RESEARCH PAPER

Anti-inflammatory activity of dental varnish prepared using Ocimum tenuiflorum and Ocimum gratissimum assisted silver nanoparticles

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ABSTRACT

Materials and Methods

Leaf extracts of Ocimum tenuiflorum and Ocimum gratissimum were utilized to produce silver nanoparticles through a green synthesis technique by reacting the extracts with a silver nitrate solution. The synthesized nanoparticles were evaluated using UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM) to confirm their formation and structural characteristics. After characterization, the prepared AgNPs were incorporated into a dental varnish base at three different concentrations: 1%, 3%, and 5%. The anti-inflammatory potential of each formulation was tested using the protein denaturation method, while diclofenac sodium was used as the reference standard. Statistical comparison between groups was performed using one-way ANOVA to identify significant differences.

Results

The AgNP-containing dental varnish demonstrated concentration-dependent anti-inflammatory activity in all experimental models. In the bovine serum albumin assay, inhibition increased from 43% at 10 µg/mL to 80% at 50 µg/mL. In the egg albumin assay, inhibition ranged from 49% to 78%, while membrane stabilization activity increased from 41% to 79% across the tested concentrations. The formulation exhibited activity approaching that of the standard anti-inflammatory drug at higher concentrations.

Conclusion

The findings suggest that dental varnish prepared with silver nanoparticles synthesized using Ocimum tenuiflorum and Ocimum gratissimum possesses strong anti-inflammatory properties. This formulation may serve as a useful natural adjunct in the management of inflammatory oral diseases such as gingivitis and periodontitis.

Keywords: Ocimum tenuiflorum, Ocimum gratissimum, silver nanoparticles, dental varnish, gingivitis, periodontitis, anti-inflammatory activity, ANOVA.

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Introduction

Inflammation is considered a major contributing factor in the development and progression of several oral disorders such as gingivitis, periodontitis, and even dental caries (Neville et al., 2018). In recent years, increasing concerns regarding antibiotic resistance, along with undesirable effects caused by many commonly used chemical agents, have encouraged researchers to explore safer and more natural alternatives (Himmelfarb and Alp Ikizler, 2018). As a result, medicinal plants have gained significant attention due to their wide range of therapeutic benefits, particularly their ability to control microbial growth and reduce inflammatory responses (Arjunan, 2017).

Among these plants, Ocimum tenuiflorum (Holy Basil/Tulsi) and Ocimum gratissimum (African Basil/Clove Basil) have long been valued in traditional systems of medicine because of their strong medicinal potential (Sneha et al., 2022). These species contain a variety of biologically active compounds, including flavonoids, eugenol, rosmarinic acid, and several other phenolic constituents, which are known to possess antimicrobial, anti-inflammatory, and antioxidant properties (Ashokkumar et al., 2024). Such phytochemicals are believed to reduce inflammation by suppressing the release of inflammatory mediators, while also supporting tissue repair, healing, and regeneration within the oral environment (Chakraborty and Ramamurthy, 2024).

In addition, the use of plant extracts for synthesizing metallic nanoparticles has recently emerged as an effective and

environmentally friendly technique, commonly referred to as green synthesis (Patra et al., 2020). Silver nanoparticles (AgNPs) have been widely studied due to their strong antibacterial action and their ability to influence inflammatory processes, making them particularly useful for biomedical and dental applications (Shanker, Rani and Hussain, 2021). During the synthesis process, the phytochemicals found in O. tenuiflorum and O. gratissimum act as natural reducing as well as stabilizing agents, allowing the formation of nanoparticles with enhanced biological activity and potentially reduced toxic effects compared to chemically synthesized products (Rao, 2012).

Dental varnishes are commonly used as topical coatings that adhere to the tooth surface and allow controlled local delivery of therapeutic agents for extended periods (Xuedong, 2016). When silver nanoparticles synthesized through plant-based methods are incorporated into a varnish system, they may offer a unique approach for limiting microbial colonization and regulating inflammatory reactions linked to oral infections (Xuedong, 2016). Such formulations may contribute to improved periodontal outcomes by lowering plaque buildup, reducing bacterial load, and supporting better oral hygiene maintenance.

This study therefore concentrates on the preparation and assessment of a dental varnish containing silver nanoparticles synthesized using Ocimum tenuiflorum and Ocimum gratissimum extracts, with special emphasis on its anti-inflammatory activity. By combining the therapeutic properties of these Ocimum species with the established antimicrobial potential of AgNPs, the research attempts to develop a biocompatible and innovative dental varnish formulation that may have significant clinical relevance in managing oral inflammatory conditions (Xuedong, 2016; Prakash, 2020).

Materials and methods

Preparation of Herbal Formulation

To prepare the herbal extract, 1 g each of Ocimum tenuiflorum and Ocimum gratissimum was added to 100 mL of distilled water and mixed thoroughly. The mixture was then heated using a heating mantle at approximately 60°C for about 15–20 minutes until it reached a boiling stage. After heating, the solution was allowed to cool slightly and was filtered slowly through filter paper to remove plant residues. The collected filtrate containing the herbal extract was preserved and used further for the synthesis of silver nanoparticles.

Green Synthesis of ZnONPs and AgNPs

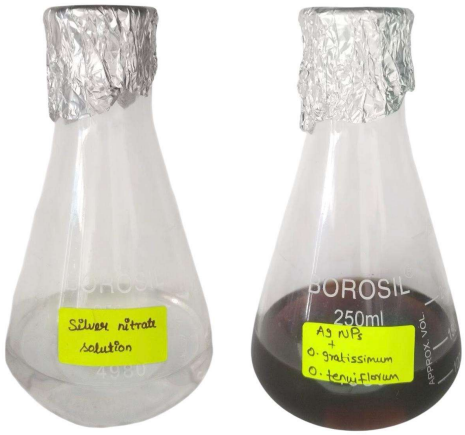
In this study, zinc oxide nanoparticles (ZnONPs) and silver nanoparticles (AgNPs) were synthesized using an environmentally safe green synthesis method involving plant extracts of African basil (Ocimum gratissimum) and black tulsi (Ocimum tenuiflorum). These plants were selected due to their high phytochemical content, which can act as natural reducing as well as stabilizing agents during nanoparticle formation.

For the preparation of ZnONPs, a 30 mM zinc nitrate solution was made by dissolving the required amount of zinc nitrate in 50 mL of distilled water. This solution was then mixed with 50 mL of the combined herbal extract obtained from both plant leaves.

For AgNP synthesis, a 1 mM silver nitrate solution was prepared using 80 mL of distilled water, and 20 mL of the filtered herbal extract was added gradually to initiate the reduction process.

After the reaction, both mixtures were centrifuged at 8000 rpm for 10 minutes. This step was essential for separating the synthesized nanoparticles from leftover reactants and plant residues. The pellets formed after centrifugation represented

the ZnONPs and AgNPs, which were then collected and stored for further characterization and experimental evaluation.



Formulation of Dental Varnish

The synthesized silver nanoparticles obtained using Ocimum tenuiflorum and Ocimum gratissimum extracts were blended into a commercially available dental varnish base. The AgNPs were added at a final concentration of 1% (w/w) and mixed continuously using a magnetic stirrer to achieve uniform dispersion throughout the varnish. After preparation, the varnish formulation was transferred into airtight containers and stored at room temperature for further experimental use.

Cytotoxicity Assay (MTT Assay)

RAW 264.7 murine macrophage cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) along with 1% penicillin–streptomycin. The cells were maintained at 37°C under humidified conditions with 5% CO_2. For the cytotoxicity study, the cells were seeded into 96-well culture plates at a concentration of 1×10^4 cells per well and allowed to attach by incubating overnight.

After incubation, the cells were exposed to varying concentrations (10, 25, 50, and 100 µg/mL) of extract obtained from the AgNP-loaded dental varnish for a period of 24 hours. Following the treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were kept for 4 additional hours to allow the formation of formazan crystals by metabolically active cells. The formed crystals were then dissolved using dimethyl sulfoxide (DMSO), and the optical density was recorded at 570 nm using a microplate reader. Cell viability was determined by comparing absorbance values, thereby evaluating the cytotoxic potential of the varnish extract.

Anti-inflammatory Activity

The anti-inflammatory effectiveness of the green-synthesized silver and zinc oxide nanocomposite dental varnish was assessed and compared with a commercially available dental varnish. The evaluation was carried out using three standard in vitro methods, namely the bovine serum albumin (BSA) protein denaturation assay, the egg albumin denaturation assay, and the red blood cell (RBC) membrane stabilization test.

Statistical Analysis

All tests were conducted in triplicate, and the obtained values were presented as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way ANOVA, followed by Tukey's post hoc analysis to identify significant variations. Differences were considered statistically significant when the p-value was below 0.05.

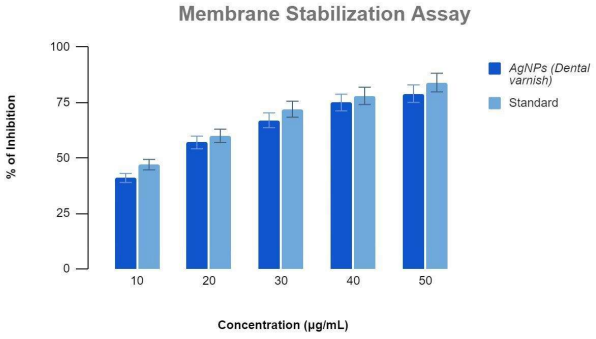
Results and Discussion

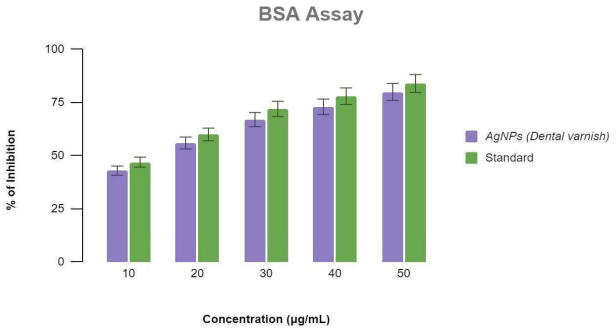
The findings of the present investigation are consistent with previous reports describing the anti-inflammatory potential of Ocimum-derived silver nanoparticles. The concentration-dependent inhibition observed in the BSA, egg albumin and membrane stabilization assays suggests that the bioactive phytochemicals present in Ocimum tenuiflorum and Ocimum gratissimum may act synergistically with silver nanoparticles. The developed varnish exhibited substantial activity across all tested concentrations and approached the effectiveness of the

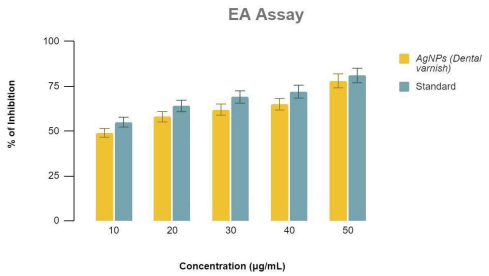
standard drug at higher concentrations. The incorporation of silver nanoparticles into a varnish delivery system may also facilitate prolonged contact with oral tissues and sustained local activity.

The anti-inflammatory potential of the dental varnish containing silver nanoparticles synthesized using Ocimum tenuiflorum and Ocimum gratissimum was evaluated using commonly accepted inhibition-based in vitro methods, including the protein denaturation assay and the membrane stabilization test (Patra et al., 2020). The formulation showed a clear dose-dependent response, with increased activity observed at higher concentrations of the nanoparticle varnish.

In the protein denaturation assay, the varnish demonstrated inhibition values ranging from nearly 50% at lower concentrations to about 80% at higher concentrations. These results were comparable to the standard anti-inflammatory drug diclofenac, which produced approximately 60% inhibition under similar conditions (Tatsuma et al., 2023). Likewise, the membrane stabilization assay revealed that the AgNP-based varnish offered protection between 35% and 75% against heat-induced hemolysis, indicating appreciable membrane-stabilizing ability, though the effect was comparable or slightly lower when compared to the standard reference compound (Müller et al., 2022).







Characterization using UV–Visible spectroscopy confirmed the successful formation of silver nanoparticles, as shown by the appearance of a characteristic surface plasmon resonance peak between 420 and 450 nm (Kumar, Kumar and Pathak, 2021). When these biologically synthesized AgNPs were incorporated into the dental varnish base, the final formulation displayed satisfactory physical properties, including uniform nanoparticle distribution, suitable viscosity, and good adhesion to tooth surfaces (Subramani and Ahmed, 2011).

The overall findings suggest that the dental varnish formulated with Ocimum tenuiflorum and Ocimum gratissimum mediated silver nanoparticles possesses notable anti-inflammatory potential (Wani, Singh and Kumar, 2022). This effect may be attributed to the combined action of silver nanoparticles and the phytochemical constituents present in the plant extracts, such as eugenol, flavonoids, and other polyphenolic compounds, which are known to exhibit anti-inflammatory activity (Tomás-Barberán, González-Sarrías and García-Villalba, 2020; Wani, Singh and Kumar, 2022). The synergistic interaction between these bioactive molecules and AgNPs may contribute to enhanced therapeutic effectiveness.

The significant inhibition observed in protein denaturation and membrane stabilization assays indicates that the formulated varnish may help in reducing inflammatory responses, which is essential for controlling gingival and periodontal inflammation (Akhtar et al., 2023). Although the activity was slightly lower when compared with the standard drug, the formulation still demonstrated considerable effectiveness and offers an added benefit of antimicrobial action due to the presence of silver nanoparticles (Var and Uzunlu, 2019).

In addition, the green synthesis method employed using Ocimum species provides an advantage by reducing the potential toxicity associated with chemically synthesized nanoparticles, while improving the biocompatibility of the varnish (Gadore et al., 2024). The anti-inflammatory results obtained in this study also support previous literature reporting the medicinal importance of Ocimum tenuiflorum and Ocimum gratissimum, which have long been utilized in traditional practices for oral health management (Anandachockalingam et al., 2024).

Overall, the incorporation of plant-mediated silver nanoparticles into dental varnish formulations appears to be a promising strategy for developing multifunctional and biocompatible oral care products. Such formulations may play a useful role in reducing oral inflammation and simultaneously

preventing bacterial colonization, thereby supporting improved oral health outcomes (Hashim, 2012).

Limitations

The present study focused primarily on the biological evaluation of the formulated dental varnish. Advanced physicochemical characterization and additional in vivo investigations were beyond the scope of the current work. Future studies incorporating comprehensive characterization and clinical validation are recommended.

Conclusion

The results of the present study indicate that the dental varnish prepared using silver nanoparticles synthesized with Ocimum tenuiflorum and Ocimum gratissimum extracts shows marked anti-inflammatory activity. This effect is likely due to the combined action of the phytochemical constituents present in both Ocimum species along with the inherent biological properties of silver nanoparticles, which together may help suppress inflammatory responses. The findings highlight the potential of this formulation as a natural and effective anti-inflammatory option for oral healthcare use. However, further in vivo investigations and well-designed clinical trials are necessary to confirm its safety, therapeutic effectiveness, and suitability for routine dental applications.

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Statement of conflict of interest:

The author declares that there was no conflict of interest in the present study

Ethical Approval:

The present investigation was an in vitro laboratory-based study involving the synthesis and evaluation of plant-mediated silver nanoparticles incorporated into a dental varnish formulation. No human participants, patient samples, or live animals were involved. Therefore, formal institutional ethical committee approval was not required.

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