Dentistry and Endodontics, Rungta College of Dental Sciences

Keywords

Grape seed extract; Nano-hydroxyapatite; Dental varnish; Streptococcus mutans; Anticariogenic; Initial enamel caries.

Abstract

Background-Dental caries is a biofilm mediated disease predominantly initiated by *Streptococcus mutans*, leading to early enamel demineralization. While fluoride based varnishes are widely used for caries prevention, the search for bioactive, fluoride free alternatives has intensified. Grape seed extract (GSE), rich in proanthocyanidins, exhibits antimicrobial properties, and nano-hydroxyapatite (nHAP) mimics enamel mineral composition, supporting remineralization.

Aim-To evaluate the antimicrobial efficacy of a novel dental varnish containing 5% Grape Seed Extract and nano-hydroxyapatite against *Streptococcus mutans*, as a preventive approach for initial enamel caries.

Materials and Methods-A novel experimental varnish incorporating nano-hydroxyapatite and 5% Grape Seed Extract was formulated. Antimicrobial activity against *Streptococcus mutans* was assessed using the agar well diffusion method. After incubation at 37°C for 48 hours, the zone of inhibition was measured in millimeters to determine bacterial sensitivity.

Results-The experimental varnish demonstrated a mean zone of inhibition of 19mm against *Streptococcus mutans*, indicating significant antibacterial activity and sensitivity of the organism to the formulation.

Conclusion- Findings suggest its potential role as an anticariogenic, fluoride free preventive agent for managing initial enamel caries through combined antimicrobial and biomimetic remineralization mechanisms.

Introduction

Dental caries remains a highly prevalent, biofilm mediated disease, primarily initiated by *Streptococcus mutans* through acidogenic and aciduric activity leading to early enamel demineralization. While fluoride based varnishes remain the gold standard for caries prevention, concerns related to fluoride exposure and the demand for bioactive, biomimetic alternatives have driven research toward natural and nano technology based agents. Grape seed extract (GSE), rich in proanthocyanidins, exhibits proven antibacterial and antiadhesive properties against cariogenic bacteria, while nanohydroxyapatite (nHAP) mimics the mineral composition of enamel and promotes remineralization. The present study evaluates a novel dental varnish incorporating 5% Grape Seed Extract and nano-hydroxyapatite, focusing on its antimicrobial efficacy against *Streptococcus mutans* as a preventive strategy for initial enamel caries.

Materials and Methods

Test group 1 – Control group using saline

Test group 2 – Novel Dental Varnish

A novel experimental varnish was formulated using nanohydroxyapatite as the remineralizing agent and 5% Grape Seed Extract as the bioactive antimicrobial component.

FORMULATION:

- Approximate weight of Varnish: 50 ml
- Formulation contains (in 50ml):
 - 5.0 % w/v (2.5gm) Grape Seed Extract use as an active ingredient
 - 5.0 % w/v (2.5gm) Nano hydroxyapatite powder

Base Content: 60-65 % Ethanol Medicated grade, 20-22 % Shellac (Natural resin, film-forming agent), 1-2 % Ethyl Cellulose (Thickening agent), 1-2% Polyethylene Glycol (Plasticizer), 0.5-1.0% Sorbitol (Sweetener, non-cariogenic), 0.5-1.0 % Flavouring agent (mint).

➤ Antimicrobial Evaluation

The antimicrobial efficacy of the experimental dental varnish was assessed against *Streptococcus mutans* (standard ATCC strain) using the agar diffusion method. *Streptococcus mutans* was cultured on *Mitis Salivarius* agar supplemented with 1% sucrose and 1% tellurite to support selective and optimal bacterial growth. A loopful of the organism from a fresh culture was inoculated into nutrient broth and incubated at 37°C for 18–24 hours. The turbidity of the bacterial suspension was standardized to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) to ensure uniform bacterial density. Sterile agar (15–20 mL) was poured into Petri dishes and allowed to solidify under aseptic conditions, after which the standardized inoculum was uniformly spread over the agar surface using a sterile cotton swab to obtain a confluent lawn culture. Wells were prepared in the agar plates using a sterile cork borer, and each well was filled with a measured quantity of the experimental dental varnish containing 5% Grape Seed Extract and Nanohydroxyapatite, while normal saline served as the negative control. The inoculated plates were incubated at 37°C for 24–48 hours under anaerobic conditions using an anaerobic jar system. Following incubation, the plates were examined for the presence of clear zones of inhibition around the wells, and the diameter of the inhibition zones was measured in millimeters (mm) using an antibiotic zone reader scale to determine antimicrobial activity.

Results

The experimental varnish containing 5% Grape Seed Extract and Nanohydroxyapatite demonstrated a clear zone of inhibition measuring 19mm against *Streptococcus mutans*. This finding indicates significant antibacterial sensitivity, suggesting effective inhibition of the primary cariogenic microorganism implicated in the initiation of enamel caries. The consistent presence of a well defined inhibition ring across samples confirms the reproducibility and antimicrobial potential of the formulation.

Discussion

The observed 19mm zone of inhibition confirms the antimicrobial efficacy of the novel varnish, supporting the anticariogenic role of 5% Grape Seed Extract against *Streptococcus mutans*. GSE's high proanthocyanidin content is known to disrupt bacterial cell walls, inhibit glucosyltransferase activity, and reduce bacterial adhesion and biofilm formation key mechanisms in early caries development. When combined with Nanohydroxyapatite, which facilitates enamel surface repair and biomimetic mineral deposition, the varnish offers a dual action approach, biological suppression of cariogenic bacteria and enhancement of enamel resistance to acid attack.

Unlike fluoride based formulations, this bioactive varnish provides a natural, fluoride free alternative with potential application in Pediatric and Preventive Dentistry, especially for early enamel lesions. The findings establish this formulation as a novel anticariogenic varnish, with promising clinical relevance for managing initial enamel caries through combined antimicrobial and remineralization mechanisms.

CERTIFICATE OF ANALYSIS OF FORMULATION

Product Name: Varnish contain Grape Seed Extract

Storage Condition: Store in cool and dry place.

PHYSICAL AND CHEMICAL PROPERTIES

Test	Result
Appearance	Smooth, uniform liquid or semi-liquid; free from clumps, bubbles, or phase separation
Odor	Mild, pleasant or neutral (mint)
pH	6.8 -7.4
Color	Brownish (golden brownish)
Viscosity	1000 - 2000 cps (it varies with evaporation of solvent)
% Volatile	Volatile (dry after exposure)
Texture	Smooth, non-gritty
Homogeneity	Uniform distribution of active ingredients with no settling or separation
Solubility	--
Spreadability	Easy to apply thinly and evenly with a brush or applicator
Foaming ability	N/A
Physical State	Liquid.
Syringeability	Should pass through a syringe tip easily without clogging
In-Vitro Analysis	N/A
Sol Gel Transaction	N/A

FORMULATION OF NOVEL VARNISH





[Fig:1-4] Procurement, identification, and authentication of Grape Seed Extract were performed, followed by preparation of the experimental dental varnish using the solvent dissolution method. The formulated varnish was then subjected to physical characterization, including viscosity measurement using a viscometer, pH evaluation using a digital pH meter, and visual inspection for homogeneity and stability.

PREPARATION OF THE AGAR MEDIA



[Fig:5-6] Pouring sterile media into petri dishes and allowing them to solidify underlaminar airflow before inoculation. Impregnation of Whatmann Filter Paper Discs with novel Varnish

ANTIMICROBIAL TESTINGS AGAINST STREPTOCOCCUS MUTANS



[Fig:7] PRE-TESTINGS:
 Positioning of discs on agar plate

[Fig:8] POST-TESTINGS:
 Zone of inhibition
 around disc with Novel Dental Varnish

RUNGTA COLLEGE OF DENTAL SCIENCES & RESEARCH, BHILAI

DEPARTMENT OF PATHOLOGY & MICROBIOLOGY

REPORT – CLINICAL MICROBIOLOGY

(PILOT STUDY)

Name of Patient: _____

Age/ Sex: _____

Sample: Test medicaments for Antibiotic Sensitivity Test against *Streptococcus mutans*

Report of Microbiological Staining: Gram positive

Morphology: Coccus in chains

Sr. No.	Antibiotic	Concentration	Result
1	Test Medicament :- Dental Varnish – 5% Grape seed extract – Antimicrobial 5% Nano Hydroxyapatite- active Ingredient Effective against Initial caries	Pure – 100% Discs made In- vitro	Intermediate Sensitive (19 mm)

Prerna Jaiswal
HEAD OF DEPARTMENT
Signature of HOD/IC-1
Rungta College of Dental Sciences & Research
Department of Microbiology,

Rungta College of Dental Sciences and Sciences,

Bhilai, Chhattisgarh

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