

In vitro antibacterial activity and oral epithelial cytocompatibility of myrrh-mediated silver nanoparticles against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Purpose

To study synthesized myrrh-mediated silver nanoparticles and characterize their physicochemical properties, evaluate the antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* alongside normal oral epithelial cells cytocompatibility.

Methods

Silver nanoparticles were synthesized with aqueous *Commiphora myrrha* extract and characterized by spectroscopic, diffraction, light-scattering, elemental, and electron-microscopic methods. Antibacterial activity was assessed by agar diffusion, broth microdilution, and bactericidal subculture. Viability of normal oral epithelial, MCF-7, and HeLa cells was assessed by tetrazolium reduction and neutral red uptake assays after 24 h.

Results

Nanoparticle formation was supported by a 416-nm plasmon band and face-centered cubic silver. Transmission electron microscopy mainly showed spherical to quasi-spherical particles measuring 4.3–39.3 nm (mean, 17.7 nm); the hydrodynamic peak was 210 nm and zeta potential was – 27.5 mV. Minimum inhibitory concentration was 10 µg/mL for both bacteria; bactericidal endpoints were 20 and 30 µg/mL, respectively. Oral epithelial-cell viability remained above 83% at 100 µg/mL, whereas both cancer cell lines showed concentration-dependent viability loss.

Conclusions

Myrrh-mediated silver nanoparticles showed antibacterial activity and comparatively favorable oral epithelial cytocompatibility under *in vitro* conditions. Oral-biofilm, clinical-isolate, and expanded oral-cell studies are required before clinical application.

Introduction

Oral and maxillofacial infections include odontogenic abscesses as well as postoperative and opportunistic infections associated with major surgical procedures (Bahl et al. 2014; Chen et al. 2023; Park et al. 2021). Although odontogenic infections are typically polymicrobial, with both aerobic and anaerobic bacteria involved (Bahl et al. 2014), specific opportunistic pathogens may be encountered in particular clinical settings. For example, *Staphylococcus aureus* has been isolated from odontogenic maxillofacial abscesses (Kang and Kim 2019). *S. aureus* and *Pseudomonas aeruginosa* have also been reported in the oral and maxillofacial region and in postoperative respiratory infections following oral and

maxillofacial surgery (Chen et al. 2023; Park et al. 2021). Both species have also been detected within oral epithelial cells (Colombo et al. 2013). These microorganisms therefore provide standardized Gram-positive and Gram-negative reference models for antimicrobial screening relevant to oral and maxillofacial infection settings.

Myrrh also has direct clinical relevance in dentistry and oral and maxillofacial surgery independent of nanoparticle formulation. In a randomized double-blind clinical trial, myrrh mouthwash was used during the first week after tooth extraction. The myrrh group showed greater improvement in surgical-site edema, tenderness, and socket-size reduction than the saline group (Al Eid 2021). The study did not evaluate myrrh-based nanoparticles. Antimicrobial resistance can reduce the effectiveness of conventional treatment and contributes to morbidity and mortality worldwide (Murray et al. 2022; Ventola 2015). AgNPs have antibacterial properties, although their effects vary according to particle size and several other factors, including surface chemistry, surface charge, dissolution, and exposure conditions (Khan et al. 2019; Kittler et al. 2010; Marambio-Jones and Hoek 2010; Tang and Zheng 2018).

For oral applications, antibacterial activity should therefore be assessed together with cytocompatibility because local formulations may contact the oral epithelium.

Plant extracts can be used in metal nanoparticle synthesis, where phytochemicals may contribute to silver-ion reduction and surface stabilization (Devanesan and AlSalhi 2021; Dikshit et al. 2021; Kurhade et al. 2021; Narayanan and Sakthivel 2011).

Myrrh is an aromatic oleogum resin obtained from *Commiphora myrrha* and contains volatile oils, resinous fractions, and water-soluble gum components (Hanuš et al. 2005; Shen et al. 2012). These constituents contribute to reported antimicrobial effects against bacterial and fungal species, including oral and opportunistic microorganisms (Dolara et al. 2000; Omer et al. 2011; Shen et al. 2012).

Myrrh-mediated AgNPs have been reported previously, including ultraviolet-assisted synthesis with antibacterial testing, broad antimicrobial screening with colon cancer-cell viability, and testing against the oral pathogen *Porphyromonas gingivalis* (AlHarthi et al. 2021; Alwhibi et al. 2020; El-Sherbiny et al. 2013). In those identified studies, normal oral epithelial cells (OECs) were not used as a nonmalignant comparator in an integrated physicochemical, minimum inhibitory concentration, minimum bactericidal concentration, and cytotoxicity assessment (AlHarthi et al. 2021; Alwhibi et al. 2020; El-Sherbiny et al. 2013).

As a consequence, the present research was undertaken to study synthesized myrrh-mediated silver nanoparticles and characterize their physicochemical properties, evaluate the antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* alongside normal oral epithelial cells cytocompatibility.

Materials and Methods

Chemicals and reagents

Silver nitrate (AgNO_3 , $\geq 99.9\%$) and neutral red were obtained from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water was produced using a water-purification system from Merck (Darmstadt, Germany). Other solvents, cell-culture reagents, microbiological media, and disposable materials were obtained from commercial suppliers recorded in the study laboratory files.

Myrrh material and aqueous extract preparation

Commiphora myrrha resin was purchased from a local market in Riyadh, Saudi Arabia, rinsed with tap water followed by Milli-Q water, air-dried at room temperature, and ground into a fine powder. An aqueous extract was prepared by dispersing 25 g of myrrh powder in 500 mL of Milli-Q water (1:20, w/v; 50 mg/mL starting-material equivalent). The suspension was heated at 65°C for 5 h under magnetic stirring at 300 rpm and then left to soak at room temperature overnight. The extract was filtered through grade 1 qualitative filter paper and centrifuged at 5,000 rpm for 15 min; the supernatant was stored at 4°C until use (AlHarthi et al. 2021; Alwhibi et al. 2020; Binsalah et al. 2022; El-Sherbiny et al. 2013).

Green synthesis of myrrh-mediated silver nanoparticles

An aqueous silver nitrate solution was prepared by dissolving 3 mM AgNO_3 in 250 mL of Milli-Q water under stirring. Fifty milliliters of aqueous myrrh extract (50 mg/mL starting-material equivalent) was added to obtain a 300-mL reaction mixture with final concentrations of 2.5 mM AgNO_3 and 8.33 mg/mL extract equivalent. The mixture was stirred at 600 rpm for 2 h at room temperature, protected from light, and incubated at 65°C . AgNPs formation was indicated by a gradual color change to light brown after approximately 8 h and a dark-brown colloidal suspension after 24 h. The suspension was sonicated in an ultrasonic bath (40 kHz, 150 W) for 15 min. It was then centrifuged at 15,000 rpm for 15 min, washed three times with Milli-Q water, dried at 40°C for 24 h, and stored at 4°C (Binsalah et al. 2022). Nanoparticle suspensions used for biological experiments were aseptically prepared.

Physicochemical characterization

Ultraviolet-visible (UV-Vis) spectra of the final myrrh-AgNPs suspension, aqueous myrrh extract, and AgNO_3 solution were recorded over 250–700 nm using an LS 55 spectrophotometer (PerkinElmer Inc., Waltham, MA, USA). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of dried myrrh extract and dried myrrh-AgNPs were collected over $4,000\text{--}500\text{ cm}^{-1}$ using an FTIR Prestige-21 instrument (Shimadzu Corp., Kyoto, Japan). X-ray diffraction (XRD) was performed with a PW-1830 instrument (Philips Analytical, Almelo, the Netherlands). Particle morphology and primary size were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) using JEOL instruments (JEOL Ltd., Tokyo, Japan). One hundred clearly distinguishable particles from six TEM micrographs were measured, excluding overlapping or aggregated particles. Energy-dispersive X-ray

spectroscopy (EDX; JEOL Ltd.) was used for elemental analysis. Dynamic light scattering (DLS) and zeta potential were measured after redispersing dried nanoparticles in Milli-Q water and sonicating immediately before measurement with a Zetasizer instrument (Malvern Instruments Ltd., Malvern, UK); the polydispersity index (PDI) was recorded.

Cell culture conditions

Human breast adenocarcinoma MCF-7 (RRID: CVCL_0031) and human cervical cancer HeLa (RRID: CVCL_0030) cell lines were provided by the Bioproducts Research Chair, King Saud University, Riyadh, Saudi Arabia. Normal human oral epithelial cells (OECs) were supplied as mycoplasma-negative cells by Nawah Scientific Inc. (Cairo, Egypt; code R-SO25430). Cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution at 37°C in a humidified 5% CO₂ atmosphere. Subculture was performed at 80–90% confluence, and cell morphology and proliferation were regularly monitored under an inverted microscope.

Nanoparticle stock and working concentrations

For biological assays, a stock suspension was prepared by dispersing 10 mg of purified dried myrrh-AgNPs in 1 mL of sterile phosphate-buffered saline (PBS; 10 mg/mL). Biological concentrations were reported according to dried nanoparticle mass. The suspension was sonicated in an ultrasonic bath (40 kHz, 150 W) for 15 min and immediately diluted into culture medium to obtain 2.5, 5, 10, 25, 50, 75, and 100 µg/mL. The unpurified reaction mixture was not used in the biological assays.

Tetrazolium reduction assay

The cytotoxic effects of myrrh-AgNPs were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann 1983). Cells were seeded at 1×10^4 cells/well in 96-well plates (100 µL medium/well) and incubated overnight. Cells were treated with 2.5–100 µg/mL myrrh-AgNPs for 24 h; untreated cells served as controls. The medium was removed, 10 µL of MTT solution (5 mg/mL) was added per well, and plates were incubated for 4 h. The solution was then replaced with 200 µL dimethyl sulfoxide (DMSO) to dissolve formazan crystals, and absorbance was measured at 550 nm using a Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Cell viability (%) was calculated as treated-group mean absorbance divided by control-group mean absorbance $\times 100$. Separate nanoparticle-only optical-interference wells were not included during interpretation.

Neutral red uptake assay

Cell viability was also evaluated by the neutral red uptake (NRU) assay (Borenfreund and Puerner 1985). Cells were seeded at 1×10^4 cells/well in 96-well plates, incubated overnight, and exposed to 2.5–100

µg/mL myrrh-AgNPs for 24 h. After washing with phosphate-buffered saline (PBS), neutral red solution (50 µg/mL) was added and incubated for 3 h at 37°C. Incorporated dye was extracted with a destaining solution containing 50% ethanol, 49% deionized water, and 1% glacial acetic acid. Absorbance was measured at 550 nm using a microplate reader. For NRU testing, the same MTT assay replication structure was used.

Morphological assessment

Morphological alterations in treated HeLa, MCF-7, and OECs cultures were examined as a qualitative complement to viability assays. Cells were seeded at 2×10^4 cells/well in 24-well plates, incubated for 24 h, and treated with myrrh-AgNPs for 24 h. Morphological changes were observed under an inverted microscope (Olympus Scientific Solutions, Tokyo, Japan) at 20× magnification. Phase-contrast morphology was interpreted as qualitative evidence of cell stress and loss of adherence.

Bacterial strains and culture conditions

Facultative anaerobic Gram-positive *Staphylococcus aureus* (ATCC 25923) and aerobic Gram-negative *Pseudomonas aeruginosa* (ATCC 50071) were used. Tryptic soy broth (TSB; Sigma-Aldrich) was used for routine growth. Overnight cultures at 37°C were diluted 1:50 in fresh TSB and incubated for 3 h to reach logarithmic growth. Suspensions were adjusted to a 0.5 McFarland standard and verified by optical density at 600 nm (OD_{600}), as applicable (Binsalah et al. 2022).

Agar well diffusion

Antibacterial activity was assessed on Mueller-Hinton agar (MHA). Nineteen grams of MHA powder were dissolved in 500 mL distilled water, sterilized at 121°C for 15 min, and poured at approximately 25 mL/plate. Plates were swabbed with bacterial suspensions adjusted to 0.5 McFarland. The 10 mg/mL myrrh-AgNPs stock was loaded at 12.5, 25, 50, 75, or 100 µL/well, corresponding to 125, 250, 500, 750, and 1,000 µg/well. A piperacillin/tazobactam control well verified bacterial responsiveness and assay performance. Plates were incubated at 37°C for 18–24 h and inhibition zones were measured in millimeters.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by broth microdilution in 96-well plates, with MTT used as an auxiliary colorimetric indicator after visual growth assessment. Final myrrh-AgNPs concentrations were 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 µg/mL. A fresh 0.5 McFarland suspension was diluted to approximately 5×10^5 colony-forming units (CFU)/mL per well. Growth controls, uninoculated sterility controls, medium-only blanks, and AgNPs-only matched blanks were included.

Piperacillin/tazobactam control wells confirmed assay responsiveness. After 24 h at 37°C, wells were inspected visually; 15 µL MTT was then added and plates were incubated for 4 h. MIC was the lowest concentration with no visible growth and no MTT reduction relative to the growth control after accounting for matched blanks.

Minimum bactericidal concentration (MBC)

The operational minimum bactericidal concentration (MBC) was determined by subculturing from myrrh-AgNPs MIC wells that showed no visible growth. A 10 μ L aliquot from each non-growth well (typically 1 $\times$, 2 $\times$, 4 $\times$, and 8 $\times$ MIC) was spotted onto MHA plates and incubated at 37°C for 18–24 h. The MBC was defined as the lowest myrrh-AgNPs concentration that yielded no colony growth on MHA ($\geq 99.9\%$ killing relative to the growth control).

Statistical analysis

All experiments were performed in triplicate. Data are presented as mean $\pm$ standard deviation. Statistical analyses were performed using GraphPad Prism version 9.3.1. One-way analysis of variance followed by Tukey's multiple-comparison test was used for multiple concentration comparisons within each assay, and two-way analysis of variance was used when two independent factors were analyzed. A p value ≤ 0.05 was considered statistically significant.

Results

Physicochemical characterization

A gradual color change from yellow to brown and then dark brown indicated AgNPs formation (Fig. 1A).

UV-Vis analysis showed a distinct surface plasmon resonance band at approximately 416 nm in the myrrh-AgNPs suspension, whereas myrrh extract and AgNO₃ controls did not show a comparable band (Fig. 1B).

ATR-FTIR showed shifts in the O-H/N-H, C=O/C=C, C-H, and C-O/C-N regions. These changes indicate the presence of myrrh-derived functional groups on the nanoparticle surface, although specific biomolecules could not be identified (Fig. 2A).

Four sharp reflections corresponding to the (111), (200), (220), and (311) planes of face-centered cubic silver were observed in the XRD pattern of the myrrh-AgNPs. The myrrh extract instead produced a broad diffuse pattern without distinct Bragg peaks, indicating a predominantly amorphous structure (Fig. 2B).

DLS showed a 210.0-nm hydrodynamic peak (PDI 0.31 ± 0.02) (Fig. 3A), and zeta potential was -27.5 mV (Fig. 3B).

EDX confirmed silver signals with carbon, oxygen, and minor elemental peaks consistent with an organic surface layer (Fig. 3C).

SEM and TEM showed predominantly spherical to quasi-spherical particles, and TEM primary-particle size was 4.3–39.3 nm (17.7 ± 10.7 nm; Fig. 4).

Cell viability and morphology

The MTT assay showed concentration-dependent viability changes in all three cell lines (Fig. 5). At 100 µg/mL, OECs viability was $85.18 \pm 0.31\%$, whereas MCF-7 and HeLa viability was $7.92 \pm 0.07\%$ and $6.84 \pm 0.01\%$, respectively. The half-maximal viability threshold was not reached for OECs within the tested range ($IC_{50} > 100 \mu\text{g/mL}$); the MTT-derived IC_{50} values were 22.2 µg/mL for MCF-7 and 29.7 µg/mL for HeLa cells.

The NRU assay showed the same overall pattern (Fig. 6). At 100 µg/mL, OECs viability was $83.17 \pm 0.01\%$, whereas MCF-7 and HeLa viability was $18.56 \pm 0.19\%$ and $14.85 \pm 0.18\%$, respectively. The NRU-derived IC_{50} was $>100 \mu\text{g/mL}$ for OECs, 23.4 µg/mL for MCF-7 cells, and 34.9 µg/mL for HeLa cells. Differences across concentrations were statistically significant by one-way analysis of variance with Tukey's multiple-comparison test ($p \leq 0.05$).

Phase-contrast microscopy showed comparatively preserved OECs morphology and more pronounced concentration-associated rounding, shrinkage, reduced spreading, and loss of adherence in MCF-7 and HeLa cells (Fig. 7). The microscopy findings were interpreted as qualitative evidence of cell stress rather than proof of a specific cell-death pathway.

Antibacterial activity

Myrrh-AgNPs produced dose-related inhibition against both reference strains (Fig. 8). No inhibition was observed at 12.5 µL/well. At 100 µL/well, inhibition zones reached $18 \pm 0.7 \text{ mm}$ for *Staphylococcus aureus* and $15 \pm 1 \text{ mm}$ for *Pseudomonas aeruginosa* (Table 1).

Clear inhibition was observed with piperacillin/tazobactam control, confirming that the assay was responsive. Both microorganisms had an MIC of 10 µg/mL. The MBC endpoint was 20 µg/mL for *Staphylococcus aureus*, compared with 30 µg/mL for *Pseudomonas aeruginosa* (Table 2).

Discussion

Previous studies have shown that myrrh can mediate AgNPs formation and that myrrh-associated AgNPs preparations can display antimicrobial activities (AlHarthi et al. 2021; Alwhibi et al. 2020; El-Sherbiny et al. 2013). The present study adds an integrated activity-safety profile to normal oral epithelium rather than a stand-alone synthesis and antimicrobial claim. Among the identified previous studies, normal OECs were not used as a nonmalignant comparator; the present work therefore contributes an oral epithelial cytocompatibility reference alongside physicochemical characterization and quantitative antibacterial endpoints.

The dental relevance of the myrrh component is supported by oral-surgery evidence independent of the nanoparticle formulation. Myrrh mouthwash used during the first postoperative week after tooth extraction improved surgical-site edema, tenderness, and socket-size reduction compared with saline (Al Eid 2021). That trial supports further investigation of myrrh-containing oral formulations, but it cannot be used as evidence that the present myrrh-AgNPs formulation.

The characterization pattern supports formation of capped, crystalline myrrh-AgNPs. The approximately 416-nm plasmon band, face-centered cubic silver XRD reflections, silver EDX signal, and spherical to quasi-spherical TEM morphology collectively support AgNPs formation. The larger DLS diameter than TEM primary-particle size is expected because DLS measures hydrodynamic objects in suspension, including the organic corona, hydration layer, intensity weighting of larger particles, and limited agglomeration (Lundqvist et al. 2008; Maguire et al. 2018; Monopoli et al. 2012). Because nanoparticle dispersions can change in proteins, salts, and assay media, the water-based DLS and zeta-potential findings are baseline characterization (Kittler et al. 2010; Lundqvist et al. 2008; Monopoli et al. 2012; Zhang et al. 2023).

The viability findings show a concentration-related separation between the malignant cell lines and normal oral epithelial cells during a 24-h exposure. This pattern is compatible with reported AgNPs-associated oxidative stress, mitochondrial or lysosomal injury, cell-cycle effects, and apoptosis-related responses in cancer cells (Gurunathan et al. 2013; Khorrami et al. 2018; Panzarini et al. 2017; Ullah et al. 2020). However, the paired MTT and NRU findings are in vitro screening evidence. AgNPs can interfere with colorimetric viability assays; the results should therefore be interpreted together with microscopy and confirmed using nanoparticle-only interference controls and dye-independent endpoints (Mello et al. 2020).

MIC and MBC provide more quantitative antibacterial information than agar diffusion because nanoparticle diffusion through agar can be affected by particle size, aggregation, surface capping, and silver-species release (Dakal et al. 2016; Zhang et al. 2023). The larger inhibition zones against *Staphylococcus aureus* than *Pseudomonas aeruginosa* are consistent with the outer-membrane barrier, efflux systems, and biofilm-related resilience of *Pseudomonas aeruginosa* (Avakh et al. 2023; Silhavy et al. 2010; Thi et al. 2020). In broth, both organisms shared a 10 µg/mL MIC, and the operational MBC values were two- to three-fold higher.

The antibacterial and cytotoxic effects must be attributed to the complete myrrh-capped AgNPs formulation because the design did not isolate the individual contributions of myrrh extract, ionic silver, and nanoparticulate silver. Future studies should include additional controls, such as myrrh extract alone, an equivalent AgNO₃ concentration, and AgNPs with different surface coatings. Stability in biological media and silver-ion release should also be evaluated. Testing against anaerobic bacteria, mixed bacterial cultures, and biofilms would provide a better representation of oral infections. These factors, together with cytocompatibility, should be considered when evaluating the antimicrobial activity of biomaterials (Zhang et al. 2025).

The microorganisms tested in this study represent only part of the bacteria encountered in oral and maxillofacial infections. Odontogenic infections are usually polymicrobial, with oral streptococci and anaerobic bacteria commonly identified (Bahl et al. 2014). *Staphylococcus aureus* has also been isolated from odontogenic maxillofacial abscesses (Kang and Kim 2019). *Pseudomonas aeruginosa* is less

typical of uncomplicated odontogenic infections but has been reported in some postoperative and other oral and maxillofacial infection settings (Chen et al. 2023; Park et al. 2021).

Detection of both species within oral epithelial cells further supports relevance to the oral microbial reservoir (Colombo et al. 2013). The current findings should therefore be interpreted as controlled screening against clinically relevant Gram-positive and Gram-negative reference organisms, not as a substitute for testing viridans *streptococci*, obligate anaerobes, *Porphyromonas gingivalis*, multispecies oral biofilms, or clinical isolates.

Taken together, myrrh-mediated AgNPs showed crystalline silver structure, nanoscale primary morphology, antibacterial activity against two reference bacteria relevant to oral and maxillofacial infection settings, and comparatively preserved OECs viability relative to MCF-7 and HeLa cells. The study extended previous myrrh-AgNPs work by integrating physicochemical characterization, MIC and operational MBC endpoints, and a normal oral epithelial comparator.

Conclusions

The present findings expand the literature on green synthesized myrrh-based silver nanomaterials by pairing quantitative antibacterial endpoints with comparative in vitro cytotoxicity in normal oral epithelial and cancer cells.

To the best of our knowledge, this is the first study to evaluate the cytotoxicity/biocompatibility of myrrh-mediated AgNPs in normal OECs. Myrrh-mediated silver nanoparticles showed antibacterial activity and comparatively favorable oral epithelial cytocompatibility under *in vitro* conditions. Oral-biofilm, clinical-isolate, and expanded oral-cell studies are required before clinical application.

Declarations

Ethical Statement

This Research was approved and registered with College of Dentistry Research Center (CDRC #PR0174) at King Saud University, Riyadh, Saudi Arabia. the present in-vitro study used commercially available established cell lines and did not involve human participants, identifiable human data, or animals.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Author Contribution

A.A., S.D., O.A., and R.K. contributed to the conceptualization of the study. A.A., S.D., and M.A. developed the methodology. A.A., S.D., M.A., N.A., and A.A.-B. carried out the investigation. A.A., S.D., N.A., and A.A.-B. were responsible for data curation. A.A. wrote the original draft of the manuscript. S.D. reviewed and edited the manuscript. R.K. and O.A. supervised the study. Funding acquisition: Not applicable.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon request.

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Tables

Table 1. Inhibition zones produced by myrrh-mediated silver nanoparticles. Values are presented as mean $\pm$ standard deviation (mm).

Bacterium	12.5 μ L (125 μ g/well)	25 μ L (250 μ g/well)	50 μ L (500 μ g/well)	75 μ L (750 μ g/well)	100 μ L (1,000 μ g/well)
<i>Staphylococcus aureus</i>	0	2 $\pm$ 0.4	13 $\pm$ 0.4	15 $\pm$ 0.7	18 $\pm$ 0.7
<i>Pseudomonas aeruginosa</i>	0	1 $\pm$ 1	10 $\pm$ 0.6	12 $\pm$ 0.4	15 $\pm$ 1

Table 2. Minimum inhibitory concentration and operational minimum bactericidal concentration endpoints. Values are presented as mean $\pm$ standard deviation (mm)

Bacterium	MIC ($\mu\text{g/mL}$)	Operational MBC * ($\mu\text{g/mL}$)
<i>Staphylococcus aureus</i>	10 ± 0	20 ± 0
<i>Pseudomonas aeruginosa</i>	10 ± 0	30 ± 0

*MBC was defined operationally as no colony growth from a 10- μL subculture spot; baseline and surviving colony-forming units were not quantified.

Figures

Figure 1A



Figure 1B

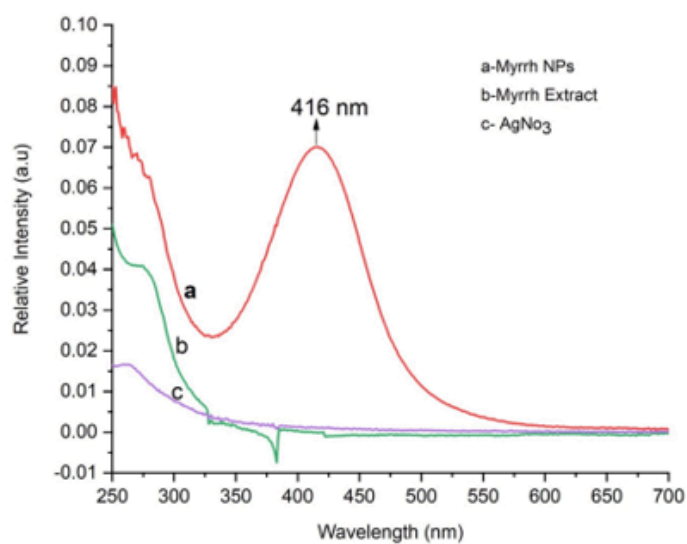


Figure 1

Synthesis and optical confirmation of myrrh-mediated silver nanoparticles.

(A) Silver nitrate solution, reaction mixture immediately after addition of aqueous myrrh extract, and the brown colloid after incubation. (B) Ultraviolet-visible spectra of myrrh-mediated silver nanoparticles, myrrh extract, and silver nitrate; the nanoparticle suspension shows a surface plasmon resonance band at approximately 416 nm

Figure 2A

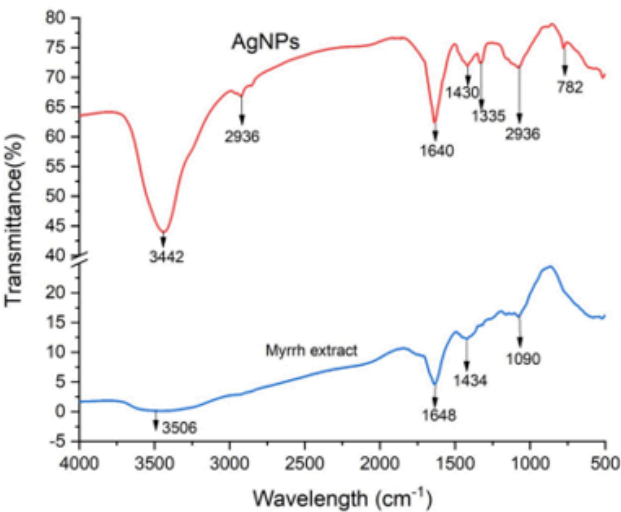


Figure 2B

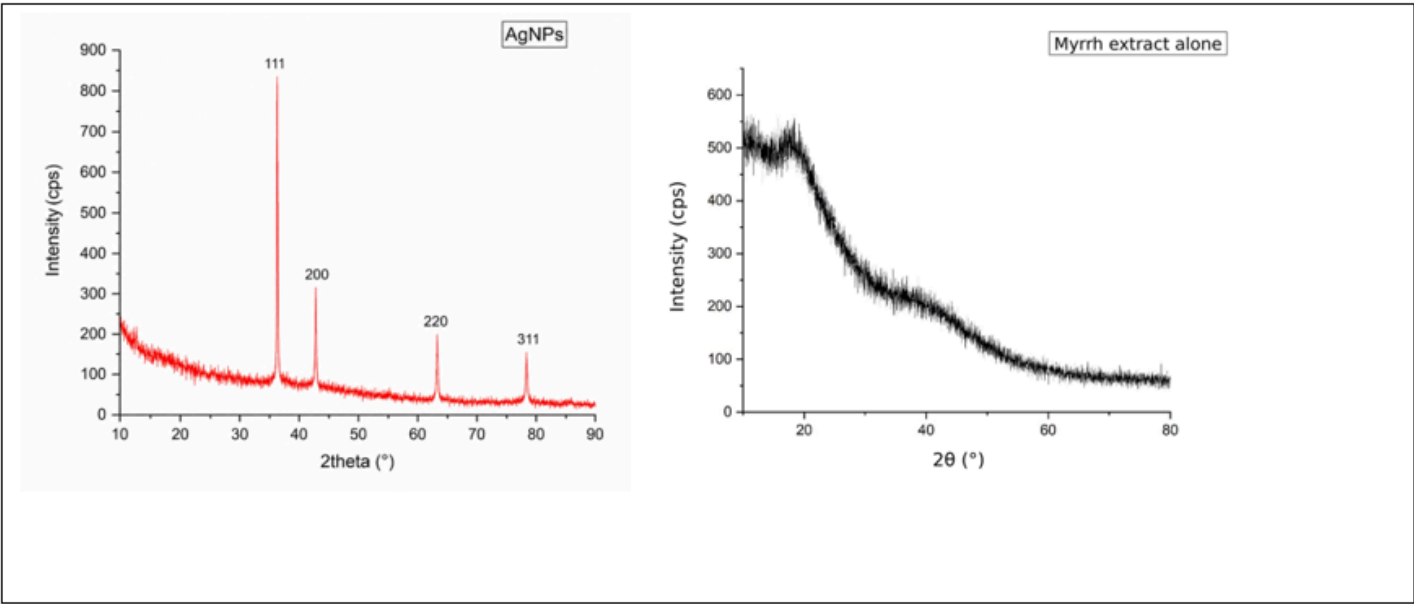


Figure 2

Spectroscopic and diffraction characterization.

(A) Attenuated total reflectance Fourier transform infrared spectra of myrrh extract and myrrh-mediated silver nanoparticles. (B) X-ray diffraction patterns of the nanoparticles and myrrh extract; silver reflections are indexed to face-centered cubic metallic silver

Figure 3A

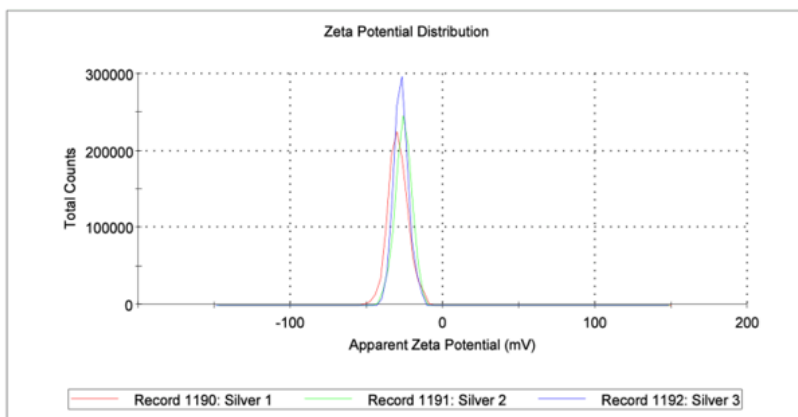


Figure 3B

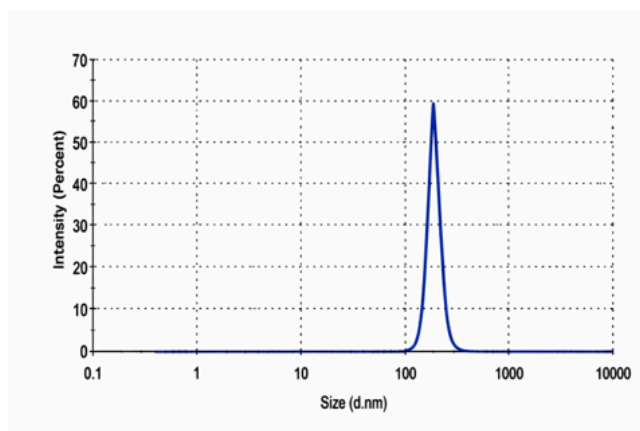


Figure 3C

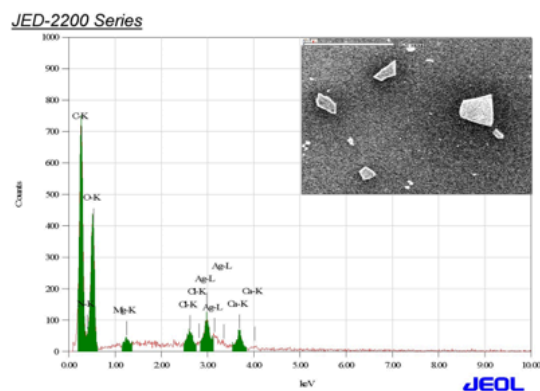


Figure 3

Colloidal and elemental characterization.

(A and B) Zeta-potential distribution and dynamic light-scattering hydrodynamic size distribution of myrrh-mediated silver nanoparticles. (C) Energy-dispersive X-ray spectrum showing characteristic silver signals with carbon, oxygen, and minor elemental peaks

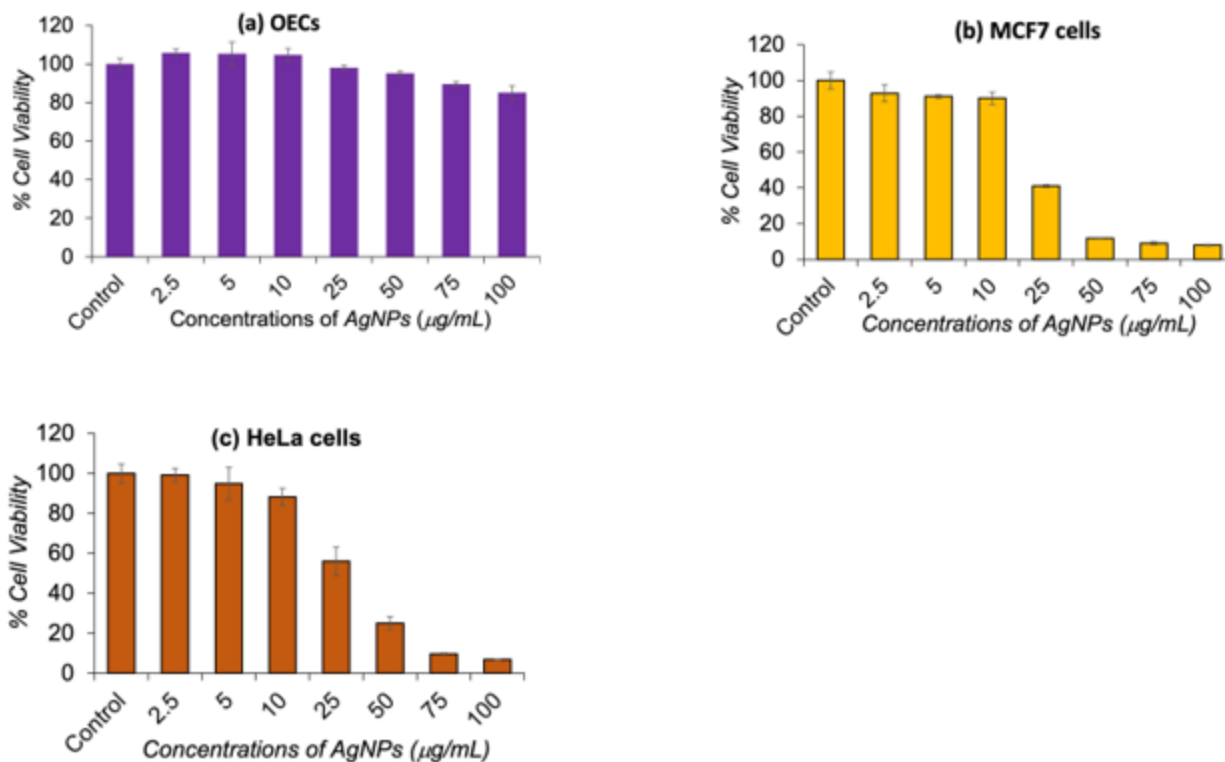


Figure 5

MTT viability after 24-h exposure to myrrh-mediated silver nanoparticles.

(A) Normal oral epithelial cells, (B) MCF-7 cells, and (C) HeLa cells.

* Bars represent the mean and error bars the standard deviation of three independent experiments, each containing three technical wells per condition. Differences across concentrations were evaluated by one-way analysis of variance followed by Tukey's multiple-comparison test ($p \leq 0.05$)

Figure 6

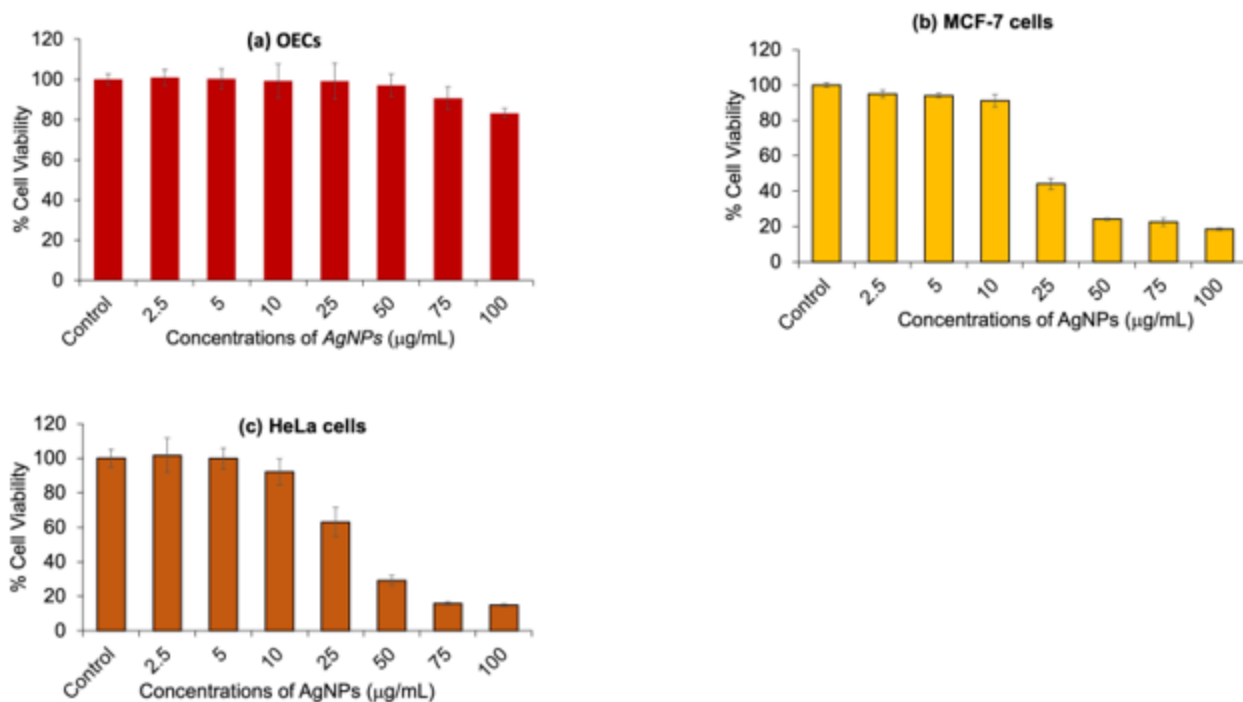


Figure 6

Neutral red uptake viability after 24-h exposure to myrrh-mediated silver nanoparticles.

(A) Normal oral epithelial cells, (B) MCF-7 cells, and (C) HeLa cells.

* Bars represent the mean and error bars the standard deviation of three independent experiments, each containing three technical wells per condition. Differences across concentrations were evaluated by one-way analysis of variance followed by Tukey's multiple-comparison test ($p \leq 0.05$).

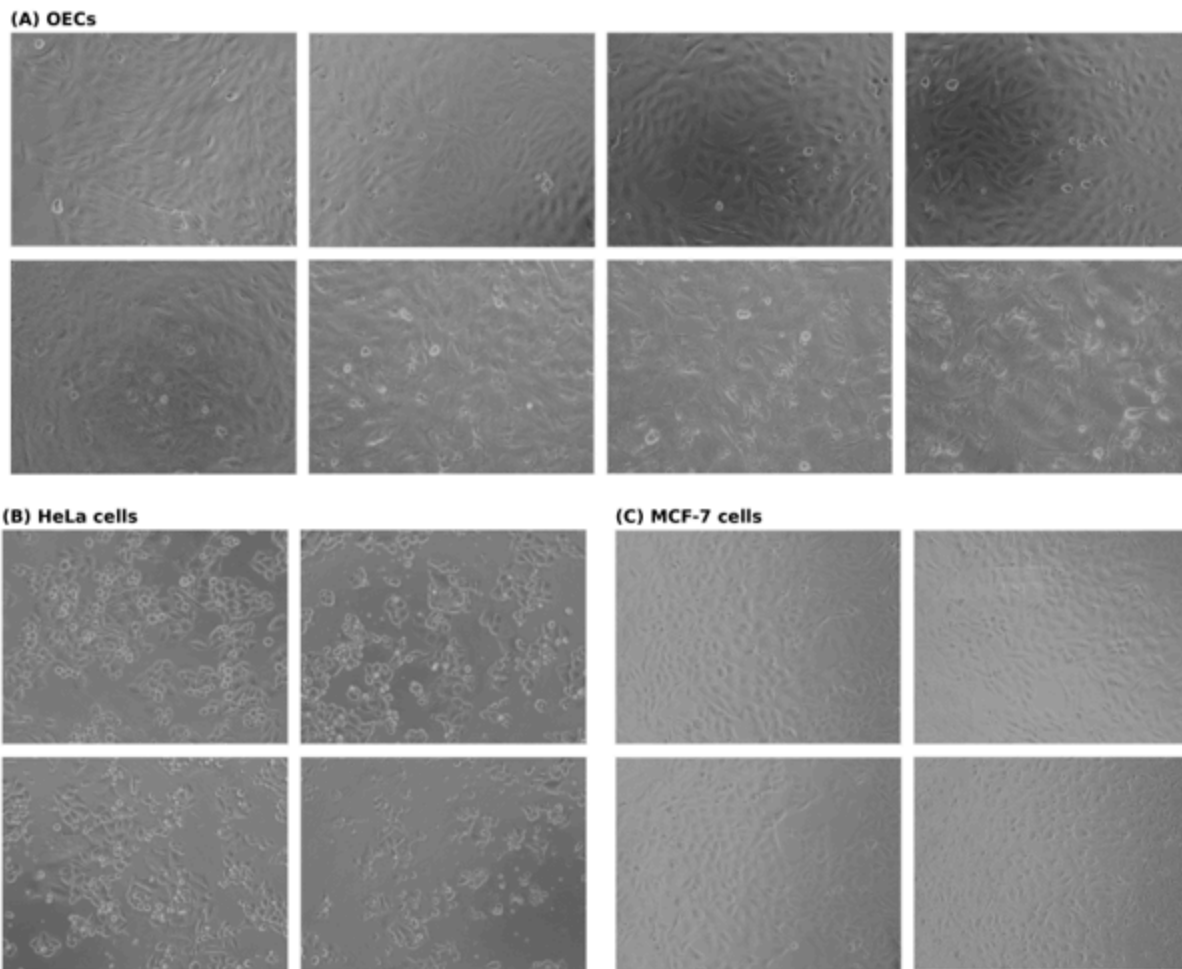


Figure 7

Phase-contrast morphology after 24-h exposure to myrrh-mediated silver nanoparticles.

Figure 7

(A) Normal oral epithelial cells at untreated control and 2.5-100 $\mu\text{g/mL}$. (B) HeLa cells at 2.5, 5, 10, and 25 $\mu\text{g/mL}$. (C) MCF-7 cells at 2.5, 5, 10, and 25 $\mu\text{g/mL}$.

* Images were interpreted as qualitative evidence of treatment-associated cell stress and loss of adherence, not as confirmation of a specific cell-death pathway. Original magnification, 20 $\times$

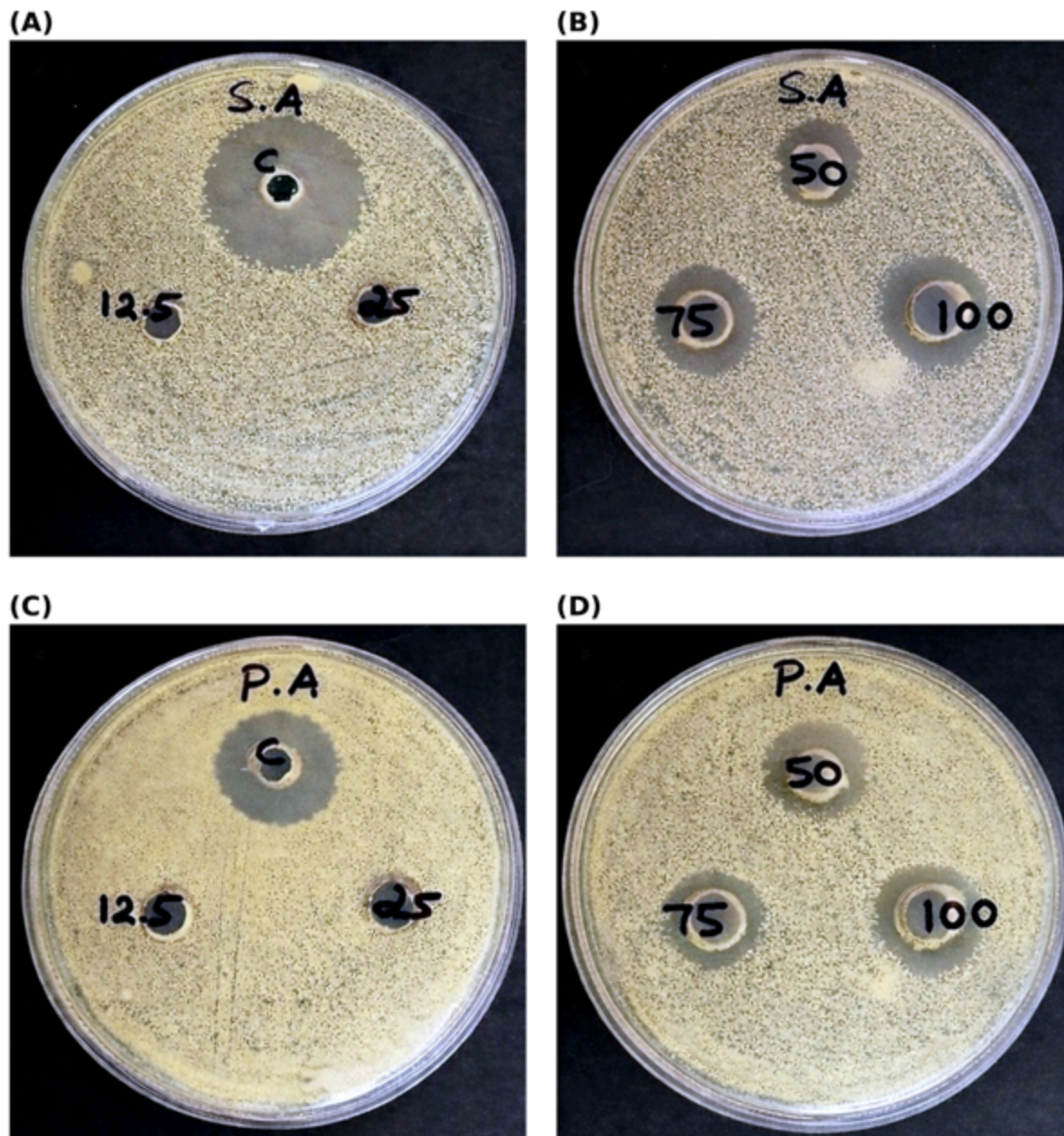


Figure 8

Agar well diffusion showing activity of myrrh-mediated silver nanoparticles against *Staphylococcus aureus* (A, B) and *Pseudomonas aeruginosa* (C, D).

* Labels 12.5-100 indicate nanoparticle suspension volume per well in microliters; C indicates the qualitative piperacillin/tazobactam assay-performance control