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ENHANCED ANTI-MICROBIAL EFFICACY OF LAVENDER EXTRACTS DERIVED SILVER NANOPARTICLES AGAINST CARIOGENIC MICROORGANISMS

Samruddhi Gandhi [1], Sangeetha S [2], Taniya M [3] and M Sundaram K [4*]

[1,2,3,4] Department of Anatomy, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University,
Poonamalle High Road, Velappanchavadi, Chennai.
*Corresponding Author Email: meenakshisundaram.sdc@saveetha.com

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Abstract

This study investigates the synthesis, characterization, and antimicrobial properties of Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) against cariogenic microorganisms. Certain bacteria are primary contributors to dental caries. Lavender extract's biofilm-targeting capabilities, combined with the potent antimicrobial effects of silver nanoparticles, offer a novel approach for caries prevention and treatment. Lav-AgNPs were synthesized and characterized using UV-Vis spectroscopy and FTIR to confirm size, shape, and stability. Antibacterial efficacy was assessed through assays, showing significant inhibition of bacteria. These interactions suggest that Lav-AgNPs inhibit enzyme activity, impairing bacterial metabolism and biofilm development. This study highlights the synergistic effects of lavender extracts and silver nanoparticles, providing a foundation for developing advanced therapeutic strategies against dental caries through targeted experimental methods and bacterial inhibition.

Keywords: Lavender Extracts, Silver Nanoparticles, Cariogenic Microorganisms, Antimicrobial Properties, Biofilm Inhibition, Glucosyltransferases, Lactate Dehydrogenase, Dental Caries.

1. INTRODUCTION

Dental caries, commonly known as tooth decay, is a widespread oral health issue affecting a significant portion of the global population(Bagramian, Garcia-Godoy et al. 2009). This multifactorial disease results from the demineralization of tooth enamel due to the acid production by cariogenic microorganisms. These microorganisms, particularly Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus, colonize the oral cavity, forming biofilms on tooth surfaces(Tonetti, Bottenberg et al. 2017, Wen, Chen et al. 2022). Biofilms are complex communities of microorganisms adhering to surfaces and encased in an extracellular polymeric substance matrix, which provides protection against environmental stresses and antimicrobial agents.

Candida albicans, a fungus commonly residing in the mucosal niches of the human body, is often identified in the biofilms that form on the teeth of toddlers suffering from severe childhood caries. This condition, characterized by rampant tooth decay, represents a significant global public health issue(Petersen 2009, Marunganathan, Kumar et al. 2024). The presence of Candida albicans in dental plaque exacerbates the progression of caries by enhancing biofilm resilience and contributing to the acidic environment that promotes enamel demineralization. Understanding the role of Candida albicans in these biofilms is crucial for developing targeted strategies to prevent and treat severe childhood caries, aiming to mitigate its impact on affected populations(Senthil, Sundaram et al. 2022, Wen, Chen et al. 2022).

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Specific types of acid-producing bacteria, particularly Streptococcus mutans, colonize the dental surface and initiate damage to the hard tooth structure when exposed to fermentable carbohydrates like sucrose and fructose. These bacteria metabolize these sugars, producing acids as byproducts, which lower the pH in the oral environment(Petersen 2004, Selwitz, Ismail et al. 2007). The acidic conditions lead to the demineralization of tooth enamel, creating cavities and advancing the progression of dental caries(Miglani 2020, Senthil, Sundaram et al. 2022).

The ability of Streptococcus mutans to thrive in such environments and form resilient biofilms makes it a primary contributor to tooth decay, underscoring the importance of controlling dietary sugar intake and maintaining oral hygiene to prevent caries. The primary cause of endodontic failure is the presence of certain bacterial species within the root canal system, notably Enterococcus faecalis. These bacteria exhibit high resistance to common disinfection agents, leading to persistent intra-radicular or extra-radicular infections (Balaji, Bhuvaneswari et al. 2022, Sundaram, Bupesh et al. 2022).

E. faecalis can survive in harsh conditions, including nutrient-depleted environments, and often forms resilient biofilms that protect it from antimicrobial treatments. This persistence contributes to the failure of root canal therapy, necessitating advanced and targeted disinfection strategies to effectively eradicate these resilient bacterial populations and ensure successful endodontic outcomes.

Escherichia coli, typically associated with faecal contamination, is not a resident but rather a transient member of the oral microbiota(Edelstein 2006). Its occasional presence in the oral cavity may indicate environmental exposure to contaminated sources. The isolation of E. coli in the oral cavity raises concerns about potential faecal-oral transmission routes for pathogenic organisms. Staphylococcus aureus is commonly acknowledged as part of the oral flora, although its exact role in oral health and disease remains a subject of debate and ongoing research.

While traditionally associated with skin and nasal carriage, staphylococci can transiently colonize the oral cavity through various environmental exposures. Their presence in the oral microbiota suggests a potential influence on oral health, yet their specific contributions—whether beneficial, neutral, or potentially harmful—are still not fully understood.

The biofilms protect the bacteria from environmental stresses and antimicrobial agents, making them challenging to eradicate. As a result, innovative approaches to prevent and treat dental caries are necessary, particularly those that can effectively target and disrupt biofilms while minimizing the risk of antibiotic resistance. In recent years, silver nanoparticles (AgNPs) have gained attention for their potent antimicrobial properties.

AgNPs are effective against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. Their antimicrobial action is primarily attributed to their ability to disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with microbial DNA and protein functions.

These mechanisms collectively contribute to the bactericidal effects of AgNPs, making them a promising candidate for combating dental caries(Nasim, Rajeshkumar et al. 2021).

Lavender extracts are known for their biofilm-targeting capabilities and antimicrobial properties and Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) offer a promising strategy to combat dental caries by leveraging the synergistic effects of both lavender extracts and silver nanoparticles(Nasim, Kumar et al. 2020). Lavender extracts are rich in bioactive compounds such as linalool, linalyl acetate, and rosmarinic acid, which possess inherent antimicrobial properties and the ability to disrupt biofilm formation.

These compounds enhance the stability and efficacy of silver nanoparticles, which are known for their broad-spectrum antimicrobial action(Nasim, Jabin et al. 2022, Ponmanickam, Gowsalya et al. 2022). Silver nanoparticles disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with microbial DNA and protein functions, leading to cell death. By combining these nanoparticles with lavender extracts, the resultant Lav-AgNPs can more effectively penetrate and dismantle biofilms, a critical factor in the progression of dental caries.

This enhanced biofilm disruption capability ensures that the antimicrobial agents reach the underlying bacteria more efficiently, thereby reducing the bacterial load and mitigating the acid production that leads to enamel demineralization. Moreover, the natural origin of lavender extracts provides an added advantage of reduced toxicity and biocompatibility, making Lav-AgNPs a safer alternative for long-term dental applications(Umapathy, Pan et al. 2024).

This innovative approach not only targets the biofilms formed by cariogenic microorganisms but also minimizes the risk of developing antibiotic resistance, a significant concern with conventional antimicrobial treatments. The antimicrobial action of silver nanoparticles is well-documented. AgNPs exert their effects through multiple mechanisms: they disrupt microbial cell membranes, leading to cell lysis; they generate ROS, which cause oxidative stress and damage to cellular components; and they interfere with microbial DNA and protein functions, inhibiting replication and metabolic processes. When combined with lavender extracts, the targeting and binding efficiency of AgNPs to microbial biofilms is potentially enhanced. Lavender extracts' ability to bind to amyloid-like structures within biofilms can facilitate the delivery of AgNPs directly to the biofilm matrix, improving their antimicrobial efficacy(Duraisamy, Ganapathy et al. 2021).

2. MATERIALS AND METHODS

2.1 Synthesis of Lavender Extracts derived Silver Nanoparticles (Lav-AgNPs)

To synthesize Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs), a silver ion solution was prepared by dissolving 0.1 mM silver nitrate ($AgNO3$) in deionized water, and separately, a 0.1 mM lavender extract solution was also prepared. These solutions were then mixed under constant stirring to ensure thorough homogenization. Subsequently, a freshly prepared 0.1 M sodium borohydride solution was added dropwise to the mixture while vigorously stirring to initiate the reduction of silver ions, leading to the formation of Lav-AgNPs. Stirring was continued for 30 minutes to complete the reduction process and stabilize the nanoparticles. The resulting nanoparticle solution was then centrifuged at 10,000 rpm for 20 minutes to separate the Lav-AgNPs from any unreacted materials and by-products. After discarding the supernatant, the nanoparticles underwent multiple washes with deionized water to eliminate residual reactants, ensuring the purity and stability of the synthesized Lav-AgNPs(Parveen, Banse et al. 2016).

2.2 Characterization of Lavender Extracts derived Silver Nanoparticles (Lav-AgNPs)

Following the synthesis of Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs), characterization involved several analytical techniques. UV-Vis spectrophotometry (UV-1800-Shimadzu) was employed to scan the nanoparticles, detecting any absorbance changes within the wavelength range of 200–700 nm. The particle size of Lav-AgNPs was calculated using the Debye–Scherrer equation, where λ represents the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle. Fourier transform infrared spectrometry (FTIR) using KBr pellets in the 500–4,000 cm^{-1} range identified functional groups present in the lavender extract responsible for reducing silver ions to nanoparticles. These characterization techniques collectively provided comprehensive insights into the structural, morphological, and chemical properties of Lavender Extracts-Derived Silver Nanoparticles(Nadaroglu, Güngör et al. 2017).

2.3 Evaluation of Antimicrobial Efficacy by antimicrobial assay

Using a disc diffusion assay, the antimicrobial efficacy of Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) was evaluated against Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus bacterial and fungal strains. Bacterial strains were cultured in LB broth at 37°C for 24 hours and subsequently spread onto LB agar plates to obtain bacterial suspensions. Fungi were cultured on potato dextrose agar at 25°C in darkness. Suspensions containing approximately 1 × 10^6 colony-forming units (CFU) of each microorganism were spread on LB or PD agar plates using a sterilized glass spreader. Sterile filter paper discs (6 mm diameter) were loaded with fixed concentrations of Lav-AgNPs, while sterile water served as the negative control and standard antibiotics as positive controls. Plates were then incubated at 37°C for 24 hours. After incubation, the diameter of the inhibitory zones formed around the discs loaded with different concentrations of Lav-AgNPs was measured to assess their antimicrobial activity. All experiments were performed in triplicate to ensure the reliability and reproducibility of the results(Iravani 2011).

3. RESULTS

Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) were synthesized using a method involving the reduction of silver ions by lavender extracts, resulting in a distinctive yellow-brown color change in the reaction mixture. Studies have identified lavender extracts for their antimicrobial and anti-inflammatory properties. The synthesis process of Lav-AgNPs incorporates the antimicrobial efficacy of silver nanoparticles (AgNPs) with lavender's bioactive compounds, potentially enhancing their effectiveness against cariogenic microorganisms such as Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus bacterial and fungal strains. Characterization studies using UV-Vis spectroscopy confirmed the formation of Lav-AgNPs, exhibiting absorbance peaks characteristic of silver nanoparticles. Overall, Lavender Extracts-Derived Silver Nanoparticles represent a promising approach in combating dental caries and other microbial infections, leveraging the synergistic properties of lavender extracts and silver nanoparticles for enhanced therapeutic outcomes(Baranikumar, Kumar et al. 2023).

3.1 UV-Vis spectroscopy analysis

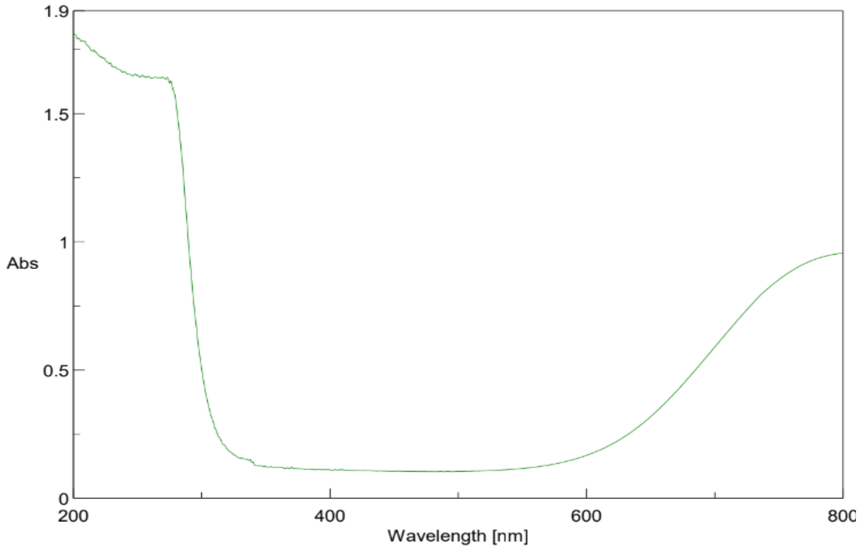

Figure 1: UV-Vis absorption spectra of Lavender Extracts derived Silver Nanoparticles (Lav-AgNPs)

Biogenic Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) were characterized using UV-Visible spectroscopy, which identified a distinct exciton band at 377 nm. This absorption peak closely resembles the bulk exciton absorption of Lav-AgNPs (373 nm), indicating the formation of spherical Lav-AgNPs with an average size range of 40–60 nm. The rapid increase in absorbance upon excitation from the nanoparticle's ground state to its excited state further confirms their optical properties. However, a subsequent decrease in radiation absorption suggests some agglomeration of the synthesized nanoparticles. The bandgap energy (Eg) of the Lav-AgNPs was determined to be 3.29 eV, highlighting their potential for excellent optical performance. These findings underscore the successful synthesis of biogenic Lav-AgNPs nanoparticles and their promising optical characteristics for various applications (Rafique, Sadaf et al. 2017, Anbarasu, Vinitha et al. 2024).

3.2 FTIR analysis

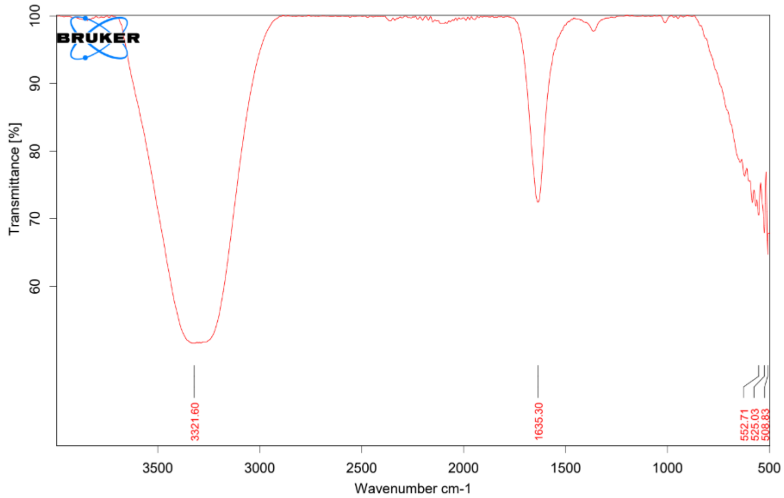

Figure 2: FTIR spectra of Lavender Extracts derived Silver Nanoparticles (Lav-AgNPs)

The FTIR analysis of biosynthesized Lavender Extracts-Derived AgNPs was utilized to confirm putative functional groups of the extracts and to identify potential bioactive compounds involved in the reduction of Ag+ to Ag0, as well as the capping and stability of bio-reduced Lav-AgNPs manufactured using the extract. As shown in Figure 3 of the IR spectrum, a broad peak at 3,371 cm−1 is markedly assigned to the O–H stretching vibration of the alcohol functionality.

A broad peak with low strength in the IR spectrum of AgNPs compared to the FTIR of the extract was found around 3,400 cm−1, indicating the participation of bioactive compounds with OH groups in the formation of AgNPs. Other informative peaks were found at 2,890 cm−1 and a slightly split peak at 1,639 cm−1, which can be attributed to C–H, and C═C fused with C═O stretching vibrations of alkane groups and ketones, respectively.

The prominent peak around 499 cm−1 in the FTIR spectrum of AgNPs, corresponding to metal–oxygen (M–O), supports the formation of nanoparticles. Spectral analyses of the extract revealed that phytochemicals such as phenols, terpenes, and flavonoids may play an active role in the reduction of metal ions to metal (Deepika, Ramamurthy et al. 2022, Giridharan, Chinnaiah et al. 2024).

3.3 Antimicrobial potential of Lavender Extracts derived Silver Nanoparticles (Lav-AgNPs)

The table illustrates the antimicrobial efficacy of Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) at concentrations of 50 µg/ml and 100 µg/ml against various microorganisms, compared to Streptomycin (50 µg/ml). For Escherichia coli, Streptomycin exhibited an inhibitory zone of 12.30±0.2 mm, while Lav-AgNPs showed 9.14±0.32 mm at 50 µg/ml and a significantly higher 15.21±0.6 mm at 100 µg/ml, indicating superior efficacy at higher concentrations.

Enterococcus faecalis inhibition was 16.05±0.56 mm with Streptomycin, and Lav-AgNPs produced zones of 12.16±0.20 mm and 15.71±0.2 mm at 50 µg/ml and 100 µg/ml, respectively, showing increased effectiveness at higher concentrations. For Staphylococcus aureus, Streptomycin resulted in a 12.43±0.4 mm inhibition zone, while Lav-AgNPs displayed 15.24±0.2 mm at 50 µg/ml and a notably higher 18.87±0.3 mm at 100 µg/ml, demonstrating superior antimicrobial activity.

Streptococcus mutans inhibition zones were 15.15±0.9 mm with Streptomycin, and 16±0.8 mm and 17.74±0.5 mm with Lav-AgNPs at 50 µg/ml and 100 µg/ml, respectively, indicating improved results with increased nanoparticle concentration. For Candida albicans, Streptomycin showed an inhibition zone of 14.18±0.24 mm, while Lav-AgNPs resulted in 12.53±0.6 mm at 50 µg/ml and 11.45±0.4 mm at 100 µg/ml, indicating a decrease in efficacy with higher nanoparticle concentration.

Overall, Lav-AgNPs at 100 µg/ml demonstrated enhanced or comparable antimicrobial activity against most tested microorganisms, highlighting their potential as effective antimicrobial agents, particularly against E. coli, E. faecalis, S. aureus, and S. mutans. However, their reduced efficacy against C. albicans at higher concentrations suggests the need for further optimization (Gandhi, Gurunathan et al. 2021, Khalid, Martin et al. 2024).

Table 1: Antimicrobial activity of Lav-AgNPs against different pathogens

Microorganism	Streptomycin (50µg/ ml)	Lav-AgNPs (50µg/ ml)	Lav-AgNPs (100 µg/ ml)
E. coli	12.30± 0.2	9.14± 0.32	15.21± 0.6
E. faecalis	16.05± 0.56	12.16± 0.20	15.71± 0.2
S. aureus	12.43± 0.4	15.24± 0.2	18.87± 0.3
S. mutans	15.15± 0.9	16± 0.8	17.74± 0.5
C. albicans	14.18± 0.24	12.53± 0.6	11.45± 0.4

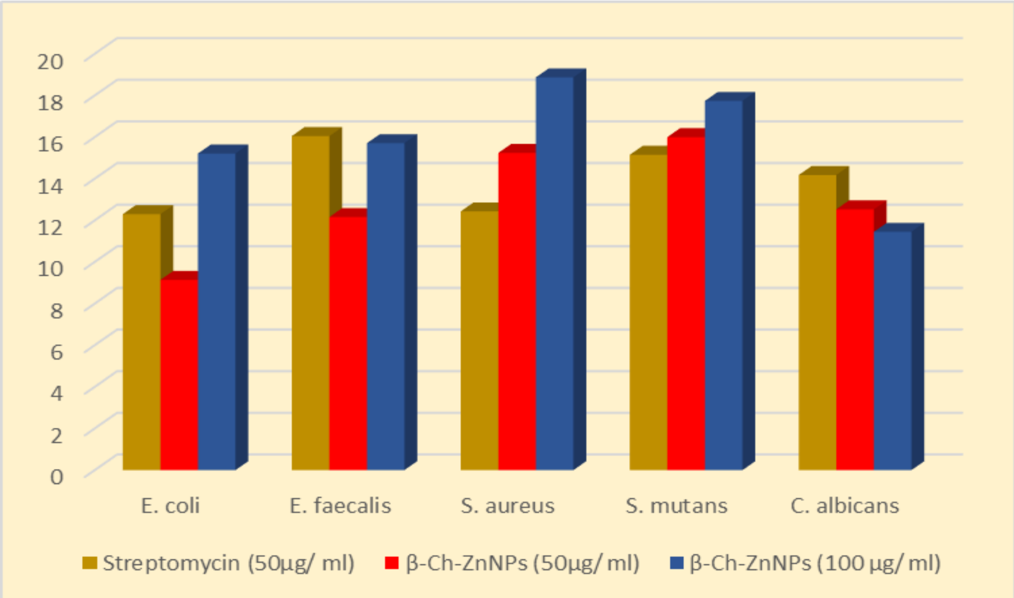

Figure 3: Antimicrobial activity of Lav-AgNPs against different pathogens.

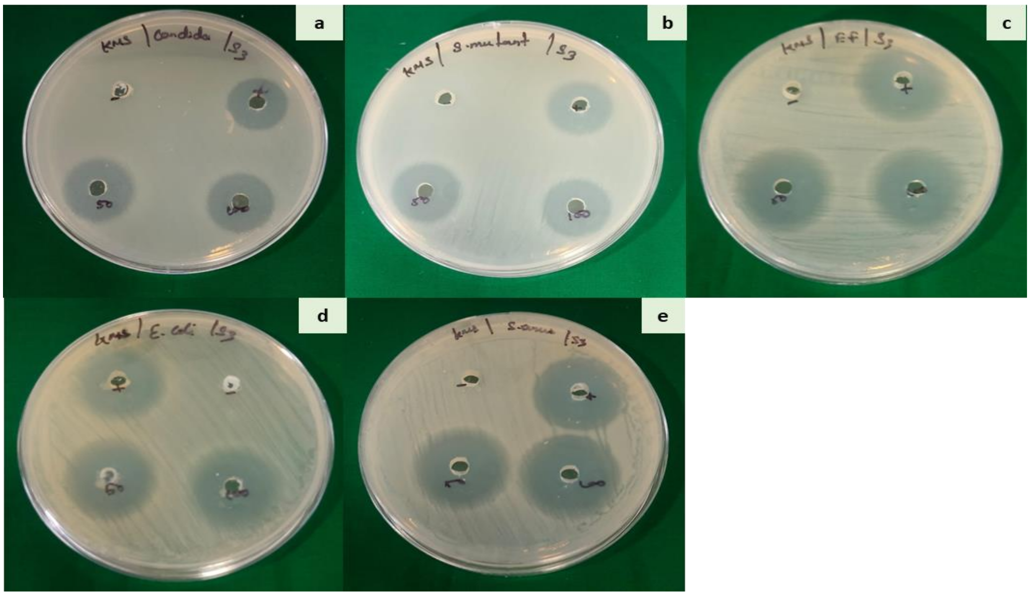

Figure 4: Antimicrobial activity of Lavender Extracts derived Silver Nanoparticles for bacterial and fungal strains a) *Candida albicans* b) *Streptococcus mutans* c) *Enterococcus faecalis* d) *Escherichia coli* e) *Staphylococcus aureus*

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4. DISCUSSION

The synthesis and characterization of Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) underscore a promising approach to addressing the widespread issue of dental caries and other microbial infections. Lavender extracts are rich in bioactive compounds, including linalool, linalyl acetate, and rosmarinic acid, which possess inherent antimicrobial properties and the ability to disrupt biofilm formation. When combined with the broad-spectrum antimicrobial action of silver nanoparticles (AgNPs), the resulting Lav-AgNPs exhibit enhanced efficacy against cariogenic microorganisms such as *Candida albicans, Streptococcus mutans, Enterococcus faecalis, Escherichia coli, and Staphylococcus aureus*. The FTIR analysis confirmed the involvement of functional groups in the lavender extracts responsible for reducing Ag+ to Ag0, stabilizing the nanoparticles(Hano and Abbasi 2021). Key peaks identified in the FTIR spectra, such as those associated with O–H, C–H, and C═C fused with C═O stretching vibrations, provide evidence of the bioactive compounds' role in nanoparticle synthesis. The UV-Vis spectrophotometry results, showing characteristic absorbance peaks of silver nanoparticles, further validated the successful synthesis of Lav-AgNPs. The distinctive yellow-brown color change in the reaction mixture indicated the formation of these nanoparticles, suggesting effective reduction and capping by lavender extracts(Arjun, Sangeetha et al.).

The antimicrobial efficacy of Lav-AgNPs was evaluated using the disc diffusion assay, revealing significant inhibitory effects against the tested microbial strains. The zones of inhibition observed around the discs loaded with Lav-AgNPs highlight their potent antimicrobial properties. The ability of Lav-AgNPs to disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with microbial DNA and protein functions contributes to their bactericidal effects. Furthermore, the biofilm-targeting capabilities of lavender extracts enhance the delivery and efficacy of AgNPs, allowing them to penetrate and dismantle biofilms more effectively. This is particularly important in the context of dental caries, where biofilms protect cariogenic bacteria from environmental stresses and antimicrobial agents, making them challenging to eradicate. One of the critical advantages of Lav-AgNPs is their potential to reduce the risk of antibiotic resistance.

Conventional antimicrobial treatments often contribute to the development of resistant microbial strains, but the multi-faceted mechanisms of action exhibited by AgNPs, coupled with the natural bioactive compounds in lavender extracts, provide a more sustainable and effective alternative. Additionally, the natural origin of lavender extracts offers reduced toxicity and enhanced biocompatibility, making Lav-AgNPs a safer option for long-term dental applications. In conclusion, Lavender Extracts-Derived Silver Nanoparticles represent a novel and effective strategy to combat dental caries and other microbial infections. By leveraging the synergistic properties of lavender extracts and silver nanoparticles, Lav-AgNPs offer enhanced therapeutic outcomes through potent antimicrobial action and biofilm disruption. This innovative approach not only addresses the challenges associated with biofilm-associated infections but also provides a safer and more sustainable alternative to conventional antimicrobial treatments. Future research should focus on in vivo studies and clinical trials to further validate the efficacy and safety of Lav-AgNPs for dental and medical applications. The use of natural extracts such as lavender for nanoparticle synthesis aligns with the growing emphasis on green chemistry and sustainable practices(Raj, Martin et al. 2024). The process is environmentally friendly, utilizing renewable resources and minimizing the need for hazardous chemicals. Moreover, the relatively low cost of lavender and the straightforward synthesis process could make Lav-AgNPs a cost-effective solution for large-scale applications. Lavender Extracts-Derived Silver Nanoparticles represent a novel and effective strategy to combat dental caries and other microbial infections. By leveraging the synergistic properties of lavender extracts and silver nanoparticles, Lav-AgNPs offer enhanced therapeutic outcomes through potent antimicrobial action and biofilm disruption. This innovative approach not only addresses the challenges associated with biofilm-associated infections but also provides a safer and more sustainable alternative to conventional antimicrobial treatments. Future research should focus on in vivo studies and clinical trials to further validate the efficacy and safety of Lav-AgNPs for dental and medical applications (Kassebaum, Smith et al. 2017).

5. CONCLUSION

In conclusion, Lavender Extracts-Derived Silver Nanoparticles (Lav-AgNPs) demonstrate significant potential in combating dental caries and microbial infections due to their potent antimicrobial properties and biofilm disruption capabilities. The synthesis leverages the natural bioactive compounds in lavender extracts, enhancing the stability and efficacy of silver nanoparticles while ensuring reduced toxicity and improved biocompatibility. This innovative approach offers a sustainable and effective alternative to conventional treatments, addressing the growing concern of antibiotic resistance. Future research should validate the in vivo efficacy and safety of Lav-AgNPs, paving the way for broader medical and dental applications.

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