

Research Article

Shanmugam Rajeshkumar*, Munusamy Tharani, Vijayarangan Devi Rajeswari, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled, Kasi Gopinath, Natesan Vijayakumar, and Marimuthu Govindarajan*

Synthesis of greener silver nanoparticle-based chitosan nanocomposites and their potential antimicrobial activity against oral pathogens

<https://doi.org/10.1515/gps-2021-0060>

received July 14, 2021; accepted September 21, 2021

Abstract: In the present investigation, silver nanoparticles (AgNPs) and silver nanoparticle-based chitosan nanocomposite were synthesized using *Cissus arnottiana* leaf extract. The biosynthesized nanoparticles and nanocomposites were characterized using SEM, TEM, and AFM to uncover the morphological characteristics such as size and shape. The SEM image depicts the size of the nanocomposite to be 30–40 nm and shape as spherical. The TEM results reveal the shape of the nanocomposite to be spherical and size around 10–60 nm. The XRD results show the crystalline nature of the AgNPs-based chitosan nanocomposite. The SAED analysis pattern seems to be concordant with the XRD results. The AFM image ensured the precise surface morphology of the synthesized silver nanocomposite

in the 3-dimensional pattern. The antimicrobial efficacy of the biosynthesized AgNPs, AgNP nanocomposite, and chitosan nanoparticles was tested against oral pathogens. The results revealed a potential antimicrobial effect, which states that it must be converted into nanomedicine to meet future biomedical needs.

Keywords: green synthesis, silver nanoparticles, chitosan nanocomposite, *Cissus arnottiana*

1 Introduction

Recently, the field of nanotechnology arised to a greater extent due to its ablazing applications in biomedical field [1]. This attracts huge number of researchers to create different nanomaterials with specific functions to cure a disease or to enhance and perform in equipments or products such as health care products, cosmetics, household products, etc. The nanolevel requirement of these nanomaterials in living cells plays a significant role in outraging the disease-causing pathogens and organisms [2].

Nowadays, the disease-causing pathogens become more resistant to upcoming antibiotic drugs. To combat this issue, metallic nanoparticles like silver nanoparticles (AgNPs) have been reported in several studies by researchers as an effective antimicrobial agent [3–5]. The AgNPs are majorly used in treating burn and open injuries to avoid contamination from wound pathogens and other nosocomial pathogens [6]. AgNPs assume a significant part in science and medication due to their desirable physicochemical properties. AgNPs are known to have antifungal, anti-inflammatory, antiviral, antibacterial, antiangiogenesis, and antiplatelet properties [7–9].

In several studies, chitosan, a natural biopolymer, has been reported to enhance the antibacterial efficacy of the metallic nanoparticles [10–12]. In this present study, chitosan was added to *Cissus arnottiana* leaf

* **Corresponding author: Shanmugam Rajeshkumar**, Department of Pharmacology, Saveetha Dental College and Hospital, SIMATS, Chennai 600077, Tamil Nadu, India, e-mail: ssrajeshkumar@hotmail.com

* **Corresponding author: Marimuthu Govindarajan**, Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalaiagar 608002, Tamil Nadu, India; Department of Zoology, Government College for Women (Autonomous), Kumbakonam 612001, Tamil Nadu, India, e-mail: drgovind1979@gmail.com

Munusamy Tharani: Department of Pharmacology, Saveetha Dental College and Hospital, SIMATS, Chennai 600077, Tamil Nadu, India

Vijayarangan Devi Rajeswari: Department of Bio-Medical Sciences, School of BioSciences and Technology, Vellore Institute of Technology (VIT), Vellore 632014, Tamil Nadu, India

Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled: Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Kasi Gopinath: Department of Packaging, Yonsei University, 1 Yonseidae-gil, Wonju-si, Gangwon-do 26493, South Korea

Natesan Vijayakumar: Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalaiagar 608002, Tamilnadu, India

extract-mediated AgNPs to attain as silver nanocomposite an increase in the potent antimicrobial efficacy of the AgNPs to a greater extent [13–15].

Cissus arnottiana is an erect woody shrub that has a place with the Vitaceae family distributed all through India. The plant roots are utilized as a remedy for rheumatic swellings. Phytochemical screening of *Cissus arnottiana* plant and fruit uncovered the presence of bioactive constituents, for example, tannins, phenols, terpenoids, flavonoids, glycosides, sugars, and saponins [16].

The AgNPs were incorporated with various polymers such as chitosan and its cross-linked polyvinyl pyrrolidone in gelatin, and polygalacturonic with hyaluronic acid-based silver nanofibers show good wound healing activity; the black berry-mediated silver, gold, and silver/gold bimetallic nanoparticles loaded with pectin show cardioprotective activity [17–19]. The AgNPs synthesized using various chemical methods show higher toxicity in animal model and cell line studies, but the AgNPs synthesized using biological agents like bacteria, plant and its parts, fungi, and algae show very good biomedical applications [20–22].

The current study deals with synthesizing AgNPs and silver chitosan nanocomposite using *Cissus arnottiana* extract as a stabilizing and reducing agent. The synthesized silver nanocomposite has been characterized using UV-double beam spectrophotometer, scanning electron microscope, transmission electron microscope, atomic force microscope, and X-ray diffraction analysis. The antimicrobial efficacy of biosynthesized silver nanocomposite was

tested against oral pathogens such as *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*.

2 Materials and methods

2.1 Chemicals

The precursor silver nitrate was purchased from Sigma Aldrich chemicals Pvt. Ltd (India). Mueller Hinton Agar was received from Hi-Media, India. The leaves of *Cissus arnottiana* plant were localized in rural areas of Vellore, Tamilnadu, India. The bacterial cultures such as *S. aureus*, *S. mutans*, *E. faecalis*, and *C. albicans* were isolated and collected from Saveetha Dental College and Hospital, Poovirunthavalli Chennai.

2.2 Preparation of *Cissus arnottiana* leaf extract

The leaves of *Cissus arnottiana* were washed thoroughly under tap water and with Milli-Q water. Then the plant was shade-dried for 3–4 days. The dried plant was grounded into a fine powder and stored in an airtight container. 1 g of the dried powdered plant was added to 100 mL of double-distilled water. The mixture was then heated using a heating

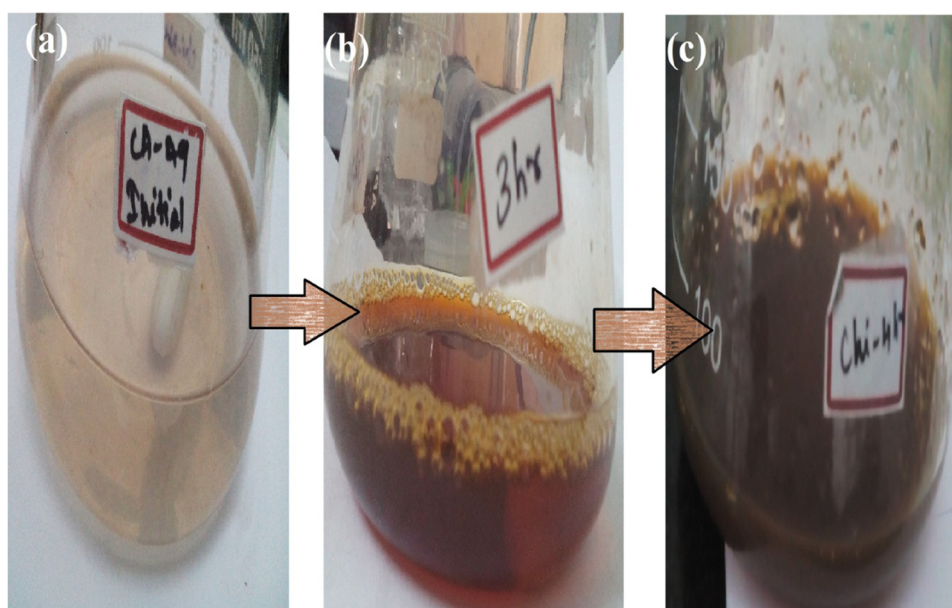


Figure 1: Visual observation: (a) silver nitrate with plant extract, (b) silver nanoparticles, and (c) AgNPs-based chitosan nanocomposites.

mantle at 70°C for 15 min. By this strategy, all phytochemical compounds present in the *Cissus arnottiana* plant get diffused in the aqueous solution. This final mixture was filtered by using filter paper (Whatman No.1) and the filtered leaf extract was stored in refrigerator for further use.

2.3 Nanoparticle synthesis

1 mM of precursor silver nitrate (AgNO_3) was added to 90 mL of deionized water. 10 mL of *Cissus arnottiana* filtered plant extract was added. The mixture was kept on a magnetic stirrer for 600–700 rpm for 48 h. UV-Vis-double beam spectrophotometer was used to analyze the synthesis of AgNPs at regular time intervals from the start wavelength 360–500 rpm (revolutions per minute). To collect pellet from the aqueous reaction mixture, bio-synthesized AgNPs were centrifuged at 8,000 rpm for 10 min. The supernatant was discarded, and pellet was

washed thrice with ethanol followed by deionized water and kept inside hot air oven at 70°C for 2 h. And powdered form of AgNPs was stored in airtight Eppendorf tube for characterization studies.

2.4 Preparation of nanocomposites

0.5 g of chitosan was dissolved in 1 mL of 1% glacial acetic acid and 49 mL of deionized water. The synthesized AgNPs were added to the chitosan solution and kept in a magnetic stirrer for 3–4 h. After adding *Cissus arnottiana*-mediated AgNPs solution, a brown nanocomposites gel was obtained. The mixture was kept again in a magnetic stirrer for 48 h. Formation of silver nanocomposites was analyzed by UV-Vis spectrometry in the wavelength range of 360–500 nm. The synthesized silver nanocomposite was allowed for centrifugation at 10,000 rpm for 10 min. The nanocomposite pellet was suspended in deionized water, centrifuged again, and lyophilized. The lyophilized silver chitosan nanocomposites were dissolved in distilled water and used for different characterization studies.

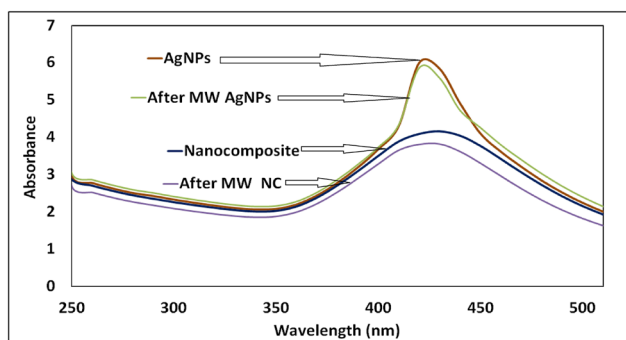


Figure 2: UV-Vis spectroscopic analysis of silver nanoparticles and AgNPs with chitosan.

2.5 Characterization of AgNPs and nanocomposites

The *Cissus arnottiana*-mediated AgNPs and nanocomposites were preliminarily characterized using UV-double beam spectrophotometer (UV-2450, Shimadzu) in the wavelength range of 360–500 nm. The morphology

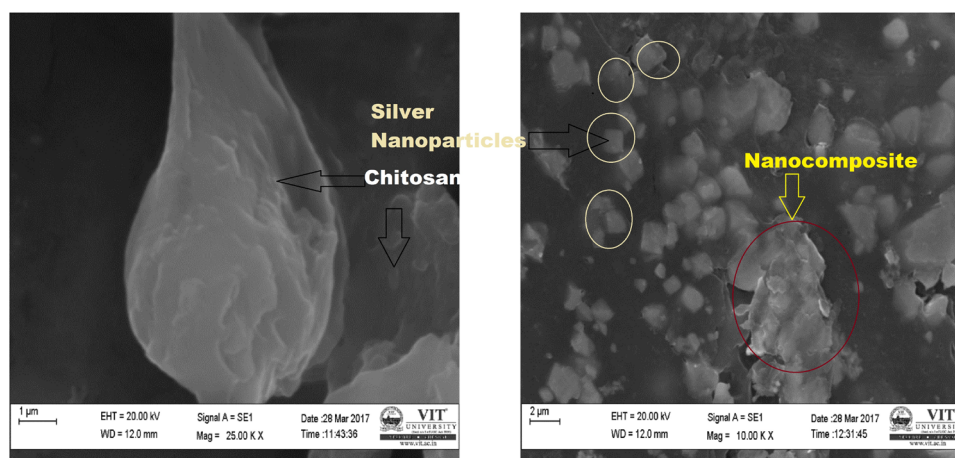


Figure 3: SEM images of chitosan and AgNPs embedded with chitosan.

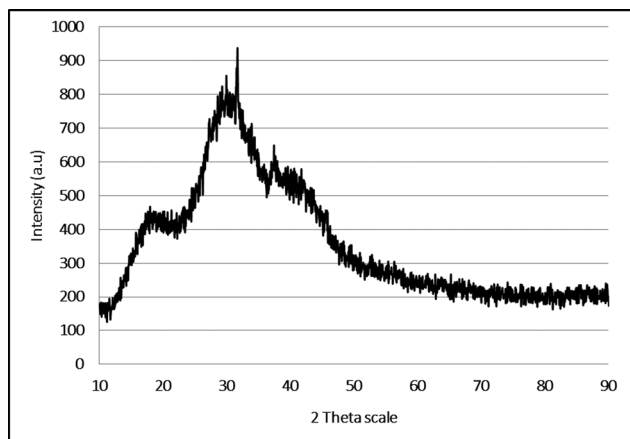


Figure 4: XRD pattern of AgNPs-based chitosan nanocomposite.

characteristics such as size and shape were studied using scanning electron microscope (SEM) and transmission electron microscope (TEM). The crystalline nature of the AgNPs-mediated chitosan nanoparticle was analyzed using X-ray diffraction. The atomic force microscope is

used to study the 3-D structure of the biosynthesized AgNPs-mediated nanocomposites with sub-nanometer resolution.

2.6 Antimicrobial activity against oral pathogens

10 μL of fresh bacterial cultures such as *S. aureus*, *S. mutans*, *E. faecalis*, and *C. albicans* were inoculated in sterile Hi-veg broth and kept above orbital shaker for 18 h at 120–150 rpm. Mueller Hinton agar was prepared, and 5 mm wells were made using a sterile polystyrene tip. The antimicrobial activity was done to enable the efficacy of *Cissus amottiana*-mediated AgNPs, biosynthesized AgNPs-mediated nanocomposite, and chitosan nanoparticle. Various concentrations such as 25, 50, and 100 μL of three samples were added to wells, and along with positive control, amox-yrite was added (except for *C. albicans*, fluconazole is used as a standard drug). The inoculated sample petriplates were kept

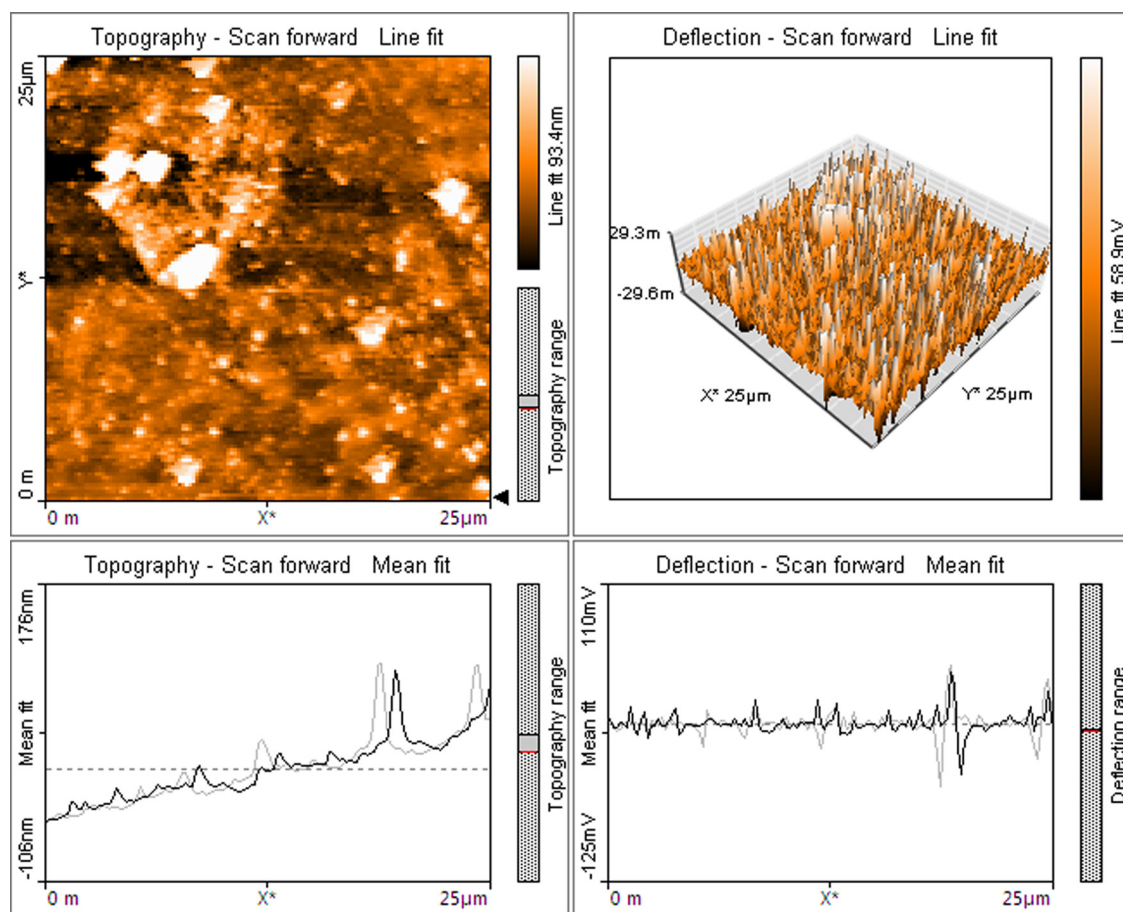


Figure 5: AFM image of AgNPs-based chitosan nanocomposite.

inside a microbial incubator at 37°C for 24 h and the zone of inhibition zone was measured to compare and study the potential effect of *Cissus amottiana*-mediated AgNP and nanocomposites and chitosan nanoparticle.

3 Results and discussion

3.1 Visual observation

Biosynthesis of nanoparticles by utilizing heterocyclic compounds acquires consideration because of their effortlessness and ecofriendly nature [23]. The color intensity of the *Cissus amottiana*-mediated AgNPs solution mixture increased by an increase in time. Also, the reduction of silver nitrate to Ag₀ by the reducing agent (*Cissus amottiana* leaf extract) was indicated by an initial light yellow to final dark brown color change (Figure 1), which was further confirmed by UV-Vis, spectrophotometry analysis. The AgNPs synthesized using orchid leaf show the brown in color confirms the nanoparticles formation [15], the AgNPs with chitosan also formed color formation confirms the nanocomposite formation [13].

3.2 Optical analysis by UV-Vis spectrophotometer

UV-Vis spectroscopy is a significant step to assess the development and stability of nanoparticles [24]. Recent work such as that of Thamilarasan *et al.* [11] analyzed concordant results as attained in the current research work. The maximum absorption peak of AgNPs was observed at 420 nm. The broad peak found around 380–460 nm confirms the silver chitosan nanocomposite (Figure 2). The small peak variation in the UV-Vis spectroscopy absorbance confirms the stability of the AgNPs after treating with microwave irradiation. The peak around 400–440 nm confirms the AgNPs synthesis using leaf extracts of *Clerodendrum inerme* and *Pedaliu murex* leaf extract [5,27].

3.3 SEM analysis

The SEM can be utilized to scan nanoscopic structures at high resolution [25]. The size of chitosan was depicted to be 90 nm and shape as pseudo-spherical. The size of *Cissus amottiana*-mediated AgNPs embedded with chitosan was found to be 30–40 nm and it reveals its shape as

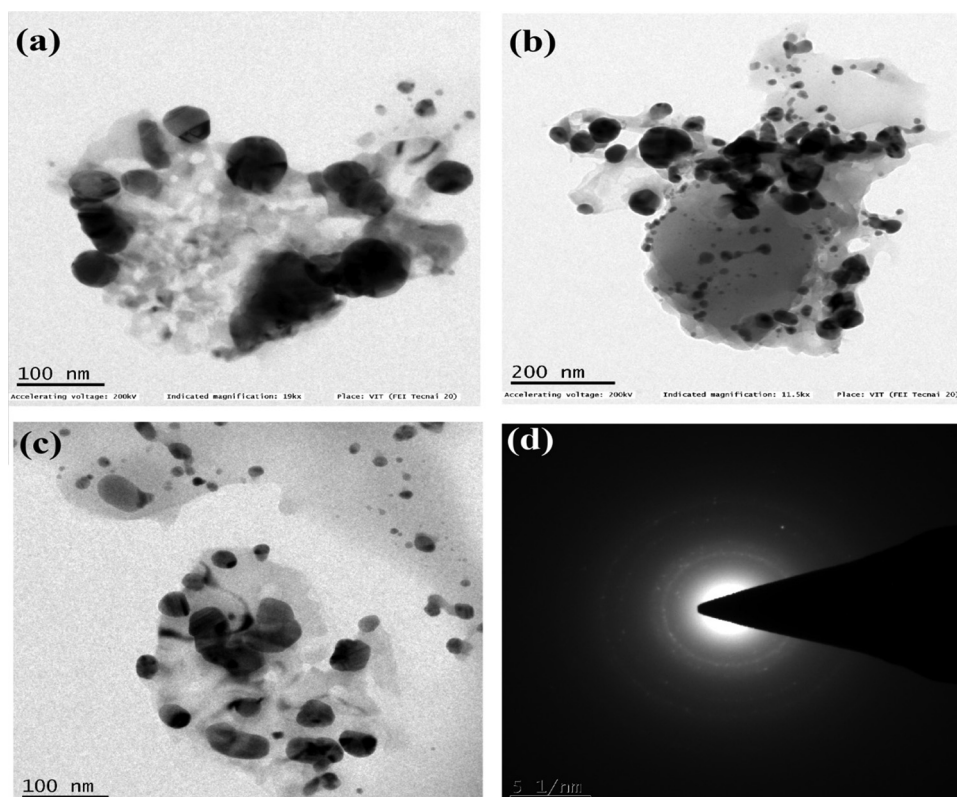


Figure 6: (a–c) TEM and (d) SAED analysis of *Cissus amottiana* mediated AgNPs based chitosan nanocomposites.

cuboidal (Figure 3). Previous studies such as those of authors of [26] correlate with the SEM result of chitosan and *Cissus arnottiana*-mediated silver nanocomposites.

3.4 XRD analysis

XRD spectra give an understanding of the crystallinity of nanoparticles. Figure 4 depicts XRD spectra of AgNPs embedded with chitosan synthesized using *Cissus arnottiana* leaf extract. The particle size was resolved using Debye–Scherrer equation and predicted as 23 nm. X-ray diffraction peaks acquired at 17.29° , 31.73° , 37.46° , and 40.64° were compared to the lattice plane of (4 6 7), (9 3 8), (6 4 9), and (5 7 0), which proposes the face-centered cubic (fcc) crystal structure of the silver nanocomposite. The stronger peaks predict presence of silver [27]. The results seem to agree with the previous studies reported [28].

3.5 AFM analysis

The AFM image in Figure 5 ensured the precise surface morphology of the synthesized silver nanocomposite. The acquired image uncovered how the synthesized nanocomposite was almost spherical without other perceptible nanostructure morphologies as affirmed by absorbance range. The particles were not profoundly mono-scattered but instead appeared non-agglomerated. Previous studies such as those of Kalaivani et al. [29] synthesized silver nanocomposite and the AFM results are concordant with the current study.

3.6 TEM analysis

Figure 6a–c represents the TEM image and Figure 6d depicts the SAED analysis image of biosynthesized AgNP-based chitosan nanocomposite. The TEM results reveal the shape

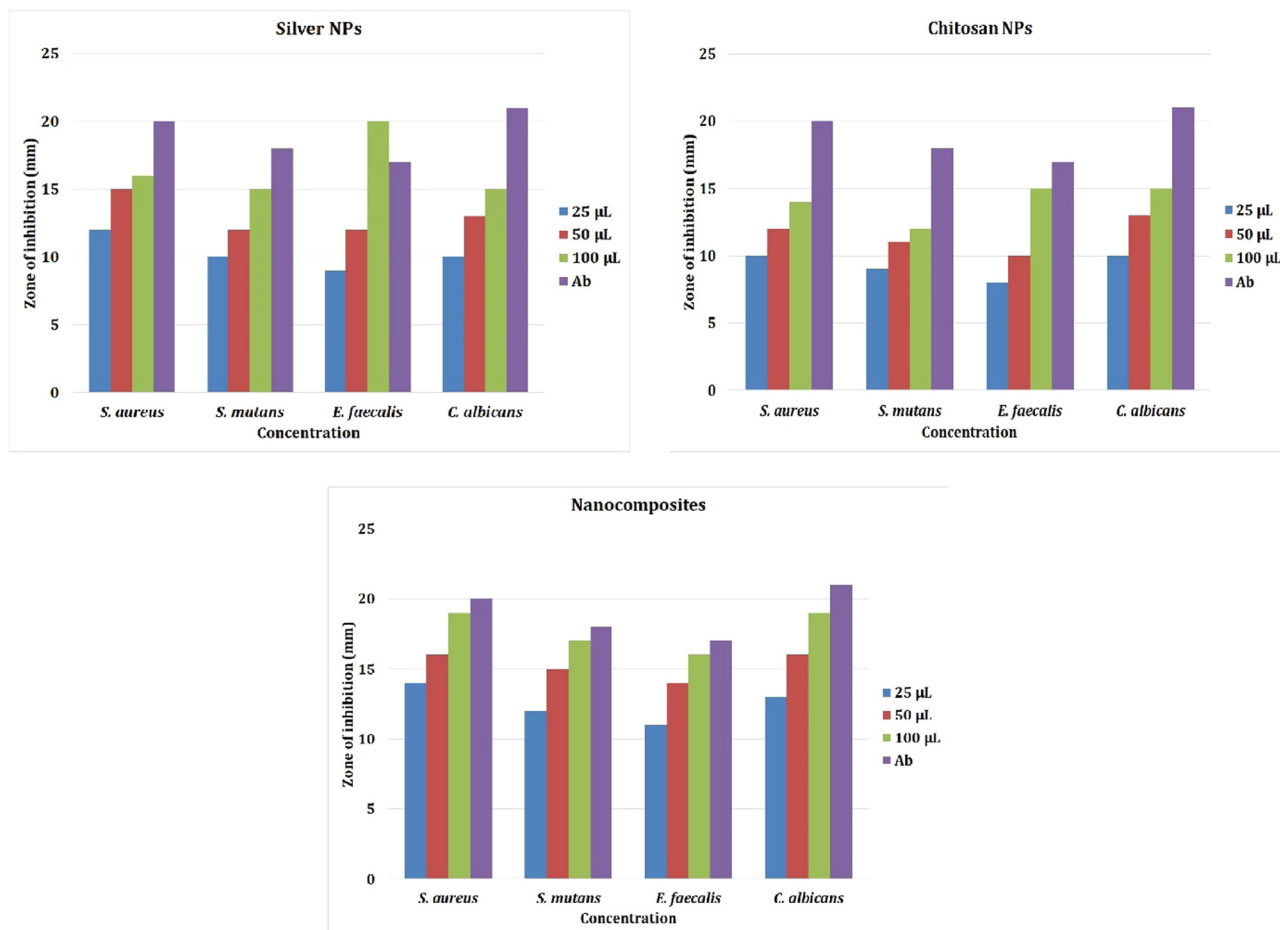


Figure 7: Antimicrobial activity of silver, chitosan nanoparticles, and nanocomposites.

of the nanocomposite to be spherical and size around 10–60 nm. The SAED analysis pattern seems to agree with the XRD results. The TEM results of AgNPs-based chitosan nanocomposites were concordant with the earlier works, such as Ghadi *et al.* [30] stating the size of the nanocomposite to be 10–80 nm.

3.7 Antimicrobial activity against oral pathogens

The antimicrobial activity of silver nanocomposite was tested by adopting agar well diffusion assay. Antibacterial effect of *Cissus amottiana*-mediated AgNPs, chitosan NP, and AgNPs-based nanocomposite was visualized against four oral pathogens such as *S. aureus*, *S. mutans*, *C. albicans*, and *E. faecalis*, which was depicted in Figure 7. Amoxycillin was used as a standard control. Results demonstrate that biosynthesized AgNPs' antibacterial efficacy, chitosan nanoparticles, and AgNP-based chitosan nanocomposite increase in a dose-dependent manner. The high inhibition zone was observed in gram-positive organism *Staphylococcus aureus* and also in opportunistic pathogenic yeast *C. albicans*. Minimum inhibition zone was noted in gram-negative organism *E. faecalis*. The biosynthesized AgNPs, chitosan nanoparticle, and AgNPs-based chitosan nanocomposite show the potent antimicrobial effect, which has to be utilized as a biomedical application in the future for desirable effects. [31,32] also reported that gram-positive organisms show higher sensitivity due to cell wall differences in bacteria. The AgNPs and its decorated cellulose and zinc oxide nanoparticles are showing lower toxicity and used in many biomedical applications [33,34].

4 Conclusion

In this research work, AgNPs and AgNP-based chitosan nanocomposite have been synthesized using *Cissus amottiana* plant extract and the synthesized AgNPs and nanocomposite showed remarkable stability. The chitosan has been utilized as a potent stabilizing agent to combine with AgNPs to obtain *Cissus amottiana*-mediated AgNPs-based chitosan nanocomposite. The results of AgNPs and nanocomposite were used as an effective antimicrobial material. The UV-Vis spectroscopy, SEM, and TEM analysis confirmed the existence of elemental silver in nanocomposite and its spherical form and size of about 30–40 nm. The synthesized AgNPs and AgNPs-based chitosan nanocomposite by *Cissus amottiana* plant extract

had been confirmed to show enhanced activity against oral pathogens. The present research is a cost-effective, eco-accommodating method for synthesizing silver nanocomposite. Therefore, the synthesized AgNPs and AgNPs-based chitosan nanocomposite can be utilized as an efficient antimicrobial material in future biomedical applications.

Acknowledgement: The authors express their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/70), King Saud University, Riyadh, Saudi Arabia.

Funding information: Researchers Supporting Project Number (RSP-2021/70), King Saud University, Riyadh, Saudi Arabia.

Author contributions: Shanmugam Rajeshkumar: conceptualization, writing – original draft; Munusamy Tharani: formal analysis; Vijayarangan Devi Rajeswari: formal analysis; Naiyf S. Alharbi: resources, funding acquisition, validation; Shine Kadaikunnan: funding acquisition, formal analysis; Jamal M. Khaled: formal analysis; Kasi Gopinath: methodology, software; Natesan Vijayakumar: formal analysis; Marimuthu Govindarajan: writing – review and editing.

Conflict of interest: Authors state no conflict of interest.

References

- [1] Govindarajan M, Benelli G. A facile one-pot synthesis of eco-friendly nanoparticles using carissa carandas: ovicidal and larvicidal potential on malaria, dengue and filariasis mosquito vectors. *J Cluster Sci.* 2017;28(1):15–36.
- [2] Goodsell DS. Bionanotechnology: lessons from nature. 2004;31–37.
- [3] Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol.* 2007;73:1712–20.
- [4] Sap-Lam N, Homklinchan C, Larpudomlert R, Warisnoicharoen W, Sereemasun A, Dubas ST. UV irradiation-induced silver nanoparticles as mosquito larvicides. *J Appl Sci.* 2010;10:3132–6.
- [5] Farooqui MA, Chauhan PS, Krishnamoorthy P, Shaik J. Extraction of silver nanoparticles from the leaf extracts of *Clerodendrum inerme*. *Dig J Nanomater Biostructures.* 2010;5:43–9.
- [6] Ip M, Lui SL, Poon VKM, Lung I, Burd A. Antimicrobial activities of silver dressings: an in vitro comparison. *J Med Microbiol.* 2006;55:59–63.
- [7] Wiley BJ, Im SH, Li ZY, McLellan J, Siekkinen A, Xia Y. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *J Phys Chem B.* 2006;110:15666–75.

- [8] Öztürk BY, Gürsu BY, Dağ İ. Antibiofilm and antimicrobial activities of green synthesized silver nanoparticles using marine red algae *Gelidium corneum*. *Process Biochem.* 2020;1(89):208–19.
- [9] Dağlıoğlu Y, Öztürk BY. A novel intracellular synthesis of silver nanoparticles using *Desmodesmus* sp.(Scenedesmaceae): different methods of pigment change. *Rend Lincei Sci Fis Nat.* 2019;30(3):611–21.
- [10] Susilowati E, Maryani A. Green synthesis of silver-chitosan nanocomposite and their application as antibacterial material. *J Phys Conf Ser.* 2019;1153:012135.
- [11] Thamilarasan V, Sethuraman V, Gopinath K, Balalakshmi C, Govindarajan M, Mothana RA, et al. Single step fabrication of chitosan nanocrystals using *Penaeus semisulcatus*: potential as new insecticides, antimicrobials and plant growth promoters. *J Clust Sci.* 2018;29:375–84.
- [12] Shamel K, Ahmad MB, Zargar M, Yunus WM, Ibrahim NA, Shabanzadeh P, et al. Synthesis and characterization of silver/montmorillonite/chitosan bionanocomposites by chemical reduction method and their antibacterial activity. *Int J Nanomedicine.* 2011;6:271–84.
- [13] Wei D, Sun W, Qian W, Ye Y, Ma X. The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. *Carbohydr Res.* 2009;344:2375–82.
- [14] Alharbi NS, Govindarajan M, Kadaikunnan S, Khaled JM, Almanaa TN, Alyahya SA, et al. Nanosilver crystals capped with *Bauhinia acuminata* phytochemicals as new antimicrobials and mosquito larvicides. *J Trace Elem Med Biol.* 2018;50:146–53.
- [15] Gopinath K, Devi NP, Govindarajan M, Bhakayaraj K, Kumaraguru S, Arumugam A, et al. One-Pot green synthesis of silver nanoparticles using the orchid leaf extracts of *Anoectochilus elatus*: growth inhibition activity on seven microbial pathogens. *J Clust Sci.* 2017;28:1541–50.
- [16] Sama K, Sivaraj R. Pharmacognostical and phytochemical screening of fruit and leaves of *Cissus arnottiana*. *Asian J Pharmaceutical Clin Res.* 2012;5:64–6.
- [17] El-Aassar MR, Ibrahim OM, Fouda MM, Fakhry H, Ajarem J, Maodaa SN, et al. Wound dressing of chitosan-based-cross-linked gelatin/polyvinyl pyrrolidone embedded silver nanoparticles, for targeting multidrug resistance microbes. *Carbohydr Polym.* 2021;255:117484.
- [18] El-Aassar MR, Ibrahim OM, Fouda MM, El-Beheri NG, Agwa MM. Wound healing of nanofiber comprising Polygalacturonic/Hyaluronic acid embedded silver nanoparticles: in-vitro and in-vivo studies. *Carbohydr polym.* 2020;238:116175.
- [19] Hussein J, El-Naggar ME, Fouda MM, Morsy OM, Ajarem JS, Almalki AM, et al. The efficiency of blackberry loaded AgNPs, AuNPs and Ag@ AuNPs mediated pectin in the treatment of cisplatin-induced cardiotoxicity in experimental rats. *Int J Biol Macromol.* 2020;159:1084–93.
- [20] Prabhu S, Poulouse EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int Nano Lett.* 2012;2(1):1.
- [21] Roy N, Gaur A, Jain A, Bhattacharya S, Rani V. Green synthesis of silver nanoparticles: an approach to overcome toxicity. *Environ Toxicol Pharmacol.* 2013;36(3):807–12.
- [22] Rana A, Yadav K, Jagadevan S. A comprehensive review on green synthesis of nature-inspired metal nanoparticles: mechanism, application and toxicity. *J Clean Prod.* 2020;14:122880.
- [23] Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci.* 2004;275:496–502.
- [24] Palem RR, Ganesh SD, Kronekova Z, Slávikov M, Saha N, Saha P. Green synthesis of silver nanoparticles and biopolymer nanocomposites: a comparative study on physico-chemical, antimicrobial and anticancer activity. *Bull Mater Sci.* 2018;4(12):1–11.
- [25] Seyforth JA. Scanning electron microscopy (SEM): an introduction to the use of SEM for characterising the surface topology and composition of matter with further applications. *Exp Tech Condens Matter Phys.* 2015;6.
- [26] Khanmohammadi M, Elmizadeh H, Ghasemi K. Investigation of Size and morphology of chitosan nanoparticles used in drug delivery system employing chemometric technique. *Iran J Pharm Res.* 2015;14:665.
- [27] Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using *Pedaliu murex* leaf extract and their antibacterial activity. *Appl Nanosci.* 2016;6(3):399–408.
- [28] Govindan S, Nivethaa EAK, Saravanan R, Narayanan V, Stephen A. Synthesis and characterization of chitosan–silver nanocomposite. *Appl Nanosci.* 2012;2(3):299–303.
- [29] Kalaivani R, Maruthupandy M, Muneeswaran T, Hameedha Beevi A, Anand M, Ramakritinan CM, et al. Synthesis of chitosan mediated silver nanoparticles (Ag NPs) for potential antimicrobial applications. *Front Lab Med.* 2018;2:30–5.
- [30] Ghadi A, Mahjoub S, Tabandeh F, Talebnia F. Synthesis and optimization of chitosan nanoparticles: potential applications in nanomedicine and biomedical engineering. *Casp J Intern Med.* 2014;5:156.
- [31] Mohsen E, El-Borady OM, Mohamed MB, Fahim IS. Synthesis and characterization of ciprofloxacin loaded silver nanoparticles and investigation of their antibacterial effect. *Res Appl Sci.* 2020;13:416–25.
- [32] Balalakshmi C, Alharbi NS, Kadaikunnan S, Khaled JM, Alanzi KF, Gopinath K, et al. Development of chitosan/agar-silver nanoparticles-coated paper for antibacterial application. *Green Process Synth.* 2020;9(1):751–9.
- [33] Abdelsalam NR, Fouda MM, Abdel-Megeed A, Ajarem J, Allam AA, El-Naggar ME. Assessment of silver nanoparticles decorated starch and commercial zinc nanoparticles with respect to their genotoxicity on onion. *Int J Biol Macromol.* 2019;133:1008–18.
- [34] Fouda MM, Abdelsalam NR, Gohar IM, Hanfy AE, Othman SI, Zaitoun AF, et al. Utilization of High throughput microcrystalline cellulose decorated silver nanoparticles as an eco-nematicide on root-knot nematodes. *Colloids Surf B.* 2020;188:110805.