RESEARCH PAPER

Green Synthesis of Silver Nanoparticles Using Trachyspermum Ammi Fruit Extract: Characterization and Antibacterial Activity

***Mohammad Yahya Hanafi-Bojd* [1,2], *Sima Hasheminezhad* [3], *Effat Alemzadeh* [4], *Sobhan Mortazavi-Derazkola* [5], *Masoud Yousefi* [6*]**

[1] *Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

[2] *Department of Pharmaceutics and Pharmaceutical nanotechnology, School of Pharmacy, Birjand University of Medical Sciences, Birjand, Iran*

[3] *Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran*

[4] *Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran*

[5] *Medical Toxicology and Drug Abuse Research Center (MTDRC), Birjand University of Medical Sciences, Birjand, Iran*

[6] *Department of Medical Microbiology, School of Medicine, Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran*

ARTICLE INFO

Article History:
Received 19 March 2023
Accepted 27 May 2023
Published 01 July 2023

Keywords:
Antibacterial activity Green synthesis
Minimum inhibitory concentration
Silver nanoparticles
Trachyspermum ammi

ABSTRACT

In recent years, bimetallic nanoparticles have gained prominence in medical science due to their biomedical properties. This research aimed to develop an eco-friendly, simple and facile process to synthesizing green silver nanoparticles (AgNPs) using *Trachyspermum ammi* fruit extract. The synthesized silver nanoparticles were characterized using FT-IR, UV-Vis spectroscopy, XRD, DLS, and TEM. The minimum inhibitory concentration (MIC) values of the synthesized AgNPs and *T. ammi* extract for five standard bacteria strains were determined by the broth microdilution method. The obtained AgNPs exhibited Surface Plasmon Resonance centered at approximately 415 nm, with an average particle size calculated to be 50 nm. The mean particle size and surface charge of biosynthesized AgNPs using *T. ammi* extract investigated by DLS and zeta potential were 26.78±1.24 nm and -13.96 mV, respectively. Furthermore, green synthesized AgNPs showed high and efficient antibacterial activity against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 9997, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212 with MIC values of 19, 19.5, 75, 150, and 39 µg/ml, respectively. Notably, the antibacterial results illustrated that green synthesized AgNPs possess significantly higher antibacterial potency than chemically produced silver nanoparticles. Our findings highlight the effective and efficient synthesis of silver nanoparticles by *T. ammi* fruit extract and its significant antibacterial activities.

How to cite this article
Hanafi-Bojd M.Y., Hasheminezhad S., Alemzadeh E., Mortazavi-Derazkola S., Yousefi M. Green Synthesis of Silver Nanoparticles Using Trachyspermum Ammi Fruit Extract: Characterization and Antibacterial Activity. J Nanostruct, 2023; 13(3):617-625. DOI: 10.22052/JNS.2023.03.002

INTRODUCTION

Infectious diseases are one of the leading causes of death worldwide, and multidrug-resistant bacterial strains as infectious agents, continue to pose a significant concern for human health. Silver has previously been used to heal

* *Corresponding Author Email: Yousefi164@gmail.com*

wounds or treat infections, but its short duration of effectiveness has limited its recognition as a viable agent in medicine. With the development of nanotechnology, the synthesis of nanoscale metals with enhanced antimicrobial properties has been explored to address this concern [1, 2].

Over the last few decades, nanomaterials have found applications in various fields of sciences including engineering, medicine, environmental, textile, and more, owing to their unique physical, chemical, and biological characteristics [3, 4]. Among the various types of nanoparticles, silver nanoparticles (AgNPs) have gained significant attention in medical science due to their wide-ranging applications in fields such as food preservatives, wound healing, water purification, biosensors, and drug delivery systems [5-8]. Extensive research has demonstrated that AgNPs act as efficient redox catalysts, requiring less activation energy, and possess high surface reactivity due to their elevated surface energy. Their antibacterial properties and tumor cell targeting capabilities have positioned AgNPs as remarkable nanomaterial in the field of nanomedicine. The antibacterial activities of nanoparticles are influenced by their physicochemical characteristics, such as the size, morphology, distribution, chemical composition, including size, morphology, distribution, chemical composition, stability, and the specific type of microorganisms they encounter. The proposed antibacterial mechanism of nanoparticles is closely associated with the generation of reactive oxygen species (ROS) [9-11].

While various techniques of nanomaterial synthesis have been explored, the growing concern over life-threatening environmental pollution has driven scientists to develop new approaches using natural substances, such as plant extracts or microorganisms, in a process referred to as "green synthesis". In green synthesis, phytochemical compounds present in the plant extracts serve as reducing, capping, and stabilizing agents [12, 13]. This method is known for its simplicity, cost-effectiveness, and eco-friendliness, as it avoids the use of toxic chemicals. Numerous studies have investigated the synthesis of AgNPs using plant extracts such as *Trigonella foenum-graecum* [14], *Moringa oleifera* [15], *Ruellia tuberosa* [16], *Nardostachys jatamansi* [17], *Cornus mas* [18], and *Madhuca longifolia* [19]. *Trachyspermum ammi* is a medicinal plant that has antimicrobial, anti-inflammatory, antispasmodic, antipyretic, analgesic, anti-nociceptive, and antioxidant properties [20].

In this research, we embarked on a journey to harness the potential of nature's gifts for a sustainable future. Our study focuses on the synthesis of silver nanoparticles (AgNPs) through both chemical and green methods, utilizing the extract derived from *Trachyspermum ammi*. These nanoparticles were then evaluated for their antibacterial properties against various bacterial strains. Our research not only contributes to the expanding field of green nanotechnology but also underscores the critical importance of sustainable and eco-friendly solutions in combating bacterial infections. This study paves the way for a hopeful future, where environmentally friendly nanoparticles may revolutionize the landscape of antibacterial treatments.

MATERIALS AND METHODS

Material and Characterization

The fruits of *T. Ammi* (Fig. 1) were collected from the areas around Birjand, Iran, and were approved by a botanist in the Agriculture Faculty at the University of Birjand. $AgNO_3$ (Silver nitrate) and $Na_3C_6H_5O_7$ (Sodium citrate) were obtained from Sigma-Aldrich Company (USA). Five standard bacteria strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 9997, *Staphylococcus aureus* ATCC 25923), and *Enterococcus faecalis* ATCC 29212 were purchased from Pasteur Institute of Iran (Tehran, Iran). Fourier transform infrared spectroscopy (FT-IR) was employed for the identification of functional groups (Perkin-Elmer, USA), Transmission electron microscopy (TEM) for direct size and morphological observation of products (Zeiss, EM10C, Germany), X-ray diffraction (XRD) for determination of crystal structure of particles (Philips X'pert PRO, Netherlands), and Dynamic light scattering (DLS) analysis for hydrodynamic diameter determination of silver nanoparticles (Nano-ZS, Malvern, UK).

Preparation of T. ammi extract

Five gram of *T. ammi* fruits were collected and dried at room temperature conditions in the shade. Subsequently, the fruit powder was mixed with 50 ml of deionized water for 24 hours. Finally, the solution was filtered using the Whatman paper and utilized for synthesis of nanoparticles.

Fig. 1. *Trachyspermum ammi* fruit.

Synthesis of silver nanoparticles

The synthesis of AgNPs was conducted through two methods: (I) chemical route: Initially, 9 mg of $AgNO_3$ was dissolved at 50 ml of distilled water. Then, 1 ml of sodium citrate (1%) was added to the above solution under vigorous stirring at room temperature. Within 1 hour, the color of the solution changed from colorless to brown, indicating the formation of AgNPs. (II) Green synthesis: In this method, 1.5 ml of *T. ammi* fruit extract was added to 30 ml of $AgNO_3$ (1 mM) under intense stirring for 24 h. Following this, the solution was centrifuged at 6000 rpm, washed three times, and then dried. The biosynthesis of AgNPs was monitored using UV-Vis absorption spectroscopy in the range of 300-500 nm.

Antibacterial Activity

In this study, the antibacterial activity of *T. ammi* aqueous extract, chemical AgNPs, and green AgNPs was investigated against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 9997, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212. The minimum inhibitory concentration (MIC) was determined using the microdilution broth method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [21]. In brief, 100 µl of two-fold serial dilutions of the test compounds in Muller Hinton broth (Merck, Germany) were suspended in sterile polystyrene 96-well microtiter plates, and then 100 µl of the bacterial suspension with a final concentration of 5×10^5 CFU/ml was added to every well of the plate. Finally, the treated plates were incubated for 18-24 hours at 37°C under ambient air conditions. The bacterial suspension with medium and only the culture medium was used for the positive and negative control, respectively. The antibacterial activity of the test compounds was compared with Tetracycline as an effective antibacterial agent.

RESULTS AND DISCUSSION

Determination of nanoparticle characterization

In our study, the following techniques were employed to determine the properties of synthesized AgNPs.

Dynamic light scattering (DLS) analysis

The stability and average particle size of biosynthesized AgNPs using *T. ammi* extract were determined using zeta potential and DLS analysis. The average particle size and zeta potential of the AgNPs synthesized through chemical and green processes were approximately 51.73±15.74 nm, 26.78±1.24 nm, -16.35 mV, and -13.96 mV, respectively. The presence of high electrostatic negative charges on the surface of nanomaterials, attributed to Van der Waals force interactions between particles, can play a crucial role in preventing the aggregation of nanomaterials [22]. The negative zeta potential of the AgNPs synthesized using natural extracts has been

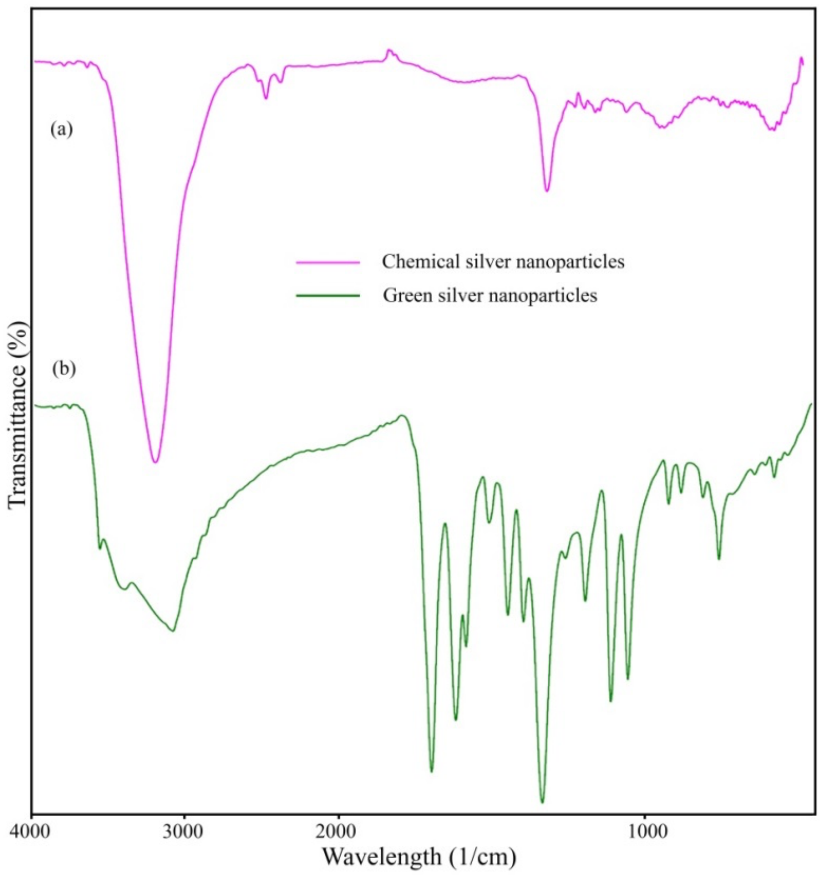

Fig. 2. FT-IR spectra of (a) chemical synthesized silver nanoparticles and (b) green synthesized silver nanoparticles using *Trachyspermum ammi* fruit extract.

previously reported in several studies [23-25]. Thus, the obtained zeta potential results confirm that the green synthesized AgNPs using *T. ammi* extract in our study possess an adequate surface charge for electrostatic stability, effectively preventing aggregation.

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR spectroscopy was performed to investigate the functional groups present in the AgNPs samples synthesized using both the chemical (Fig. 2a) and green (Fig. 2b) routes. The strong broadband at about 3400 cm^{-1} was assigned to the O-H stretching vibration group of alcohols [26]. The band about 2900 cm^{-1} can be related to the C-H stretching vibration band [27]. In the case of AgNPs synthesized using the extract, the extract is deposited on the surface of the nanoparticles. This phenomenon was clearly observed in the FT-IR spectrum of AgNPs synthesized using *T. ammi* extract (Fig. 2b). Several peaks at 1652, 1397, and 1076 cm^{-1} were associated to -C=O, C-N stretching vibration of amines, and -C-O bending vibrations group, respectively [28, 29]. All FT-IR results revealed that the chemical compounds present in the *T. ammi* extract were responsible for the biosynthesis of AgNPs.

Transmission electron microscopy (TEM)

TEM analysis was employed to confirm the formation and morphological characteristics of the synthesized AgNPs using both the chemical (Fig. 3a) and green (Fig. 3b) processes. Oval and spherical morphologies, along with homogeneity were prominent features of the AgNPs. These homogeneous structures may be due to the presence of biomolecules from *T. ammi* extract, which was the surface covering AgNPs. Furthermore, the various morphologies and sizes of the AgNPs could be due to the presence of reducing agents in the *T. ammi* extract [30, 31]. A closer examination of the TEM images revealed

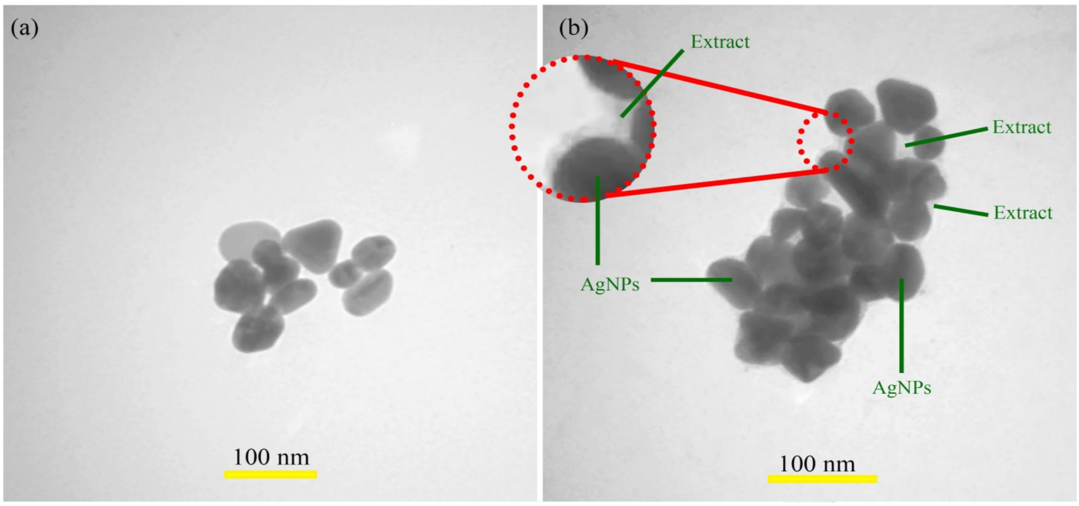

Fig. 3. Transmission electron microscopy (TEM) image of (a) silver nanoparticles synthesized using chemical method and (b) synthesized silver nanoparticles using *Trachyspermum ammi* fruit extract.

that the size of nanoparticles synthesized using the chemical and green methods was approximately 75 and 50 nm, respectively.

X-ray diffraction (XRD)

XRD analysis was employed to determine the crystallinity and structural phase of the samples. The XRD pattern of green-synthesized AgNPs using *T. ammi* extract is depicted in Fig. 4, and revealing distinct 2theta peaks at values of 38°, 44°, 67°, and 77°. According to the XRD results, the cubic structure of AgNPs exhibited respective planes (111), (200), (220), and (311), which closely matched the standard JCPDS No: 01-087-0717. Furthermore, the XRD pattern of AgNPs showed no evidence of impurities, indicating the presence

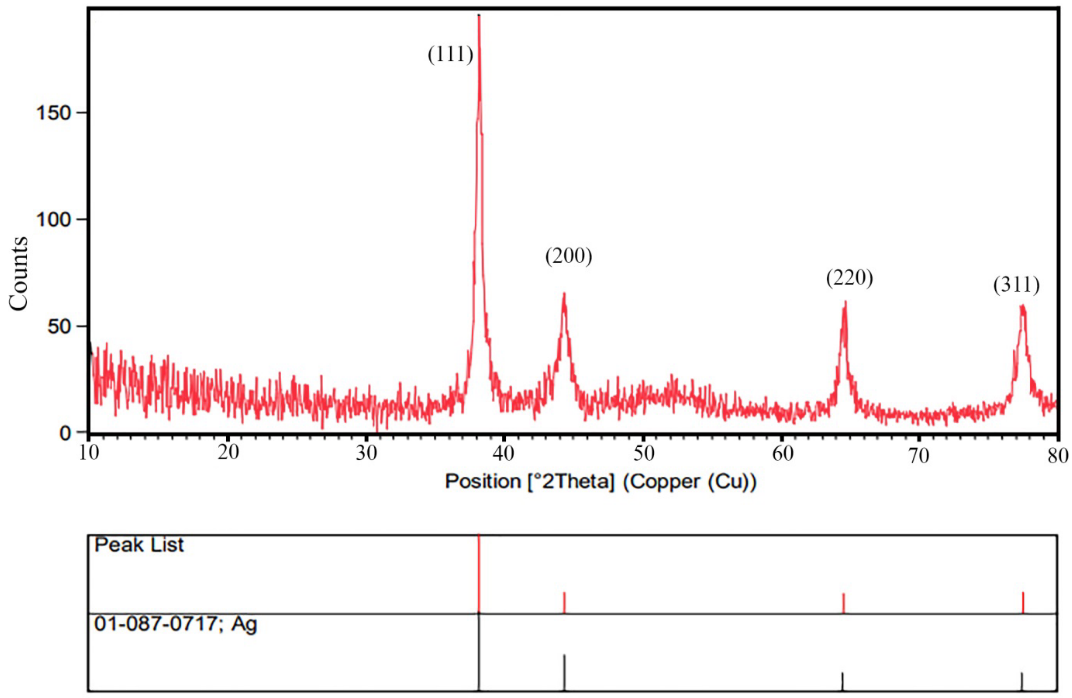

Fig. 4. XRD pattern of biosynthesized AgNPs using *Trachyspermum ammi* fruit extract.

Table 1. Antibacterial activity (MIC values in mg/ml) of chemically synthesized silver nanoparticles (AgNPs) and *Trachyspermum ammi* fruit extract.

Bacteria strains	*T. ammi* extract	Chemically synthesized AgNPs	
		Conc. 1%	Conc. 2%
E. coli ATCC 25922	> 40	> 20	>10
P. aeruginosa ATCC 27853	> 40	20	10
K. pneumonia ATCC 9997	> 40	> 20	> 10
S. aureus ATCC 25923	> 40	> 20	> 10
E. faecalis ATCC 29212	> 40	> 20	> 10

Table 2. Antibacterial activity (MIC values in µg/ml) of green synthesized silver nanoparticles (AgNPs) using *Trachyspermum ammi* fruit extract and Tetracycline.

Bacteria strains	Tetracycline	Green synthesized AgNPs	
		Conc. 1%	Conc. 2%
E. coli ATCC 25922	0.97	19	9
P. aeruginosa ATCC 27853	3.9	19.5	9.7
K. pneumonia ATCC 9997	7.8	75	37.5
S. aureus ATCC 25923	0.97	150	75
E. faecalis ATCC 29212	7.8	39	19.5

of a single-phase. The presence of low-intensity peaks may be attributed to the crystallization of bioorganic phases on the surface of the AgNPs [32]. These findings are consistent with prior research [33].

Antibacterial activity

The MIC values for AgNPs synthesized using a chemical reagent and the *T. ammi* extract are presented in Tables 1 and 2. The results indicate that chemical AgNPs and the *T. ammi* extract

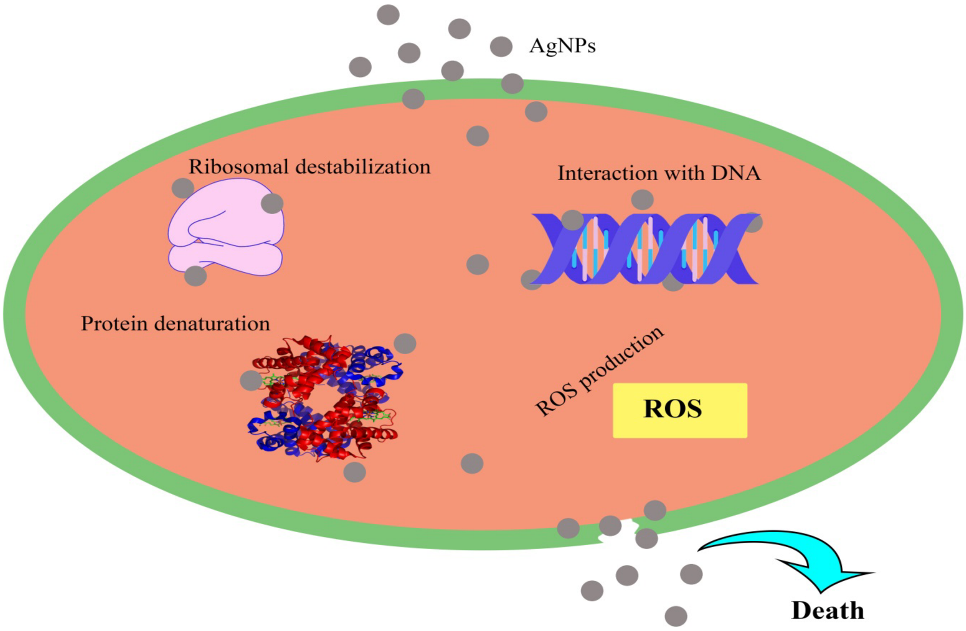

Fig. 5. The antibacterial mechanism of silver nanoparticles.

exhibited no antibacterial activity. Notably, AgNPs synthesized using the *T. ammi* extract displayed significant antibacterial activity compared to those synthesized with a chemical reagent. Also, the results showed a direct relationship between the concentration of nanoparticles and antibacterial activity, so with increasing the concentration of nanoparticles, antibacterial activity increases. As the concentration of AgNPs increased from 1% to 2%, the MIC value was reduced by almost half. In our study, the antibacterial activity of AgNPs synthesized using *T. ammi* extract seems to be higher against Gram-negative than Gram-positive bacteria. A similar result was obtained in previous studies for AgNPs biosynthesized with various plant extracts [34-36]. These results can be explained by the difference in bacterial cell wall composition and the thickness of the peptidoglycan layer in Gram-positive bacteria, which provide protection against chemical agents. Additionally, the higher electrostatic attraction of AgNPs to Gram-negative bacteria may influence changes in cell wall composition, affecting its permeability [35, 37]. Although the precise mechanism of AgNPs' antibacterial effect is not yet fully understood, a potential mechanism is depicted in Fig. 5.

CONCLUSION

In this study, *T. ammi* fruit extract was introduced as an excellent reducing, capping, and stabilizing agent in the green synthesis of AgNPs. Our findings underscore the effective and efficient synthesis of AgNPs by *T. ammi* extract and its significant antibacterial activities. Based on the study results, it is concluded that AgNPs synthesized using *T. ammi* extract exhibit significant antibacterial properties, particularly against gram-negative bacteria.

ACKNOWLEDGMENT

This research was supported by Birjand University of Medical Sciences, Birjand, Iran (grant number 455935).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Al-Radadi NS. Laboratory scale medicinal plants mediated green synthesis of biocompatible nanomaterials and their versatile biomedical applications. Saudi J Biol Sci. 2022;29(5):3848-3870.
2. Jadhav K, Dhamecha D, Bhattacharya D, Patil M. Green and ecofriendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns. J Photochem Photobiol B: Biol. 2016;155:109-115.
3. Kavitha G, Vinoth kumar J, Devanesan S, Asemi NN, Manikandan V, Arulmozhi R, et al. Ceria nanoparticles anchored on graphitic oxide sheets (CeO_2-GOS) as an efficient catalyst for degradation of dyes and textile

effluents. Environ Res. 2022;209:112750.
4. García-Quintero A, Palencia M. A critical analysis of environmental sustainability metrics applied to green synthesis of nanomaterials and the assessment of environmental risks associated with the nanotechnology. Sci Total Environ. 2021;793:148524.
5. Abdellatif AAH, Sameh Tolba N, Al Rugaie O, Alhumaydhi FA, Mousa AM. WITHDRAWN: Green synthesis of silver nanoparticles for enhancing wound healing activity in rats. Saudi Pharmaceutical Journal. 2022.
6. Gul AR, Shaheen F, Rafique R, Bal J, Waseem S, Park TJ. Grass-mediated biogenic synthesis of silver nanoparticles and their drug delivery evaluation: A biocompatible anti-cancer therapy. Chem Eng J. 2021;407:127202.
7. Hanif Z, Khan ZA, Choi D, La M, Park SJ. One-pot synthesis of silver nanoparticle deposited cellulose nanocrystals with high colloidal stability for bacterial contaminated water purification. Journal of Environmental Chemical Engineering. 2021;9(4):105535.
8. Soundharraj P, Dhinasekaran D, Aruna P, Ganesan S. Facile synthesis of biomass silica-silver colloidal nanoparticles and its application as highly sensitive fluorescent biosensor. Surfaces and Interfaces. 2021;23:101010.
9. Ye H-L, He X-W, Li W-Y, Zhang Y-K. Two-photon-excited tumor cell fluorescence targeted imaging based on transferrin-functionalized silicon nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2022;267:120450.
10. Lenaz G. The Mitochondrial Production of Reactive Oxygen Species: Mechanisms and Implications in Human Pathology. IUBMB Life. 2001;52(3-5):159-164.
11. Khan MS, S R, S H. Synthesis and characterization of Kappaphycus alvarezii derived silver nanoparticles and determination of antibacterial activity. Materials Chemistry and Physics. 2022;282:125985.
12. Singh S, Maurya IC, Tiwari A, Srivastava P, Bahadur L. Green synthesis of TiO_2 nanoparticles using Citrus limon juice extract as a bio-capping agent for enhanced performance of dye-sensitized solar cells. Surfaces and Interfaces. 2022;28:101652.
13. Amaliyah S, Pangesti DP, Masruri M, Sabarudin A, Sumitro SB. Green synthesis and characterization of copper nanoparticles using Piper retrofractum Vahl extract as bioreductor and capping agent. Heliyon. 2020;6(8):e04636.
14. Awad MA, A. Hendi A, Ortashi KM, Alzahrani B, Soliman D, Alanazi A, et al. Biogenic synthesis of silver nanoparticles using Trigonella foenum-graecum seed extract: Characterization, photocatalytic and antibacterial activities. Sensors and Actuators A: Physical. 2021;323:112670.
15. Mehwish HM, Rajoka MSR, Xiong Y, Cai H, Aadil RM, Mahmood Q, et al. Green synthesis of a silver nanoparticle using Moringa oleifera seed and its applications for antimicrobial and sun-light mediated photocatalytic water detoxification. Journal of Environmental Chemical Engineering. 2021;9(4):105290.
16. Seerangaraj V, Sathiyavimal S, Shankar SN, Nandagopal JGT, Balashanmugam P, Al-Misned FA, et al. Cytotoxic effects of silver nanoparticles on Ruellia tuberosa: Photocatalytic degradation properties against crystal violet and coomassie brilliant blue. Journal of Environmental Chemical Engineering. 2021;9(2):105088.
17. Muthuraman MS, Nithya S, Vinoth kumar V, Christena LR, Vadivel V, Subramanian NS, et al. Green synthesis of silver nanoparticles using Nardostachys jatamansi and evaluation of its anti-biofilm effect against classical colonizers. Microb Pathog. 2019;126:1-5.
18. Rostamizadeh E, Iranbakhsh A, Majd A, Arbabian S, Mehregan I. Green synthesis of Fe_2O_3 nanoparticles using fruit extract of Cornus mas L. and its growth-promoting roles in Barley. Journal of Nanostructure in Chemistry. 2020;10(2):125-130.
19. Patil MP, Singh RD, Koli PB, Patil KT, Jagdale BS, Tipare AR, et al. Antibacterial potential of silver nanoparticles synthesized using Madhuca longifolia flower extract as a green resource. Microb Pathog. 2018;121:184-189.
20. Siddiqui M, Aslam A, Nokhala A, Peerzada S, Ahmed S, Khan T. Evaluation and comparison of Trachyspermum ammi seed extract for its anti-inflammatory effect. Journal of Pharmacy And Bioallied Sciences. 2020;12(6):777.
21. Sahu C, Jain V, Mishra P, Prasad KN. Clinical and laboratory standards institute versus European committee for antimicrobial susceptibility testing guidelines for interpretation of carbapenem antimicrobial susceptibility results for Escherichia coli in urinary tract infection (UTI). Journal of Laboratory Physicians. 2018;10(03):289-293.
22. Tong Kong Y, Veloo Kutty R. Physical characterization of propolis encapsulated vitamin E TPGS as nanomedicine. Materials Today: Proceedings. 2022;48:807-810.
23. Nagar N, Devra V. A kinetic study on the degradation and biodegradability of silver nanoparticles catalyzed Methyl Orange and textile effluents. Heliyon. 2019;5(3):e01356.
24. Beldjilali M, Mekhissi K, Khane Y, Chaibi W, Belarbi L, Bousalem S. Antibacterial and Antifungal Efficacy of Silver Nanoparticles Biosynthesized Using Leaf Extract of Thymus algeriensis. Journal of Inorganic and Organometallic Polymers and Materials. 2019;30(6):2126-2133.
25. Rajkumar R, Ezhumalai G, Gnanadesigan M. A green approach for the synthesis of silver nanoparticles by Chlorella vulgaris and its application in photocatalytic dye degradation activity. Environmental Technology; Innovation. 2021;21:101282.
26. Zare-Bidaki M, Aramjoo H, Mizwari ZM, Mohammadparast-Tabas P, Javanshir R, Mortazavi-Derazkola S. Cytotoxicity, antifungal, antioxidant, antibacterial and photodegradation potential of silver nanoparticles mediated via Medicago sativa extract. Arabian Journal of Chemistry. 2022;15(6):103842.
27. Shirzadi-Ahodashti M, Mizwari ZM, Hashemi Z, Rajabalipour S, Ghoreishi SM, Mortazavi-Derazkola S, et al. Discovery of high antibacterial and catalytic activities of biosynthesized silver nanoparticles using C. fruticosus (CF-AgNPs) against multi-drug resistant clinical strains and hazardous pollutants. Environmental Technology & Innovation. 2021;23:101607.
28. Rolim WR, Pelegrino MT, de Araújo Lima B, Ferraz LS, Costa FN, Bernardes JS, et al. Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity. Appl Surf Sci. 2019;463:66-74.
29. Banumathi B, Vaseeharan B, Suganya P, Citarasu T, Govindarajan M, Alharbi NS, et al. Toxicity of Camellia sinensis-Fabricated Silver Nanoparticles on Invertebrate and Vertebrate Organisms: Morphological Abnormalities and DNA Damages. J Cluster Sci. 2017;28(4):2027-2040.
30. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638.
31. Patil MP, Kim G-D. Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. Applied Microbiology and Biotechnology. 2016;101(1):79-92.
32. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J Nanopart Res. 2011;13(7):2981-2988.

33. Singla S, Jana A, Thakur R, Kumari C, Goyal S, Pradhan J. Green synthesis of silver nanoparticles using Oxalis griffithii extract and assessing their antimicrobial activity. OpenNano. 2022;7:100047.
34. Nayem SMA, Sultana N, Haque MA, Miah B, Hasan MM, Islam T, et al. Green Synthesis of Gold and Silver Nanoparticles by Using Amorphophallus paeoniifolius Tuber Extract and Evaluation of Their Antibacterial Activity. Molecules. 2020;25(20):4773.
35. Hemlata, Meena PR, Singh AP, Tejavath KK. Biosynthesis of Silver Nanoparticles Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines. ACS Omega. 2020;5(10):5520-5528.
36. Javan bakht Dalir S, Djahaniani H, Nabati F, Hekmati M. Characterization and the evaluation of antimicrobial activities of silver nanoparticles biosynthesized from Carya illinoinensis leaf extract. Heliyon. 2020;6(3):e03624.
37. Okafor F, Janen A, Kukhtareva T, Edwards V, Curley M. Green Synthesis of Silver Nanoparticles, Their Characterization, Application and Antibacterial Activity. International Journal of Environmental Research and Public Health. 2013;10(10):5221-5238.