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Green Synthesis of Silver Nanoparticles Using Ocimum tenuiflorum and Ocimum gratissimum: Development of a Dental Varnish with Enhanced Antibacterial Properties

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Abstract **Background:** The growing resistance of oral pathogens to conventional antimicrobial agents has created a demand for innovative, natural alternatives in dental care. *Ocimum tenuiflorum* and *Ocimum gratissimum* are widely recognized for their medicinal properties, particularly their antimicrobial potential. This study investigates the green synthesis of silver nanoparticles (AgNPs) using these herbal extracts and their incorporation into a dental varnish. The varnish underwent comprehensive characterization using Fluorescent analysis, NMR analysis, Zeta potential studies and UV-Vis spectroscopy to evaluate its structural and physicochemical properties. **Methods:** Silver nanoparticles were synthesized using *Ocimum tenuiflorum* and *Ocimum gratissimum* herbal extracts via a green synthesis approach. The synthesized AgNPs were incorporated into a dental varnish and their antibacterial efficacy was tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Escherichia coli*. The antibacterial activity was evaluated using the agar well diffusion method and time-kill curve assay. Inhibition zones were measured at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL and results were compared with a commercially available dental varnish. **Results:** The AgNP-based dental varnish demonstrated superior antibacterial activity across all tested concentrations. The inhibition zones for *S. aureus* were 10 mm, 14 mm and 26 mm at 25 µg/mL, 50 µg/mL and 100 µg/mL, respectively, outperforming the commercial varnish's 9 mm inhibition zone. For *S. mutans*, the Ocimum-based varnish showed inhibition zones of 24 mm, 27 mm and 34 mm, which significantly exceeded the standard varnish's 9 mm. *Lactobacillus* sp. exhibited inhibition zones of 20 mm, 22 mm and 31 mm, while *E. faecalis* showed 9 mm, 11 mm and 14 mm. *E. coli* displayed the highest inhibition zones of 27 mm, 30 mm and 35 mm, compared to the 9 mm zone produced by the commercial varnish. The time-kill curve assay demonstrated that the Ocimum-based varnish at 100 µg/mL effectively reduced bacterial counts of *E. coli* and *S. mutans* to 10 CFU/mL within 5 hours. Similar reductions were observed for *S. aureus*, *Lactobacillus* sp. and *E. faecalis*, indicating potent bactericidal activity with increasing concentrations. **Conclusion:** The Ocimum-mediated AgNP dental varnish exhibited superior antibacterial efficacy compared to the commercial dental varnish, particularly at 100 µg/mL. The enhanced antibacterial activity, combined with the eco-friendly green synthesis process, suggests that *Ocimum tenuiflorum* and *Ocimum gratissimum*-based AgNPs hold promising potential as effective and safe alternatives in dental care for combating oral pathogens. Further *in vivo* studies are recommended to evaluate the varnish's clinical performance and long-term safety.

Key Words *Ocimum tenuiflorum*, *Ocimum gratissimum*, silver nanoparticles, dental varnish, antibacterial activity, oral pathogens, green synthesis

INTRODUCTION

Traditional dental varnishes, particularly fluoride-based formulations, have long been employed to prevent dental caries and enhance enamel remineralization. However, these conventional varnishes present several limitations that impact their clinical effectiveness and patient acceptability. One

common drawback is the temporary yellow discoloration they can leave on teeth, which, while useful for ensuring complete coverage during application, may be aesthetically displeasing to patients [1,2]. Additionally, fluoride varnishes can cause oral discomfort, with some patients reporting a burning sensation if the varnish contacts gingival tissues. The sticky texture and unpleasant taste further reduce patient compliance, particularly among children and individuals with sensory sensitivities.

Moreover, fluoride varnishes are known to pose allergic risks, particularly in individuals sensitive to colophony, a common component in many varnishes. Dental professionals may also develop sensitivities over time due to repeated exposure to colophony-based varnishes, posing occupational risks [3]. Another limitation is the technical precision required for varnish application. Maintaining uniform coverage demands manual dexterity, which can be challenging for some practitioners, potentially compromising application quality. To sustain its preventive effects, periodic reapplication is necessary, which can be inconvenient for patients and may reduce long-term treatment compliance [4,5]. These limitations underscore the need for improved dental varnish formulations that overcome these challenges while maintaining strong antibacterial properties and patient comfort.

In recent years, nanoparticle-based dental varnishes have emerged as promising alternatives to traditional fluoride varnishes, offering enhanced antimicrobial efficacy and improved clinical performance. Among these, silver nanoparticles (AgNPs) have demonstrated potent antimicrobial effects against key oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*. AgNPs possess unique antibacterial properties that allow them to penetrate bacterial cell walls, disrupt biofilms and inhibit microbial growth more effectively than conventional antimicrobial agents [6,7].

Research indicates that incorporating AgNPs into dental varnish formulations not only enhances antibacterial activity but also promotes dental remineralization, especially when combined with fluoride. This combination has shown superior efficacy in reversing early carious lesions compared to fluoride varnish alone [8,9]. Furthermore, AgNPs have been shown to be biocompatible with mammalian cells when used in controlled doses, making them a safe and effective alternative for dental materials [10]. Beyond varnishes, AgNPs and other nanoparticles like titanium dioxide (TiO) have successfully been incorporated into dental composites, implants, endodontic sealers and orthodontic adhesives, highlighting their versatile applications in dentistry [11,12].

A particularly promising innovation involves the green synthesis of silver nanoparticles using herbal formulations such as *Ocimum tenuiflorum* and *Ocimum gratissimum*. These medicinal herbs, known for their potent antimicrobial properties, provide an eco-friendly, sustainable method for synthesizing AgNPs with enhanced antibacterial potential [13]. Unlike traditional dental varnishes, which may struggle to combat diverse oral pathogens, Ocimum-mediated AgNPs have demonstrated superior efficacy in inhibiting microbial growth. The natural bioactive compounds present in these herbal extracts contribute to a synergistic effect, improving the stability and antimicrobial strength of the synthesized nanoparticles [14].

In this present study, green synthesized AgNPs using *Ocimum tenuiflorum* and *Ocimum gratissimum* were incorporated into a dental varnish formulation. The antibacterial activity of the formulated varnish was evaluated using the agar well diffusion method and time-kill curve assay to assess its efficacy against common oral pathogens. This study aims to explore the potential of Ocimum-mediated AgNPs as a promising alternative to conventional dental varnishes, providing improved antimicrobial action with enhanced biocompatibility and sustainability.

METHODS

Preparation of Ocimum tenuiflorum and Ocimum gratissimum Herbal Formulation

Fresh leaves of Ocimum tenuiflorum and Ocimum gratissimum were collected and thoroughly washed with distilled water to remove surface contaminants. The leaves were shade-dried at room temperature until fully dehydrated to preserve their bioactive compounds. Once dried, the leaves were finely powdered using a mechanical grinder.

A solution was prepared by combining 1 g of each powdered leaf with 100 mL of distilled water. The mixture was heated at 60°C for 15-20 minutes using a heating mantle to facilitate the extraction of active phytochemicals. After boiling, the mixture was gradually filtered using sterile filter paper to obtain a clear herbal formulation for further use.

Synthesis of Ocimum tenuiflorum and Ocimum gratissimum Herbal Formulation Mediated Silver Nanoparticles and its Dental Varnish

For the green synthesis of silver nanoparticles (AgNPs), a 1 mM silver nitrate (AgNO) solution was prepared by dissolving silver nitrate in 80 mL of distilled water. Subsequently, 20 mL of the filtered herbal extract was added to this solution. The mixture was stirred continuously and centrifuged at 8000 rpm for 10 minutes to collect the AgNP pellet. The obtained pellet was washed with distilled water to remove impurities and stored for dental varnish preparation. The dental varnish formulation was prepared by combining 7 mL of chitosan solution, 2.5 mL of ethanol and 500 µL of the synthesized AgNPs solution. This mixture was blended using a vortex mixer for 1-2 hours to ensure uniform dispersion of the nanoparticles in the varnish matrix.

Physico-Chemical Diagnostic Studies

Fluorescence Spectroscopy

The structural and fluorescence properties of the synthesized AgNPs were evaluated using a JY Fluorolog-3-11 spectrophotometer. The 3D Excitation-Emission Matrix (EEM) spectra were recorded with excitation and emission wavelengths ranging from 200-600 nm at 10 nm intervals to generate a contour plot of the fluorescent properties.

Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) Spectrum

The NMR spectra were obtained using a Bruker Avance 500 MHz spectrometer. For analysis, 10 µL of the AgNP sample was dissolved in 0.5 mL of CDCl solvent and loaded into a 5-mm NMR tube for spectral acquisition.

Zeta Potential and Particle Size

Zeta potential analysis was performed using a Zeta Sizer to evaluate the colloidal stability of the synthesized nanoparticles. The particle size distribution was determined using Dynamic Light Scattering (DLS) to assess the uniformity and dispersion of the AgNPs.

UV-Vis Spectroscopy

A Double Beam Scanning UV-Vis Spectrophotometer was used to confirm the formation and stability of the synthesized AgNPs. Measurements were conducted across the 200-1100 nm wavelength range to assess the characteristic surface plasmon resonance peak of AgNPs.

Antibacterial Activity

Agar Well Diffusion Technique

The antibacterial efficacy of the Ocimum-mediated AgNP dental varnish was tested against common oral pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Escherichia coli*.

A standardized bacterial inoculum (approximately 10 CFU/mL) was evenly spread onto sterile Mueller Hinton agar plates. Wells of 9 mm diameter were created using a sterile cork borer and 100 µL of the AgNP-based dental varnish at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL were dispensed into the respective wells. A commercial dental varnish was used as a standard control. The plates were incubated at 37°C for 24 hours and the resulting zones of inhibition were measured in millimeters to assess the antibacterial efficacy.

Time-Kill Curve Analysis

A time-kill curve assay was conducted to evaluate the bactericidal effect of the AgNP-based dental varnish on *S. mutans* and *Lactobacillus* sp. over a 5-hour period.

An inoculum standardized to 10 CFU/mL was treated with the AgNP-based dental varnish at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL. A commercial dental varnish served as the control, alongside an untreated bacterial inoculum as the negative control.

Aliquots were collected at 0, 1, 2, 3, 4 and 5 hours, serially diluted and their Optical Density (OD) was measured using an ELISA plate reader at 600 nm. The bacterial reduction data obtained allowed for the plotting of time-kill curves to compare the bactericidal efficiency of the nanoparticle-based dental varnish, the commercial varnish and the untreated control.

Statistical Analysis

All experiments were performed in triplicate to ensure data reliability and reproducibility. Results are presented as mean values with Standard Deviation (SD) indicated by error bars to illustrate data variability.

For statistical analysis, Analysis of Variance (ANOVA) was employed to determine significant differences among groups, followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc comparisons. A p-value < 0.05 was considered statistically significant to confirm meaningful differences in antibacterial efficacy and other measured parameters.

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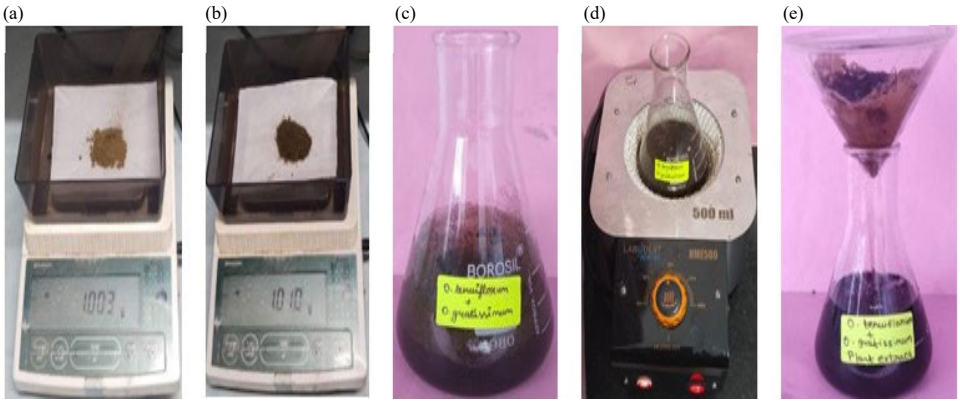

Figure 1(a-e): Preparation of herbal formulation based oral rinse, (a) *Ocimum tenuiflorum* powder, (b) *Ocimum grattissimum* powder, (c) Addition of both powders in 100 g distilled water, (d) Boiled using heating mantle and (e) Filtered herbal formulation

Figure 2(a-e): Green synthesis of AgNPs using *Ocimum tenuiflorum* and *Ocimum grattissimum* herbal formulation, (a) Herbal formulation, (b) Silver nitrate solution, (c) Green synthesized silver nanoparticles solution, (d) AgNPs pellet and (e) Green synthesized AgNPs based dental varnish

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Time-Kill Curve Analysis

A time-kill curve assay was conducted to evaluate the bactericidal effect of the AgNP-based dental varnish on *S. mutans* and *Lactobacillus* sp. over a 5-hour period.

An inoculum standardized to 10 CFU/mL was treated with the AgNP-based dental varnish at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL. A commercial dental varnish served as the control, alongside an untreated bacterial inoculum as the negative control.

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For statistical analysis, Analysis of Variance (ANOVA) was employed to determine significant differences among groups, followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc comparisons. A p-value <0.05 was considered statistically significant to confirm meaningful differences in antibacterial efficacy and other measured parameters.

RESULT

Antibacterial Activity

Agar Well Diffusion Technique

The antibacterial activity of the *Ocimum tenuiflorum* and *Ocimum gratissimum* herbal formulation-based dental varnish was systematically evaluated against various oral pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Escherichia coli*, utilizing the agar well diffusion technique (Figure 3, 4).

Inhibition zones were precisely measured in millimeters at different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) and compared with a commercial dental varnish serving as the standard control. The herbal formulation-based dental varnish demonstrated significant antibacterial efficacy, with *S. aureus* showing inhibition zones of 10 mm, 14 mm and 26 mm at concentrations of 25 µg/mL, 50 µg/mL and 100 µg/mL, respectively, outperforming the commercial varnish, which exhibited a 9 mm inhibition zone.

For *S. mutans*, the inhibition zones were measured at 24 mm, 27 mm and 34 mm across the same concentrations, substantially exceeding the 9 mm inhibition zone observed for the standard varnish. Similarly, *Lactobacillus* sp. showed inhibition zones of 20 mm, 22 mm and 31 mm, markedly larger than the 9 mm zone produced by the standard. *E. faecalis* displayed inhibition zones of 9 mm, 11 mm and 14 mm, with the highest concentration reaching the 9 mm zone of the commercial varnish.

Notably, *E. coli* demonstrated the most significant inhibition zones, measuring 27 mm, 30 mm and 35 mm, substantially surpassing the 9 mm zone of the standard dental varnish. These results indicate that the *Ocimum tenuiflorum* and *Ocimum gratissimum* herbal formulation-based dental varnish possesses potent antibacterial properties, with the highest efficacy observed at 100 µg/mL.

This highlights its potential as a natural and effective alternative to conventional commercial dental varnishes, offering a promising approach to enhancing oral health through the inhibition of pathogenic bacteria.

Time Kill Curve Assay

The antimicrobial efficacy of the AgNPs-based dental varnish was assessed using time-kill curve analysis against a range of oral pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp., *Enterococcus faecalis* and *Escherichia coli* which was represented in Figure 4. The effectiveness of different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) of the dental varnish was compared against a standard commercial product and a control by measuring the optical density (OD) over a period of 5 hours.

The optical density measurements for *S. aureus* (Figure 5a) indicated a progressive decrease in bacterial growth with increasing concentrations of the AgNPs-based dental varnish. The 100 µg/mL concentration showed a

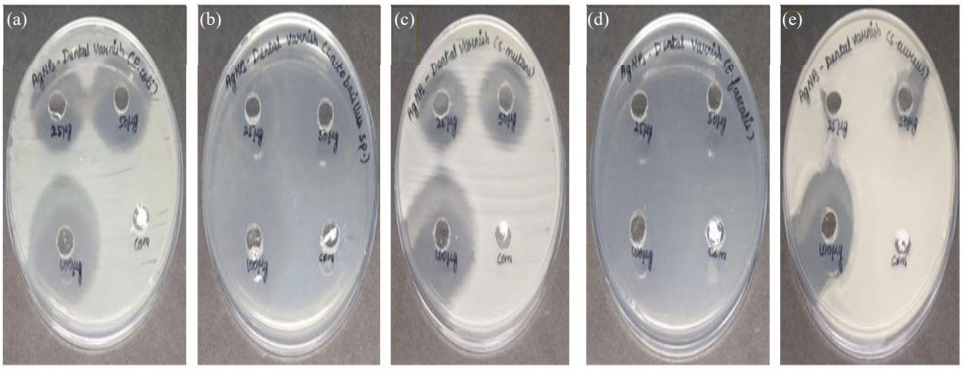

Figure 3(a-e): Antibacterial activity of AgNPs based dental varnish tested using agar well diffusion technique, (a) *E. coli*, (b) *Lactobacillus* sp., (c) *S.mutans*, (d) *E. faecalis* and (e) *S. aureus*

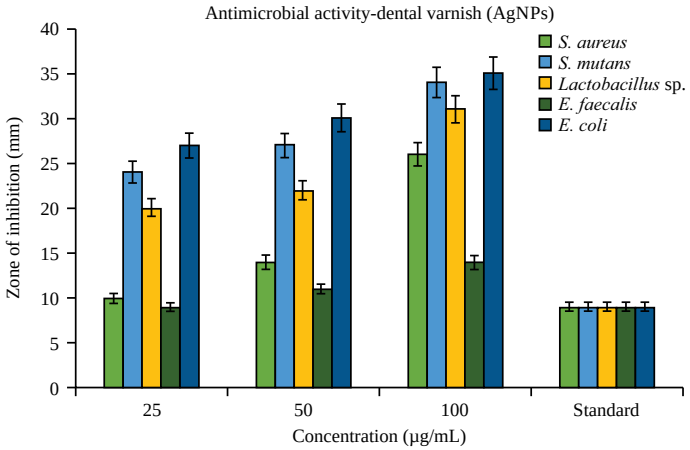

Figure 4: Zone of inhibition of green synthesized AgNPs based dental varnish treated against different oral pathogens

significant reduction in OD over time, closely aligning with the standard treatment. Specifically, the OD values decreased from 0.65 at the start to 0.52 after 5 hours for the 100 µg/mL concentration. In contrast, the control maintained a stable OD, increasing slightly to 0.72 after 5 hours, indicating ongoing bacterial growth.

The AgNPs-based dental varnish demonstrated effective inhibition of *S. mutans* (Figure 5b), with the 100 µg/mL concentration showing the greatest reduction in optical density, similar to the standard. The OD values for the 100 µg/mL concentration reduced from 0.70 at the start to 0.61 after 5 hours. The control group showed consistent bacterial growth with OD values increasing to 0.74 over the same period.

Against *Lactobacillus* sp., (Figure 5c) the dental varnish exhibited a marked bactericidal effect at higher concentrations. The 100 µg/mL concentration reduced the OD from 0.68 to 0.55 over 5 hours, while the standard also showed a similar reduction in bacterial density. Lower concentrations (25 µg/mL and 50 µg/mL) displayed moderate decreases in OD. The control group's OD remained relatively stable, indicating continuous bacterial proliferation.

The time-kill curve analysis for *E. faecalis* (Figure 5d) revealed that the 100 µg/mL concentration of AgNPs-based varnish significantly reduced OD from 0.67 to 0.52 over 5 hours, closely paralleling the standard treatment's efficacy. Lower concentrations (50 µg/mL and 25 µg/mL) also resulted in reduced OD values but to a lesser extent. The control group's OD increased to 0.73, highlighting ongoing growth.

In the case of *E. coli* (Figure 5e), the AgNPs-based dental varnish at 100 µg/mL concentration demonstrated substantial antibacterial activity, reducing OD from 0.68 at the start to 0.54 after 5 hours. This was comparable to the standard treatment. The control maintained a stable OD around 0.75, indicating no inhibition of bacterial growth.

Among all the pathogens, E. coli and S. mutans exhibited the highest inhibition, particularly at the 100 µg/mL concentration, showing significant reductions, revealing the potent bactericidal effect of the herbal dental varnish.

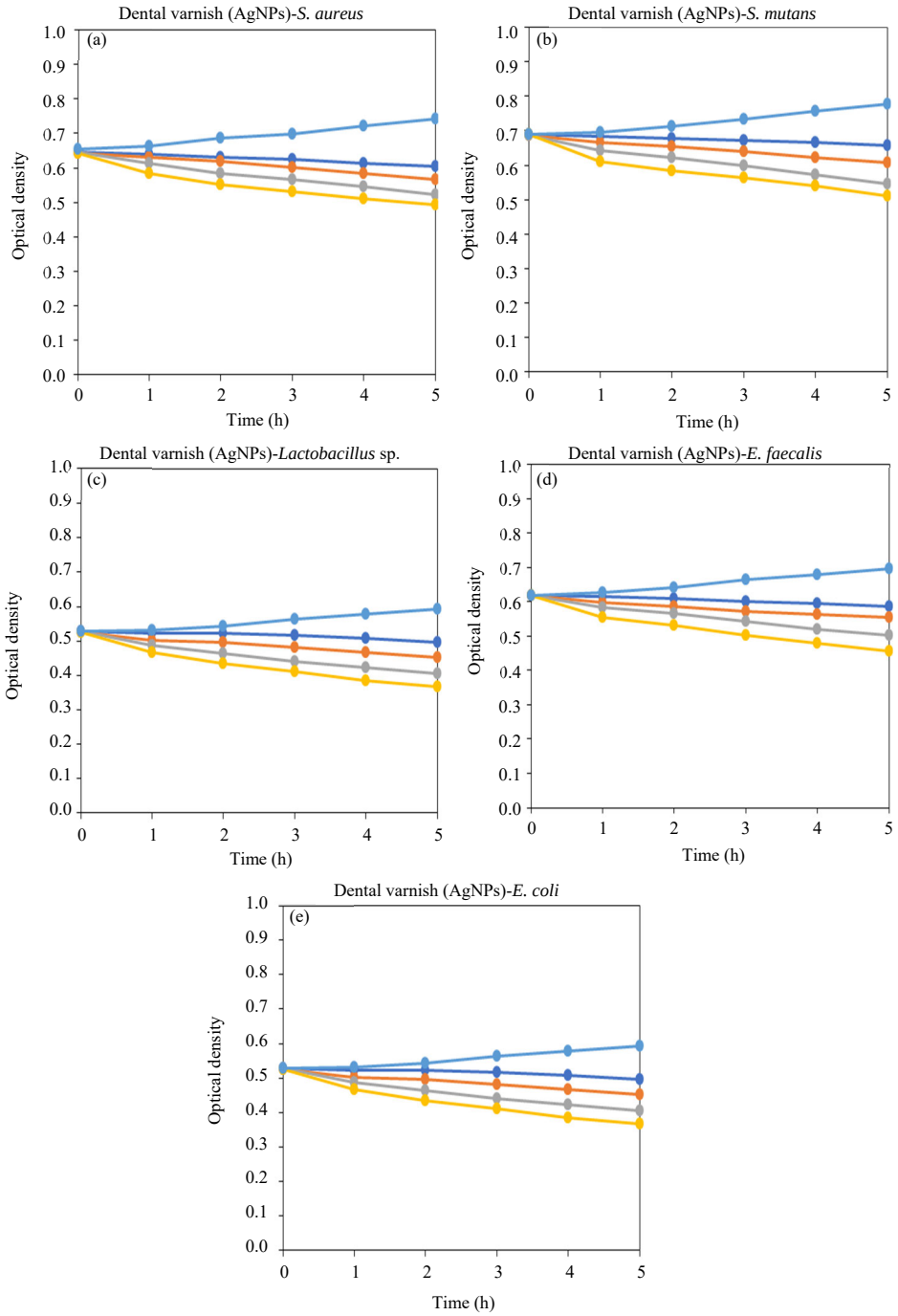

Figure 5: Time kill curve analysis of AgNPs based dental varnish against different spectrum of oral pathogens, (a) *S. aureus* (b) *S. mutans* (c) *Lactobacillus* sp., (d) *E. faecalis* and (e) *E. coli*

These results indicate that the herbal formulation demonstrates strong bactericidal activity across all tested pathogens, particularly at higher concentrations, showcasing its potential as a natural and effective alternative to commercial dental varnishes in reducing pathogenic bacterial counts over time.

Figure 6 shows Excitation Emission spectrum for Sample in the variable range of 200 to 600 nm. The 3D contour plot from the excitation-emission matrix suggests the presence of multiple photophysical processes within the ZnO-Ag nanoparticle system, though these interpretations remain tentative and require further confirmation.

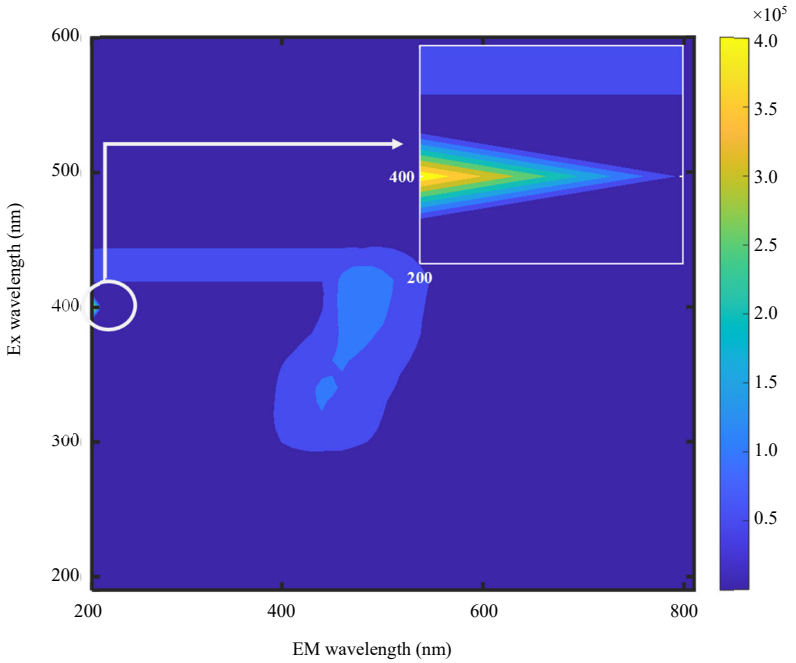

Figure 6: Excitation emission matrix of the sample

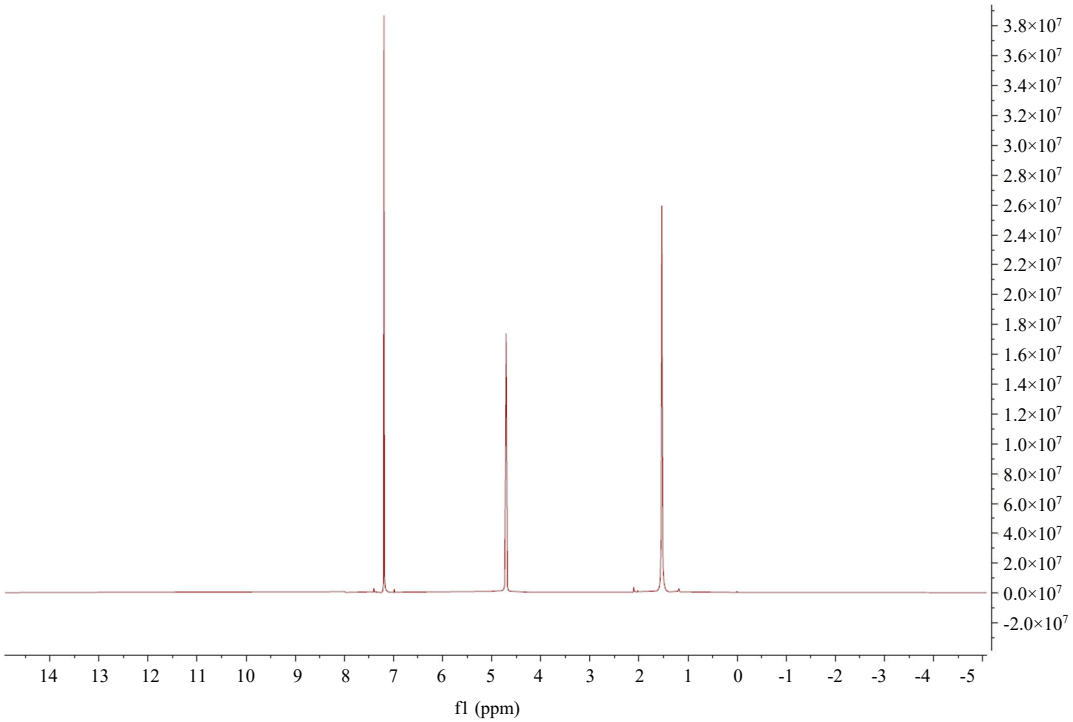

Figure 7: ^{1}H NMR of sample

Figure 7 represents ^{1}H NMR recorded on a Bruker Avance 500 MHz spectrometer in $CDCl_3$ solvent at room temperature.

The observed zeta potential of -10 mV (as shown in Table 1) corresponds to low colloidal stability, indicating a tendency for aggregation. Figure 8 represent particle size

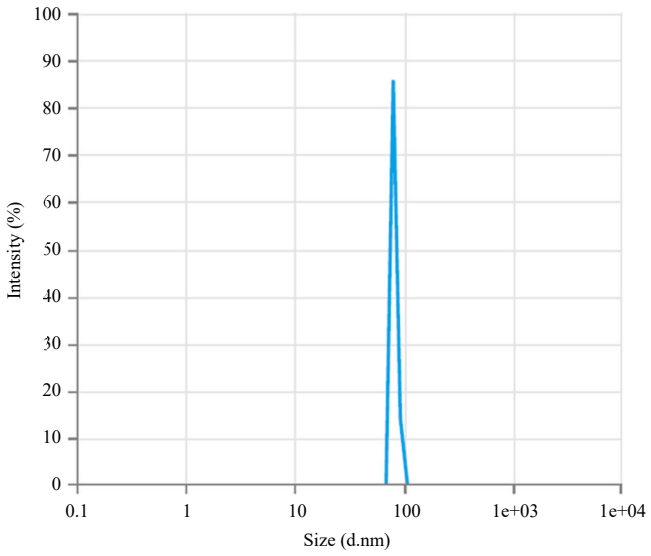

Figure 8: Particle size distribution for the sample

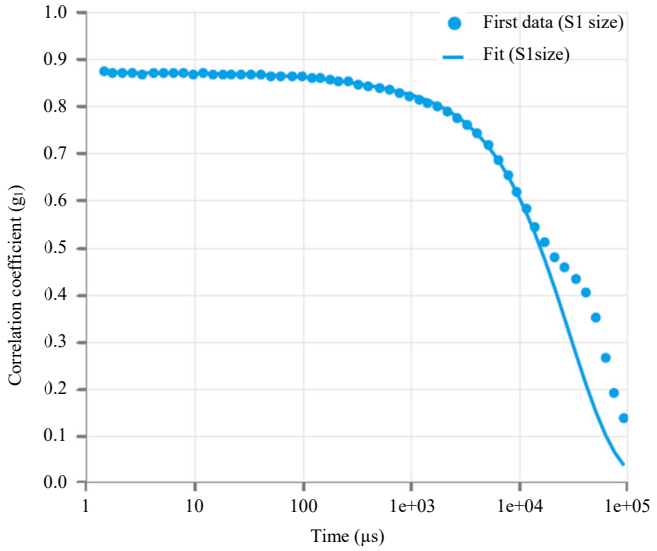

Figure 9: Correlation coefficient (g_1) for the sample

Table 1: Zeta potential, particle size, conductivity and polydispersity index of the sample

S. no.	Parameter	Mean value
1.	Zeta Potential (mV)	-10.63
2.	Conductivity (mS/cm)	0.6713
3.	Particle Size(µm)	7.98
4.	Polydispersity Index (PI)	0.7732

distribution of the sample with average mean particle size of 7.98 µm. This reflects significant agglomeration of the nanoparticles in the sample.

Figure 9 shows a correlation coefficient indicating a polydispersity index (PDI) of 0.7732, reflecting a highly polydisperse system.

Figure 10 presents the UV-visible absorption spectrum of the sample, recorded in the range of 200-600 nm, showing a distinct absorption peak at 223 nm.

DISCUSSION

The present study evaluated the antibacterial efficacy of a dental varnish formulated with *Ocimum tenuiflorum* and *Ocimum gratissimum* mediated silver nanoparticles (AgNPs) against key oral pathogens. Using both the agar well diffusion method and the time-kill curve assay, the results demonstrated that the AgNP-based dental varnish exhibited significant antibacterial activity against *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* sp.,

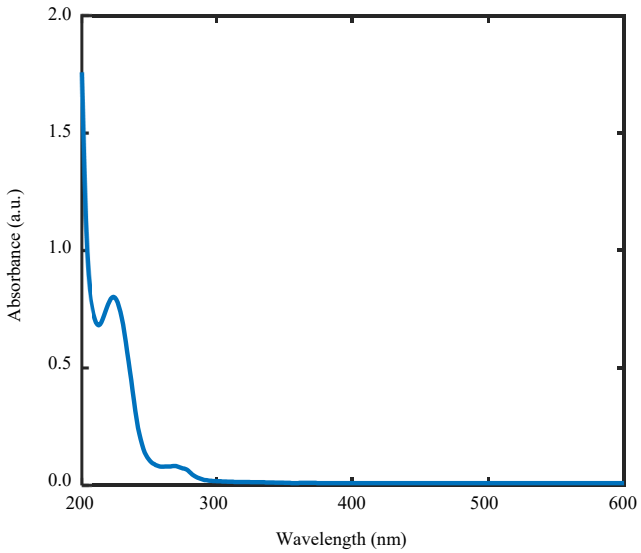

Figure 10: UV-Vis absorbance spectra for the sample

Enterococcus faecalis and *Escherichia coli*. The AgNP varnish consistently showed larger zones of inhibition than the commercial varnish across all tested concentrations, indicating enhanced antibacterial efficacy [15]. Notably, the highest concentration of 100 µg/mL resulted in the most substantial inhibition zones, reinforcing the potent bactericidal potential of the green-synthesized AgNP varnish [12].

The enhanced antibacterial activity can be attributed to the unique properties of silver nanoparticles. AgNPs synthesized using *Ocimum tenuiflorum* and *Ocimum gratissimum* release silver ions that induce oxidative stress, disrupt bacterial cell membranes and interfere with essential cellular functions. These actions ultimately compromise bacterial cell integrity and promote bacterial death. The strong antibacterial potential of AgNPs against oral pathogens such as *S. mutans*, *E. faecalis* and *S. aureus* is consistent with previous findings that highlight AgNPs' superior antimicrobial capabilities [16].

Compared to conventional antibacterial agents, silver nanoparticles present a valuable alternative due to their ability to combat antibiotic resistance, lower cytotoxicity and strong antibacterial efficacy even at low concentrations [17]. The combined action of silver ions, reactive oxygen species (ROS) generation and nanoparticle-induced membrane disruption makes AgNPs an effective tool against oral pathogens [18].

The findings of this study align with earlier research on fluoride varnishes, which have demonstrated antimicrobial efficacy against *S. mutans* and *Lactobacillus acidophilus*. However, while fluoride varnishes primarily target caries-related pathogens, the Ocimum-mediated AgNP varnish effectively inhibited a broader spectrum of bacteria, showcasing its potential as a comprehensive antibacterial solution in dental care [19]. Herbal formulations such as *Ocimum tenuiflorum* and *Ocimum gratissimum* have demonstrated antimicrobial potential in previous studies and their incorporation into silver nanoparticles further strengthens their antibacterial capabilities [20,21].

The present study's findings are consistent with research highlighting AgNPs' antimicrobial role in dental materials. Studies incorporating green-synthesized AgNPs in pit and fissure sealants have demonstrated significant antibacterial effects against cariogenic bacteria such as *S. mutans* [22]. This reinforces the potential of Ocimum-mediated AgNPs in improving oral health outcomes through enhanced antimicrobial action in dental varnish formulations [23].

The fluorescent analysis provided additional insights into the material's photophysical properties. The first contour observed at excitation and emission wavelengths of approximately 422 nm and 200 nm may indicate surface or quantum effects influenced by the interaction of ZnO and Ag nanoparticles [24]. A second contour at excitation 422 nm and emission 448 nm, with reduced intensity, is suggestive of defect-related luminescence, possibly due to oxygen vacancies or zinc interstitials within the ZnO structure [25]. These observations highlight the intricate interactions between ZnO and Ag nanoparticles in modifying fluorescence properties. While these findings point to potential energy transfer processes, complementary analysis is required to confirm these mechanisms [26].

In the NMR study, ZnO and Ag, being inorganic, did not generate direct NMR signals. However, their presence influenced the chemical environment of surrounding molecules. The observed spectrum showed a prominent signal at approximately 4.8 ppm, corresponding to the protons of water (H_2O). This peak may reflect interactions between water molecules and the surfaces of ZnO or Ag nanoparticles,

possibly influenced by hydrogen bonding or electrostatic interactions. Such environmental effects are consistent with the ability of nanoparticles to influence proton signals in NMR spectra.

The analysis of particle size distribution revealed significant variation, indicating a polydisperse system with notable heterogeneity. This inconsistency may be linked to nanoparticle aggregation, insufficient stabilization, or variability in the synthesis process. Future refinement of the synthesis method may improve particle size uniformity to enhance the dental varnish's consistency and performance.

The UV-Vis spectroscopy analysis demonstrated a characteristic absorption peak at 223 nm, which is commonly associated with ZnO nanoparticles. The presence of this peak indicates electronic transitions linked to ZnO's wide bandgap, although potential Ag-ZnO interactions may have also influenced this result. Additional studies are recommended to explore these interactions further and confirm the origins of the observed spectral features.

CONCLUSION

This study demonstrated that a dental varnish formulated with Ocimum-mediated silver nanoparticles exhibits substantial antibacterial activity against a range of oral pathogens, outperforming a commercially available dental varnish in bacterial inhibition. The AgNP-infused varnish, particularly at the highest concentration of 100 µg/mL, displayed significant zones of inhibition against *S. aureus*, *S. mutans*, *Lactobacillus* sp., *E. faecalis* and *E. coli*. The time-kill curve assay further confirmed the varnish's bactericidal efficacy, showing substantial reductions in bacterial counts within a short period.

The fluorescence analysis indicated possible surface interactions and quantum effects related to ZnO and Ag nanoparticles. NMR analysis revealed proton shifts suggesting nanoparticle-induced environmental changes, while UV-Vis spectroscopy identified characteristic absorption peaks associated with ZnO.

The findings collectively highlight the potential of Ocimum-mediated AgNPs as a powerful and natural alternative to conventional dental varnishes. This novel formulation not only demonstrates superior antibacterial properties but also aligns with sustainable green synthesis practices, offering a safer and more effective dental care solution.

Overall, the Ocimum-derived AgNP dental varnish represents a significant advancement in antimicrobial dental materials, reinforcing the importance of innovative, eco-friendly strategies in improving oral healthcare outcomes. Further research will help unlock its full potential for commercial dental applications and long-term patient benefits.

Implications and Future Research

The promising results from this study suggest that the Ocimum-mediated AgNP dental varnish holds significant potential as a natural and effective alternative to conventional fluoride varnishes. Its strong antibacterial properties, particularly at higher concentrations, highlight its ability to enhance oral health by inhibiting key pathogens responsible for dental caries and periodontal infections.

Future research should focus on exploring the varnish's long-term stability, cytotoxicity and safety in clinical applications. In vivo studies are essential to assess the varnish's efficacy in a real-world setting, ensuring it maintains its antimicrobial properties without adverse effects. Investigating the varnish's impact on biofilm formation, enamel remineralization and its potential for sustained antibacterial action over time would further support its clinical relevance.

Additionally, understanding the precise mechanisms driving the antibacterial activity of Ocimum-mediated AgNPs could provide valuable insights into their behavior in oral environments. Examining the effects of varying nanoparticle sizes, concentrations and formulation parameters will help optimize the varnish's efficacy and ensure safety for long-term use.

Ethical Considerations

The study was conducted following the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethical Committee. Written informed consent was obtained from all participants prior to sample collection. Participants were provided with detailed information regarding the study's objectives, methods and potential risks. Confidentiality of participant data was strictly maintained throughout the research process.

Conflict of Interest

The authors declare no conflict of interest related to this study.

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