Original Article

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Dentistry Section

Morin-Mediated Silver Nanoparticles: In-vitro Synthesis, Antimicrobial Activity and Characterisation using Fourier Transform Infrared, Ultraviolet-visible, X-ray Diffraction

R SUKANTH[1], PT PRIYANGHA[2]

ABSTRACT

Introduction: Nanotechnology enables precise molecular manipulation, providing nanoparticles as versatile tools for medicine and other fields. Green synthesis methods for nanoparticles utilise natural sources, such as plant extracts and flavonoids. Silver nanoparticles, known for their antimicrobial and anti-inflammatory properties, have significant potential in biomedical applications. Despite these advances, studies utilising morin as a green reducing and stabilising agent remain limited. Owing to its intrinsic antimicrobial and anti-inflammatory potential, morin offers a promising approach for nanoparticle synthesis.

Aim: To synthesise Morin-mediated Silver Nanoparticles (M-AgNPs) and evaluate their in-vitro properties, such as Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible (UV-Vis) spectrum, X-ray Diffraction (XRD), antibiofilm, and antimicrobial activities.

Materials and Methods: This in-vitro experimental study was conducted in Green Lab, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India, between August 2024 and September 2024 to synthesise and evaluate morin-mediated silver nanoparticles (M-AgNPs). Morin flavonoid was used as a reducing and stabilising agent for nanoparticle synthesis by reacting with a 1 mM silver nitrate ($AgNO_3$) solution under controlled alkaline conditions. The formation of M-AgNPs was confirmed through colour change and subsequently characterised using Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet–Visible (UV-Vis) spectroscopy, and X-ray Diffraction (XRD) analysis to determine functional groups, optical properties, and crystalline structure.

The antibacterial and antibiofilm activities of the synthesised nanoparticles were evaluated against oral biofilm-forming microorganisms using standard agar well diffusion and biofilm inhibition assays. All experiments were performed in triplicate. International Business Machine (IBM) Statistical Packages of Social Sciences (SPSS) Statistics version 21.0 was used to perform one-way Analysis of Variance (ANOVA), with a p-value of less than 0.05 considered statistically significant.

Results: The FTIR results revealed a broad peak around 3300 cm^{-1} corresponding to O-H stretching from hydroxyl groups in Morin, while the peak near 2900 cm^{-1} indicates C-H stretching from its organic components. Peaks below 1000 cm^{1} suggest specific interactions between silver nanoparticles and Morin's functional groups, confirming successful surface functionalisation. UV-Vis spectrum showed a Surface Plasmon Resonance (SPR) peak at 400-450 nm. The XRD analysis revealed a strong peak at $2\theta=32°$, along with additional peaks at 38°, 44°, and 64°. The sharp peak at 32° indicates a high degree of crystallinity, while slight peak broadening suggests a small crystallite size. The antibiofilm assay revealed that the control sample, an untreated mixed biofilm grown for three days, showed 100% live bacteria, indicating no antibacterial effect. The test sample was treated with M-AgNPs for 24 hours, resulting in significant antibiofilm activity. After treatment, only 0.5% of the bacteria remained alive, while 99.5% were dead. M-AgNPs demonstrated antibacterial activity comparable to standard antibiotics, showing Zones Of Inhibition (ZOI) of 14 mm for *Staphylococcus aureus* (Gentamycin: 14 mm), 16 mm for *Streptococcus mutans* (Amikacin: 16 mm), and 13 mm for *Escherichia coli* (Chloramphenicol: 14 mm) and the results were statistically significant for the test group (p-value<0.05). In contrast, Morin-mediated yttrium nanoparticles (M-YtNPs) showed less effective antibacterial activity.

Conclusion: Thus, M-AgNPs showed effective antibiofilm and antibacterial properties, offering a promising, eco-friendly advancement for enhancing periodontal disease treatments.

Keywords: Antibacterial agents, Biofilms, Flavonoids

INTRODUCTION

Nanotechnology involves engineering materials with particle sizes ranging from 1 to 100 nanometers [1]. Their nanoscale dimensions enable precise interactions with biological systems, allowing innovative uses in targeted therapy, diagnostics, and material science. Nanoparticle synthesis employs multiple techniques, including sol-gel, chemical reduction, and hydrothermal methods [2]. Though effective, nanoparticle synthesis methods often involve toxic chemicals, posing environmental and health risks, and also involve a complex synthesis process.

Therefore, green synthesis of nanoparticles has gained prominence since it uses natural sources like plant extracts, flavonoids, and microorganisms and offers an eco-friendly alternative [3]. This approach also leverages the inherent biological properties and biocompatibility of natural materials.

Flavonoids, a diverse group of plant compounds, are widely recognised for their role in promoting cardiovascular health by improving blood circulation [4]. In addition, flavonoids also have anti-inflammatory, antiviral, and anticancer properties. They are commonly found in dietary sources, and their versatility makes

them a significant area of research and application across multiple fields.

Silver nanoparticles (Ag-NPs) have a wide array of applications, such as cost-effectiveness, safety, and biocompatibility [5]. In the medical field, they are widely used for their antimicrobial effects and incorporated into wound dressings to reduce the risk of infections and act as catalysts to facilitate various chemical reactions. They also possess anti-inflammatory properties.

The synthesis of nanoparticles using flavonoids has become popular as it is safe and versatile. Flavonoids, extracted from plant sources, act as reducing agents by donating electrons to metal salts and converting them into nanoparticles [6]. This green synthesis approach has sustainable potential applications in medical fields.

Morin is a flavonoid found in various plants, including the Morus (mulberry) species, and is recognised for its diverse biological activities [7]. It is abundant in onions, guava leaves, and seaweeds. Morin has potent antioxidant properties, which involve scavenging oxyradicals and deactivating enzymes responsible for generating free radicals [8]. Morin also exhibits potential anticancer effects and antimicrobial properties.

Limited literature exists on the utilisation of Morin flavonoid in nanoparticle synthesis and development, thus prompting further investigation. Since the flavonoid has many potential therapeutic properties, exploring its application becomes pertinent in periodontal therapy [9]. Consequently, the current study focuses on synthesising and characterising silver nanoparticles using Morin as a facilitating agent, with an emphasis on evaluating their in-vitro properties. The primary objective of the study was to synthesise and characterise M-AgNPs. The secondary objectives were to assess their antimicrobial activity against selected bacterial strains and to evaluate their antibiofilm potential. The study was conducted under the following hypotheses: the null hypothesis (H_0) stated that M-AgNPs do not exhibit significant antimicrobial and r antibiofilm activity compared to Morin-mediated yttrium nanoparticles and the untreated mixed biofilms, while the alternative hypothesis (H_1) proposed that M-AgNPs do exhibit significant antimicrobial and antibiofilm activity compared to Morin-mediated yttrium nanoparticles and the untreated mixed biofilm.

MATERIALS AND METHODS

This is an in-vitro study conducted in Green Lab, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. The Institutional Scientific Research Board (SRB/SDC/FACULTY/24/PERIO/236) approval was obtained before the start of the study. Sample size was not calculated as this is an in-vitro study; antibacterial assays were performed in triplicate (n=3) for each bacterial strain to ensure reproducibility and reliability.

Inclusion and Exclusion criteria: Since the present study is an *in-vitro* investigation using M-AgNPs and standard bacterial strains (*S. aureus*, *S. mutans*, *E. coli*), only pure chemicals from certified suppliers, freshly cultured and verified bacterial strains, and solutions prepared under sterile conditions were included.

Experiments using contaminated cultures, impure reagents, improperly prepared solutions, or conducted outside the defined temperature, pH, or stirring conditions were excluded. Only samples suitable for FTIR, UV-Vis, XRD characterisation, and proper biofilm formation for CLSM analysis were used.

Study Procedure

Preparation of Morin-mediated Silver Nanoparticles (M-AgNPs)

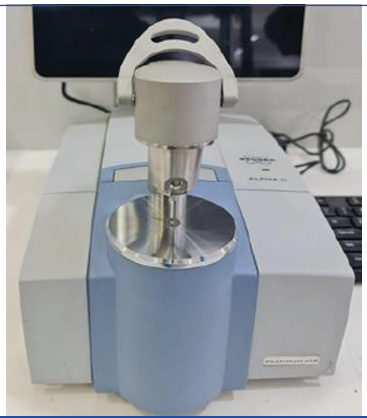
Ten grams of commercially available Morin in powdered form (Sigma-Aldrich (Merck Life Science India Pvt., Ltd.,), Mumbai) were accurately weighed and dissolved in 100 millilitres of distilled water. The dissolution process was aided by continuous manual stirring to ensure that the Morin was completely dissolved in the water. This procedure resulted in a clear solution where the concentration of Morin was 100 milligrams per millilitre.

Consequently, 17 milligrams of $AgNO_3$ obtained in solid powder form (Sigma-Aldrich (Merck Life Science Pvt., Ltd.,), Mumbai) were accurately weighed and added to a container containing 100 millilitres of distilled water. The silver nitrate readily dissolved in the water, forming a homogeneous and clear solution. The concentration of this solution was calculated to be 1 millimolar (mM), indicating that there is 1 millimole of $AgNO_3$ per litre of solution, which is equivalent to 0.001 moles per litre [10].

Then, in a beaker, the prepared Morin solution was added to the 10 mL of 1 mM silver nitrate solution and stirred continuously using a magnetic stirrer in a volume ratio of 1:1.The pH of the solution was adjusted to 10.5, and 0.1 M Sodium Hydroxide (NaOH) was used to further increase the pH as Morin-mediated synthesis often occurs at a slightly alkaline pH. Then the solution was continuously stirred at a room temperature of 40-60°C for four hours. The colour changed slightly from yellow to brown, indicating the formation of silver nanoparticles. After the reaction was complete, the solution was centrifuged at a high speed of 10,000 rpm for 20 minutes to separate the nanoparticles. The supernatant was discarded, and the nanoparticles were redispersed in distilled water. The size and shape of M-AgNPs were about 10-50 nm. The centrifugation was repeated, and adequate washing was done to remove the unreacted Morin and silver ions [Table/Fig-1] [11].

[Table/Fig-1]: Morin-mediated Silver Nanoparticles (M-AgNPs) solution.

Characterisation of the Morin-mediated Silver Nanoparticles (M-AgNPs) FTIR Analysis: A Bruker ALPHA II FTIR Spectrometer, Bruker Optics (a division of Bruker Corporation), Ettlingen, Germany, was used to investigate the cross-linking between the Morin and the silver nanoparticles [Table/Fig-2]. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectra were recorded using dry films. To create these films, the formulated M-AgNPs substance was poured into a polystyrene petri plate and left to dry for three days [12].



[Table/Fig-2]: Fourier Transform Infrared (FTIR) Spectrophotometer (Bruker Alpha II) used for characterisation of Morin-mediated nanoparticles.

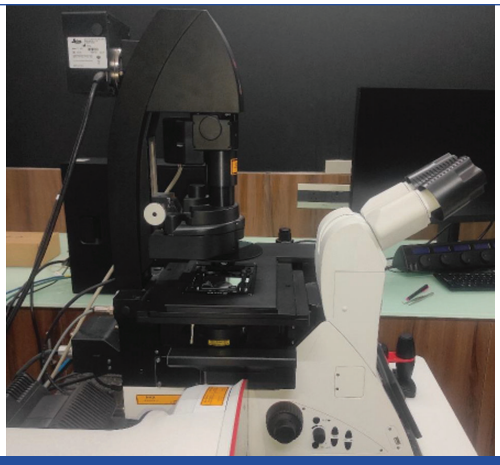
UV-Vis Spectrum: The UV-Vis spectrum is a graphical representation of a sample's absorbance or transmittance of ultraviolet and visible light as a function of wavelength and is measured by a Visible Spectrophotometer (ASCO V-730 JASCO Corporation, Tokyo, Japan) [Table/Fig-3]. It is widely used to characterise the optical properties of nanoparticles. The SPR peak is a feature in the UV-Vis spectrum that represents the collective oscillation of conduction electrons on the surface of metallic nanoparticles, such as silver nanoparticles, in response to incident light [13].

[Table/Fig-3]: UV-Visible Spectrophotometer (JASCO V-730) used for optical characterisation of Morin-mediated nanoparticles.

XRD analysis: The XRD was employed to examine the material's crystal structure and phase composition using a X-ray diffractometer (Bruker Corporation D8 ADVANCE, AXS GmbH, Karlsruhe, Germany) [Table/Fig-4]. The diffraction pattern revealed peaks corresponding to particular atomic planes in the crystal lattice. Analysis of the positions and intensities of these peaks enabled the determination of the material's essential crystal structure characteristics [14].

[Table/Fig-4]: X-ray diffractometer (Bruker D8 Advance) used for crystalline structure analysis of Morin-mediated nanoparticles.

Antibiofilm activity: Biofilms are structured communities of bacteria that are attached to a surface and embedded in a self-produced extracellular matrix. Antibiofilm activity refers to the ability of a substance to prevent the formation and disruption of established biofilms. In the study, biofilms were first allowed to form on appropriate surfaces (e.g., glass or polystyrene) for a specified duration. After treatment with the test substance, the biofilms were gently washed to remove planktonic cells. They were then stained using fluorescent dyes- (Green fluorescent nucleic acid stain) for live cells (green) and propidium iodide for dead cells (red). Confocal Laser Scanning Microscopy (CLSM) was used to scan the biofilm layer by layer with a laser, producing detailed three-dimensional images [Table/Fig-5]. These images were examined visually to observe biofilm architecture and the distribution of live and dead cells, allowing evaluation of the anti-biofilm effect of the tested substance. CLSM was employed to detect the anti-biofilm activity [15]. It provides detailed 3D images of biofilms and also differentiates between live and dead cells using fluorescent stains. The reported values were derived from visual



[Table/Fig-5]: Confocal Laser Scanning Microscope (Leica Stellaris 5) used for fluorescence imaging of Morin-mediated nanoparticles. A side view of the Leica Stellaris 5 Confocal Laser Scanning Microscope set-up, showing the motorised stage, laser scanning head, and optical components used for high-resolution fluorescence imaging.

interpretation of the CLSM images obtained after staining. Multiple representative regions of each biofilm were examined, and the relative proportions of green and red signals were estimated to provide an approximate percentage of live and dead cells. These values are therefore semi-quantitative approximations intended to reflect the overall trend in biofilm disruption, rather than statistically computed measurements. This approach demonstrates the presence or absence of biofilm disruption and qualitatively assesses the potency of antibiofilm agents.

Antibacterial activity: For assessing antimicrobial activity, the agar well diffusion method was employed. Morin-mediated Yttrium Nanoparticles (M-YtNPs) commercially procured from Nanochemazone were utilised alongside the test silver nanoparticles as a comparative control. The antibacterial activity of M-AgNPs and M-YtNPs was evaluated using the agar well diffusion method, which provides a qualitative and semi-quantitative measure through the ZOI., The antimicrobial activity of Morin-mediated AgNPs was evaluated at a single concentration of 50 µg/mL against *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli*.

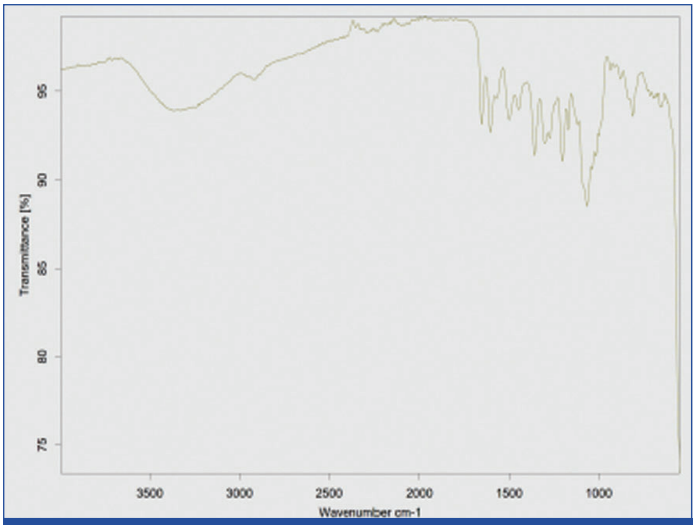
Morin-mediated yttrium nanoparticles at a concentration of 50 µg/mLwere used alongside the test sample for better comparison of efficacy [16]. The antibiotics mentioned, Gentamycin (10 µg/mL), Amikacin (30 µg/mL), and Chloramphenicol (30 µg/mL) obtained from (HiMedia Laboratories Pvt., Ltd., Mumbai, India), served as positive controls because they are known to inhibit bacterial growth. Gentamycin was used as the antibiotic control for S. aureus, Amikacin for S. mutans, and Chloramphenicol for E. coli, respectively. The fresh microbial suspension was dispersed on Mueller-Hinton agar plates, and solutions were added to wells before incubation at 37°C for 24 hours. The zone of inhibition was measured, and the results were interpreted accordingly [15].

STATISTICAL ANALYSIS

The collected data were entered into Microsoft Excel 2017, and statistical analysis was conducted using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were reported as mean±Standard Deviation (SD). For the antibacterial activity analysis, a One-way ANOVA was performed to compare the ZOI among the antibiotics, Morin-mediated yttrium oxide nanoparticles, and M-AgNPs groups.

RESULTS

FTIR analysis: The functional group of the synthesised M-AgNPs was identified using FTIR analysis. As illustrated in [Table/Fig-6], the broad Peak around 3300 cm^{-1} was attributed to the O-H stretching vibrations from Morin, which is a flavonoid with hydroxyl groups,

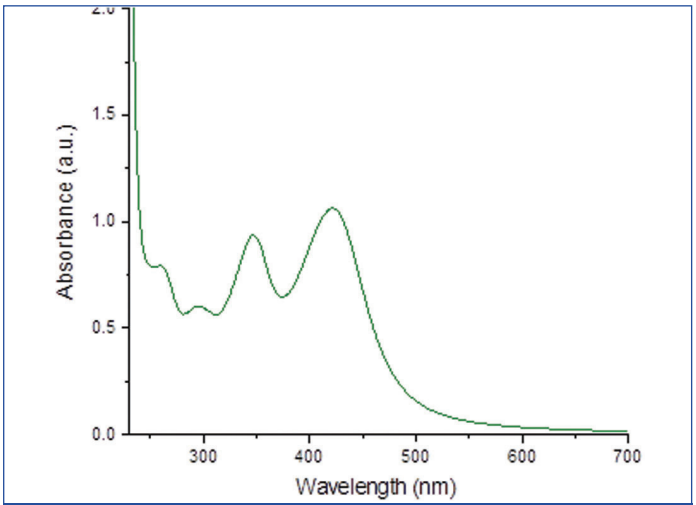


[Table/Fig-6]: FTIR spectrum of M-AgNPs.

and the region around 2900 cm^{-1} corresponds to C-H stretching vibrations from the organic components of Morin.

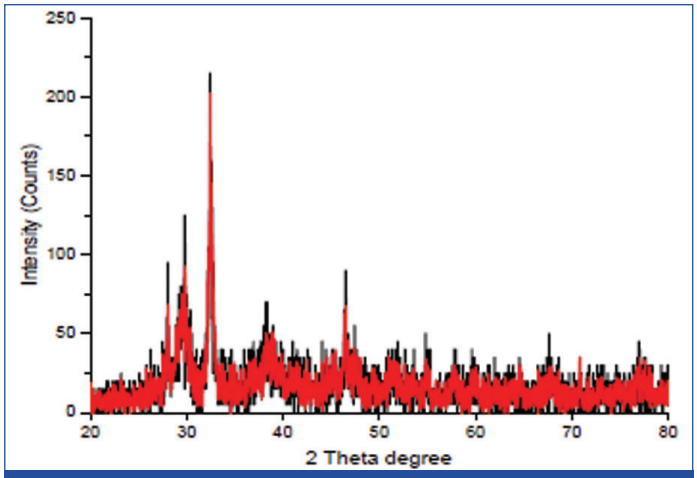
The region around 1700 cm^{-1} typically indicates C=O stretching vibrations present in the flavonoid structure, while the region around 1600-1500 cm^{-1} was due to C=C stretching vibrations from the aromatic rings in the Morin structure. The region below 1000 cm^{1} indicates specific interactions between the silver nanoparticles and the functional groups of Morin. Overall, the spectrum suggested that Morin was present on the surface of the silver nanoparticles, indicated by the characteristic peaks corresponding to its functional groups.

UV-Vis spectrum: In the UV-Vis spectrum of M-AgNPs, silver nanoparticles exhibited a characteristic SPR peak of 400-450 nm, indicative of the collective oscillation of electrons on the nanoparticle surface in response to light [Table/Fig-7]. Observations from the graph showed a major shift towards shorter wavelengths, suggesting smaller particle sizes or specific interactions with Morin. Broader SPR peaks indicated the polydispersity in nanoparticle size.



[Table/Fig-7]: UV-Vis spectrum of M-AgNPs.

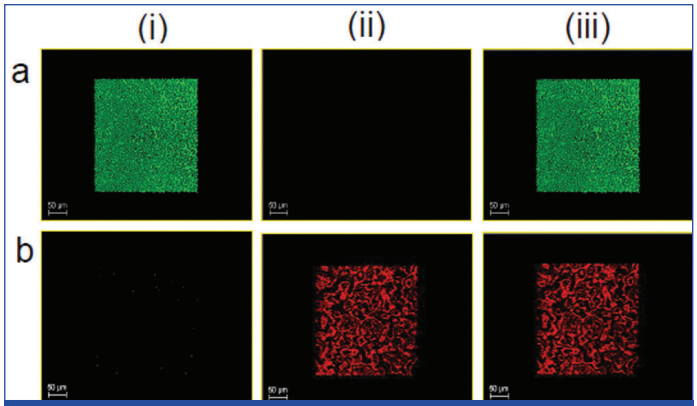
XRD Analysis: The XRD pattern obtained for M-AgNPs showed distinct peaks at different 2θ values [Table/Fig-8]. A strong and sharp diffraction peak was observed around 2θ=32°, corresponding to the (111) plane of face-centred cubic (fcc) silver. Another prominent peak appeared near 2θ=38°, which could be indexed to the (200) plane of fcc silver. A weak shoulder was noted around 44°, suggesting only a minor contribution from the (220) plane. In addition, a less intense peak was observed near 64°, corresponding to the (311) plane of fcc silver. These characteristic peaks confirm the crystalline nature of the synthesised silver nanoparticles, while the broadening of some peaks indicates a smaller crystallite size. Additional peaks that do not correspond



[Table/Fig-8]: XRD pattern of M-AgNP.

to the standard silver peaks were also observed, indicating the presence of other phases or impurities, likely from the Morin or unreacted precursors. The presence of well-defined peaks also indicated that Morin not only mediated the reduction of silver ions but also served as a stabilising agent by capping the nanoparticles and preventing their aggregation.

Antibiofilm activity: The antibiofilm activity of M-AgNPs against a mixed biofilm was observed using CLSM [Table/Fig-9].



[Table/Fig-9]: Confocal Laser Scanning Microscopy (CLSM) images showing the antibiofilm activity of Morin-mediated Silver Nanoparticles (M-AgNPs) against mixed biofilm. (a) Untreated mixed biofilm of *S. aureus*, *S. mutans*, and *E.coli* after three days; (b) M-AgNPs treated mixed biofilm after 24 h; (i) Live cell population (green fluorescence); (ii) Dead cell population (red fluorescence); (iii) Merged live/dead image.

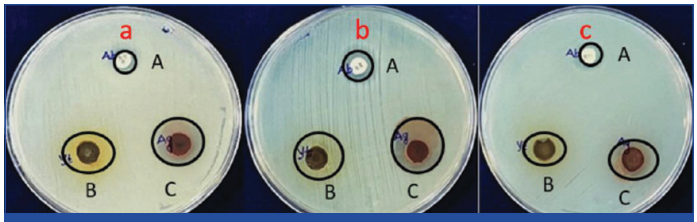
The control sample was the untreated mixed biofilm after three days of growth, showed 100% live bacteria, indicating robust and healthy biofilm formation without any antibacterial intervention.

The test sample was treated with M-AgNPs, and the biofilm treated with M-AgNPs for 24 hours demonstrated significant antibiofilm activity. After 24 hours of treatment, only 0.5% of the bacteria remained alive, while 99.5% were dead. This significant reduction in live bacteria indicated the potent antibiofilm activity of the M-AgNPs.

Antibacterial activity: The antibacterial activity of M-AgNPs and Morin-mediated Yttrium nanoparticles (M-YtNPs) was tested against *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli*, comparing their ZOI to standard antibiotics Gentamycin, Amikacin, and Chloramphenicol as a control [Table/Fig-10].

M-AgNPs demonstrated antibacterial activity comparable to standard antibiotics against *S. aureus* and *S. mutans*, but slightly lower activity against *E. coli*. In contrast, M-Yt-NPs showed less effective antibacterial activity with a ZOI of 14 mm for *Staphylococcus aureus*, 16 mm for *Streptococcus mutans*, and 13 mm for *Escherichia coli*.

The ZOI measurements for the three groups against the tested microorganisms, summarised with their respective mean values,



[Table/Fig-10]: Antibacterial activity (ZOI) of Morin-mediated yttrium oxide (M-Y2O3NPs) and silver nanoparticles (M-AgNPs) against *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli*. a) Control antibiotic (Gentamycin, Amikacin, Chloramphenicol); b) Morin-mediated yttrium oxide nanoparticles; c) Morin-mediated silver nanoparticles.

SD, and p-values, have been depicted in [Table/Fig-11]. The results indicate statistically significant differences ($p<0.05$) among the groups for *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli*.

In conclusion, M-AgNPs exhibited strong antibacterial activity comparable to that of standard antibiotics, particularly against *Staphylococcus aureus* and *Streptococcus mutans*, and were nearly as effective against *Escherichia coli*. On the other hand, Morin-mediated yttrium oxide nanoparticles showed less effective antibacterial activity against the tested bacterial strains, indicating that they may not be suitable for use as antimicrobial agents under the conditions tested.

Micro-organisms	Control antibiotic (Gentamycin, Amikacin, Chloramphenicol)	Morin-silver nanoparticles	Morin - Yttrium nanoparticles	p-value	F value
a) *Staphylococcus aureus*	14±1	14±0.5	9±1	0.00056*	33.34
b) *Streptococcus mutans*	16±1	16±0.5	10±1	0.00020*	48.00
c) *Escherichia coli*	14±0.5	13±1	8±1	0.00031*	41.32

[Table/Fig-11]: Antibacterial activity (ZOI in mm) of M-Y2O3NPs and M-AgNPs.
Statistical test applied: One-way ANOVA; $p<0.05$ considered statistically significant. Abbreviations: M-AgNPs: Morin-mediated silver nanoparticles; M-YONPs: Morin-mediated yttrium oxide nanoparticles

DISCUSSION

The rise of green synthesis in nanoparticle production represents a key advancement in nanotechnology and environmental sustainability. Flavonoids serve as effective reducing and stabilising agents, minimising environmental impact and improving the functionality of nanoparticles for biomedical uses [17]. The bioactive properties of flavonoids add further value, endowing nanoparticles with additional therapeutic benefits. Thus, flavonoid-mediated green synthesis has rapidly become a preferred method for producing multifunctional nanomaterials.

Nanotechnology is revolutionising a wide range of industries by manipulating matter at the atomic and molecular scale, typically between 1 and 100 nanometers [18]. Despite its potential, nanotechnology raises safety concerns, requiring responsible development. The study focuses on green synthesis to reduce environmental risks.

Morin, a natural polyphenol predominantly found in the Moraceae family, is extracted from various plant parts such as leaves, fruits, stems, and branches [19]. It has demonstrated a wide range of beneficial effects, including antioxidant, antidiabetic, antiinflammatory, antitumoral, and antibacterial. Notably, it exhibits low toxicity and is well-tolerated with even chronic use. Studies on the efficacy of Morin as a potential therapeutic phytocomponent have yielded positive results, providing valuable insights into its mechanism of action [20,21].

The objective of the study was to assess the in-vitro characteristics of the formulated M-AgNPs. In the FTIR analysis, peaks were detected within a range from 1000 cm^{-1} to 3300 cm^{-1}, corresponding to specific interactions between the silver nanoparticles and the functional groups of Morin. The FTIR analysis confirms that Morin is not only present on the surface of the synthesised silver nanoparticles but also actively contributes to their stabilisation and functionalisation. This suggests that the nanoparticles retain the bioactive properties of Morin, enhancing their potential for biomedical and therapeutic applications.

The UV-Vis spectrum of Morin-mediated silver nanoparticles reveals a characteristic SPR peak in the 400-450 nm range, confirming the presence of silver nanoparticles and their ability to exhibit collective electron oscillations. These findings highlight Morin's influence on nanoparticle characteristics and help understand the structural and optical properties of the synthesised nanoparticles.

In the XRD analysis, the significance of the XRD pattern for Morin-mediated silver nanoparticles lies in its confirmation of the nanoparticles' crystalline structure. The presence of strong, sharp peaks at 2θ values of 32°, 38°, 44°, and 64° indicates that the silver nanoparticles are well-crystallised and exhibit the typical fcc structure of silver.

The study also evaluated the anti-biofilm and anti-microbial activity of Morin-mediated silver nanoparticles through CLSM imaging. The CLSM images revealed M-AgNPs' effectiveness in disrupting mixed biofilms, shifting from mostly live in the control to mostly dead in treated samples, demonstrating their strong anti-biofilm potential. Similar to present study, another study exploring Morin as an anti-quorum-sensing agent against resistant strains of *Staphylococcus aureus* concluded that Morin significantly inhibited biofilm formation and decreased extracellular polymeric substance production in these resistant strains [22].

The significance of the antibacterial activity of M-AgNPs lies in their equivalent antibacterial activity to Gentamycin and Amikacin, against *Staphylococcus aureus* and *Streptococcus mutans*. This indicates that M-AgNPs demonstrate strong antibacterial properties comparable to traditional antibiotics. The near-equivalence highlights their potential for use in clinical applications, especially in combating antibiotic-resistant bacteria. Present study aligns with research on Morin from mulberry fruits, which demonstrated strong antioxidant activity and moderate inhibition of *Streptococcus mutans* in disc diffusion assays [23].

Since the antibiofilm assay revealed that treatment with M-AgNPs reduced bacterial viability to only 0.5%, compared to 100% viability in the untreated control. In the antibacterial assay, M-AgNPs produced clear Zone of Inhibition (ZOI) comparable to those of standard antibiotics, with statistically significant differences (p-value<0.05). Based on these findings, it can be concluded that M-AgNPs possess significant antibacterial and antibiofilm effects; therefore, the null hypothesis (H_0) is rejected and the alternative hypothesis (H_1) is accepted.

In a separate study, researchers investigated the anticarcinogenic effects of the dietary flavonoid Morin and concluded that it can reduce oxidative stress and downregulate p-Akt and NF-κBexpression [24]. On the other hand, silver nanoparticles are always known for their antibacterial and antimicrobial activity [25]. Silver nanoparticles were also synthesised using other eco-friendly sources, such as curcumin [26]. Various other nanoparticles were also synthesised using greener methods, like gold nanoparticles with pomegranate peel extract and copper oxide nanoparticles with Aloe Vera gel [27]. Thus, greener methods for synthesising nanoparticles are gaining popularity these days, like green synthesis of copper oxide nanoparticles using Cymbopogon citratus and Zingiber officinale, reflecting a growing emphasis on environmentally friendly practices [28].

The structural and functional characteristics of M-AgNPs, as revealed by FTIR, UV-Vis, and XRD analyses, along with their antimicrobial and anti-biofilm activities, suggest their potential as therapeutic agents, particularly for oral and systemic infections caused by antibiotic-resistant pathogens. Their ability to disrupt biofilms indicates possible applications in preventing infections on medical devices or dental surfaces. Further in-vivo studies are required to assess safety, optimise dosage, and evaluate delivery methods. Translating these nanoparticles into clinical practice presents opportunities, such as novel and effective antimicrobial agents, as well as challenges, including ensuring stability, biocompatibility, and regulatory approval. These considerations underscore the potential clinical relevance of the current study findings.

Green synthesis is preferred due to its simplicity and biocompatible nature. Morin, a polyphenolic flavonoid, can be used for silver nanoparticle synthesis due to its multiple hydroxyl groups. These hydroxyl groups act as reducing agents, converting silver ions (Ag^+) into silver nanoparticles (Ag^0), and can stabilise the formed nanoparticles, preventing agglomeration and ensuring uniform particle size distribution. The bioactive nature of Morin imparts beneficial properties to the silver nanoparticles, such as enhanced antioxidant, antimicrobial, and anti-inflammatory effects, making them suitable for various biomedical applications. Thus, in the current study, Morin was employed to produce silver nanoparticles.

Exploring the realm of nanoparticle synthesis with natural sources like flavonoids offers a promising approach to developing eco-friendly materials. Flavonoids can serve as effective reducing and stabilising agents in nanoparticle synthesis. This integration allows for the creation of bioactive nanoparticles with enhanced functionality. The fusion of nanotechnology with natural flavonoids thus holds significant potential for advancing sustainable and biocompatible innovations.

Limitation(s)

While Morin-mediated nanoparticles show antibiofilm and antibacterial potential, more research is needed to assess their broad-spectrum efficacy and cytotoxicity. Further clinical studies are essential to validate these findings. For the antibacterial activity, the study did not determine the Minimum Inhibitory Concentration (MIC); instead, it focused on comparing antibacterial activity across different concentrations. Future studies could include broth microdilution or macrodilution methods to determine the exact MIC of M-AgNPs against each bacterial strain.

CONCLUSION(S)

In conclusion, Morin-facilitated silver nanoparticles exhibited effective antibiofilm and antibacterial properties. M-AgNPs, which demonstrated strong antibiofilm activity, markedly reduced bacterial viability in the mixed biofilm. Its antibacterial activity was comparable to standard antibiotics, demonstrating a maximum ZOI of 16 mm against *Streptococcus mutans*. Although M-AgNPs demonstrated therapeutic potential, further in-vivo studies are needed to validate their clinical efficacy.

REFERENCES

[1] Vijayaram S, Razafindralambo H, Sun YZ, Vasantharaj S. Applications of green synthesised metal nanoparticles: a review. Biol Trace Elem Res. 2024;202(1):360-86. Doi: 10.1007/s12011-023-03645-9.

[2] Gavas S, Quazi S, Karpinski TM. Nanoparticles for cancer therapy: current progress and challenges. Nanoscale Res Lett. 2021;16(1):173. Doi: 10.1186/s11671-021-03628-6.

[3] Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, Hong J. Green synthesis of nanoparticles: current developments and limitations. Environ Technol Innov. 2022;26:102336. Doi: 10.1016/j.eti.2022.102336.

[4] Dobrzynska M, Napierala M, Florek E. Flavonoid nanoparticles: a promising approach for cancer therapy. Biomolecules. 2020;10(9):1268. Doi: 10.3390/biom10091268.

[5] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76–83. Doi: 10.1016/j.biotechadv.2008.09.002.

[6] Aiello P, Consalvi S, Poce G, Raguzzini A, Bernardi M, Peluso I. Dietary flavonoids: nano delivery and nanoparticles for cancer therapy. Semin Cancer Biol. 2021;69:150–165. Doi: 10.1016/j.semcancer.2019.08.029.

[7] Sinha K, Ghosh J, Sil PC. Morin and its role in chronic diseases. In: Anti-inflammatory Nutraceuticals and Chronic Diseases. 2016;928:453–471. Doi: 10.1007/978-3-319-41334-1_19.

[8] Al-Numair KS, Chandramohan G, Alsaif MA, Veeramani C, Newehy AS. Morin, a flavonoid, on lipid peroxidation and antioxidant status in experimental myocardial ischemic rats. Afr J Tradit Complement Altern Med. 2014;11(3):14–20. Doi: 10.4314/ajtcam.v11i3.3.

[9] Adamczyk J, Chwalowska M, Gebka N, Stos K, Jakubowicz N, Gabrys-Kwolek A, Roj R. The role of flavonoids in the treatment of periodontal diseases: a literature review. J Pre Clin Clin Res. 2022;16(4):153–156. Doi: 10.26444/jpccr/156979.

[10] Ramalingam N, Venkatachalam P, Vinodhkumar V, Vichitra C. Morin hydrate mediated biosynthesis of silver nanoparticles and its antimicrobial activity. Int J Drug Deliv Technol. 2024;14(1):140–144. Doi: 10.25258/ijddt.14.1.20.

[11] Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plant extract-mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res. 2016;7(1):17–28. Doi: 10.1016/j.jare.2015.02.007.

[12] Zayed MF, Abdel-Monem YK, Arafa AA, Eisa WH. Mass production of morin-stabilised silver nanoparticles: characterisation, antioxidant, and antimicrobial activities. Microsc Res Tech. 2024;87(1):149–158. Doi: 10.1002/jemt. 24419.

[13] Xu G, Chen Y, Tazawa M, Jin P. Surface plasmon resonance of silver nanoparticles on vanadium dioxide. J Phys Chem B. 2006;110(5):2051–2056. Doi: 10.1021/jp055744j.

[14] Klug HP, Alexander LE. X-ray diffraction procedures for polycrystalline and amorphous materials. 2nd ed. New York: Wiley, 1974. Doi: Not available.

[15] Sushma B, Ragavendran C. Antibacterial and antibiofilm activity of dental pathogens using leaf extract-derived palladium nanoparticles from Andrographis. Cuest. Fisioter. 2025;54(3):2964–2974. Doi: 10.48047/nw10kp18.

[16] Rajakumar G, Gomathi T, Anbazhagan S, Rahuman AA, Karthikeyan S, Velayutham K, et al. Yttrium oxide nanoparticle synthesis: an overview of antibacterial applications and biomedical prospects. Appl Sci. 2021;11(5):2172. Doi: 10.3390/app11052172.

[17] Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:47. Doi: 10.1017/jns.2016.41.

[18] Malik S, Muhammad K, Waheed Y. Nanotechnology: a revolution in modern industry. Molecules. 2023;28(2):661. Doi: 10.3390/molecules28020661.

[19] Kong LX, Peng Z, Li SD, Bartold PM. Nanotechnology and its role in the management of periodontal diseases. Periodontol 2000. 2006;40(1):184–196. Doi: 10.1111/j.1600-0757.2005.00143.x.

[20] Goyal A, Dubey N, Agrawal A, Verma A, Sharma R. Therapeutic potential of morin: special focus on cognitive dysfunction, diabetes mellitus, and cancer. Pharmacol Res Mod Chin Med. 2023;9:100318. Doi: 10.1016/j.prmcm.2023.100318.

[21] Cakmak F, Kucukler S, Gur C, Comakli S, Ileriturk M, Kandemir FM. Morin provides therapeutic effects by attenuating oxidative stress, inflammation, endoplasmic reticulum stress, autophagy, apoptosis, and oxidative DNA damage in testicular toxicity caused by ifosfamide in rats. Iran J Basic Med Sci. 2023;26(10):1227. Doi: 10.22038/IJBMS.2023.71702.15580.

[22] Rajput SA, Raza A, Shamsi S, et al. Morin hydrate: a comprehensive review on therapeutic applications and future perspectives. Int J Mol Sci. 2021;22(8):4215. Doi: 10.3390/ijms22084215.

[23] Chemmugil P, Lakshmi PT, Annamalai A. Exploring morin as an anti-quorum-sensing agent against resistant strains of Staphylococcus aureus. Microb Pathog. 2019;127:304–315. Doi: 10.1016/j.micpath.2018.12.007.

[24] Yang N, Lee JY, Lee HS. Evaluation of antioxidant and antibacterial activities of morin isolated from mulberry fruits (Morus alba L.). J Korean Soc Appl Biol Chem. 2012;55:485–489. Doi: 10.1007/s13765-012-2110-9.

[25] Manna SK, Aggarwal RS, Sethi G, Aggarwal BB, Ramesh GT. Morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) abolishes nuclear factor-κB activation induced by various carcinogens and inflammatory stimuli, leading to suppression of nuclear factor-κB–regulated gene expression and up-regulation of apoptosis. Clin Cancer Res. 2007;13(7):2290–2297. Doi: 10.1158/1078-0432.CCR-06-2394.

[26] Dharman S, Maragathavalli G, Shanmugam R, Shanmugasundaram K. Biosynthesis of turmeric silver nanoparticles: its characterisation and evaluation of antioxidant, anti-inflammatory, antimicrobial potential against oral pathogens: an in vitro study. J Indian Acad Oral Med Radiol. 2023;35(3):299–305. Doi: 10.4103/jiaomr.jiaomr_309_22.

[27] Dharman S, Maragathavalli G, Rajeshkumar K, Shanmugasundaram K. Eco-friendly synthesis, characterisation and antibacterial activity of curcumin mediated silver nanoparticles. Int J Dent Oral Sci. 2021;8(4):2314–2318. Doi: 10.19070/2377-8075-21000457.

[28] Selvaraj K, Venkatesan LS, Ganapathy D, Sathishkumar P. Treatment of dental biofilm-forming bacterium Streptococcus mutans using tannic acid-mediated gold nanoparticles. Microb Pathog. 2024;189:106568. Doi: 10.1016/j.micpath.2024.106568.

PARTICULARS OF CONTRIBUTORS:
1. Dentist, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.
2. Senior Lecturer, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. PT Priyangha,
Senior Lecturer, Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.
E-mail: priyanghapt.sdc@saveetha.com

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