



Biogenic synthesis, characterization, antibacterial and antioxidant activities of silver nanoparticles mediated from *Tamarindus indica* Linn fruit pulp extract

Kehinde Oluwakemi Fagbemi^{1*}, Olufunmiso Olusola Olajuyigbe¹, Roger Coopoosamy²¹Department of Microbiology, School of Science Technology, PMB 4005, Babcock University, Ilisan Remo, Ogun State, Nigeria²Department of Nature Conservation, Faculty of Natural Science, Mangosuthu University of Technology, P.O. Box 12363, Jacobs, 4026, Durban, South Africa

ARTICLE INFO

Article Type:
Original Article**Article History:**
Received: 13 April 2022
Accepted: 30 June 2022**Keywords:**
Biogenic activity
Oxidative stress
Functional group
Silver nanoparticles

ABSTRACT

Introduction: Due to the numerous potentials discovered from biologically synthesized silver nanoparticles (SNPs), the interest of many researchers has been stirred up. *Tamarindus indica* fruit has many therapeutic potentials attributed to fruit. Hence, the objective of this study was to synthesize, characterize, and evaluate the *in vitro* antioxidant and antibacterial activities of SNPs mediated from *T. indica* fruit pulp extract.**Methods:** The bioreduction of silver nitrate was performed using methanol extract of *T. indica* fruit pulp. UV-vis spectrophotometry studies at 480 nm confirmed the synthesis of SNPs. The synthesized nanoparticles were characterized using Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), X-ray diffraction (XRD), and energy-dispersive X-ray spectroscopy (EDX). The antioxidant properties were assessed using methods of ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antibacterial potential was evaluated using the agar well technique.**Results:** FTIR spectroscopy revealed that the presence of various functional groups was responsible for reduction and stabilization during the biosynthesis process. With the aid of ImageJ software, the size of the nanoparticles was determined to be in the range of 18-50 nm. The anti-oxidation activity assays showed a strong reducing potential towards the radicals tested. Lastly, strong antibacterial activities were observed when the nanoparticles were tested on some pathogenic bacteria through the agar well method.**Conclusion:** This biological method of synthesizing SNP from *T. indica* has shown significant enhancement in its biological activities in terms of antibacterial and antioxidant properties; thus, it might be considered a therapeutic agent.

Implication for health policy/practice/research/medical education:

The synthesized SNPs mediated from *T. indica* showed significant radical scavenging potentials and a strong inhibitory effect on some pathogenic organisms. This study can be expanded to include an *in vivo* assay to acquire robust evidence on the effectiveness of SNP mediated from this fruit in the biological environment. The findings could be used as an alternate solution to herbal medicine to treat oxidative stress-related health issues and for the production of antibiotics.**Please cite this paper as:** Fagbemi KO, Olajuyigbe OO, Coopoosamy R. Biogenic synthesis, characterization, antibacterial and antioxidant activities of silver nanoparticles mediated from *Tamarindus indica* Linn fruit pulp extract. J Herbm Pharm. 2023;12(4):459-468. doi: 10.34172/jhp.2023.43430.

Introduction

Nanotechnology signifies a groundbreaking path for scientific development that deals with the various structures of matter having dimensions of a billionth of a meter (One billion times smaller than a meter) (1). The fascinating and unique properties exhibited by nanoparticles are due to their definite characteristics:

finite-size effects and surface effects. Several metals such as silver, gold, lead, titanium, zinc, cerium, iron, copper, and platinum are used for the synthesis of nanoparticles (2). Among all these metals, silver nanoparticles (SNPs) are among the most commercialized nanoparticles globally (3). Their usage is usually attributed to their antibacterial properties. Moreover, possessing unique

*Corresponding author: Kehinde Oluwakemi Fagbemi,
Email: olakemik4u.kf@gmail.com

optical and catalytic activities makes them quite exciting for manufacturing innovative and cutting-edge functional materials (4). For the synthesis of SNPs, three primary methods are employed. Chemical, physical, and biological methods have been applied in the synthesis of metallic nanoparticles; however, the biological method has been reported to be cheap and environmentally friendly. The method used must be able to regulate the size of the SNP synthesized when all other parameters are optimized. The cost and toxicity associated with the chemical and physical methods have discouraged researchers from the use of this method (5). The biological method focuses on the use of plants, bacteria, and fungi. However, plants are considered the best for the synthesis of nanoparticles due to the presence of biologically active molecules like proteins, phenols, and flavonoids, which act as reducing and capping components of the nanoparticles (6,7). Many extracts from various fruits have been used in the synthesis of nanoparticles, such as carambola (8), *Emblica officinalis* (9), *Citrus limon* (10), *Andean blackberry* fruit (11), clammy cherry (12), and grape (13).

Tamarindus indica Linn is a tropical plant globally used for its nutraceutical and medicinal values; it is used as a cherished ingredient in folkloric practice and for culinary purposes (14). The leaves, fruits, bark, and seeds have biological potential. *T. indica* has been used to treat many diseases and ailments such as wound healing, abdominal pain, diarrhea, dysentery, parasitic infestation, fever, malaria, and respiratory disorder (15). However, there is a paucity of information on these fruit's antioxidant and antibacterial potential after synthesizing nanoparticles using methanol as the solvent. This study aims to synthesize, characterize, and assess the antioxidant and antibacterial activities of the *T. indica* fruit pulp synthesized SNPs obtained from methanol solvent.

Materials and Methods

Tamarindus indica, purchased from an utilized center in Adamawa state, Nigeria was botanically identified by Dr. Nodaz George (University of Lagos). A voucher specimen (No. LUH: 8771) was deposited there. Methanol, silver nitrate, Molybdate reagent solution, acetate buffer (300 mM pH 3.6), and 2, 4, 6-Tri (2-pyridyl)-s-triazine (TPTZ) used were of analytical grade.

Preparation of the fruit extract

The fruits of *T. indica* were cut into small pieces to remove the seeds. The seeds were manually removed, and the pulp was aseptically sundried. A total of 120 g of the dried pulp were added to 550 mL of 70% methanol, and intermittently agitated for 72 hours. The mixture was filtered with a Whatman No. 1 filter paper, and the filtrate was oven-dried at 40°C and kept in the refrigerator at 4°C for further use (16).

Synthesis of SNPs using *Tamarindus indica* fruit pulp extract

Green synthesis of SNP was carried out under standard laboratory conditions from the methanol extract of *T. indica* fruit pulp. Briefly, 10 mL of Tamarind fruit pulp extract was gradually added to 90 mL of aqueous 10 mM AgNO₃ solution and continuously stirred (17). The reacting mixture was observed for any virtual color changes, indicating nanoparticle synthesis. After observing a noticeable color change, the reaction mixture was centrifuged for 30 minutes at 10000 rpm. The sediment was washed four times with until obtaining a clear solution. Subsequently, the washed sediments were dried at 45°C and kept in an airtight vial for further investigation.

Characterization of biosynthesized SNPs

The physical, chemical, and morphological characteristics of the green synthesized nanoparticles were evaluated using various analytical methodologies, such as UV-VIS spectroscopy (Millipore Spectroquant Pharo 300 spectrophotometer), Fourier transform infrared spectroscopy (FT-IR), energy-dispersive X-ray (EDX) analysis, scanning electron microscopy (SEM), and X-ray powder diffraction (XRD) analysis (18). All these analyses were carried out for the fruit extract, which served as the control.

UV-VIS spectral analysis

Preliminary characterization of the biosynthesized SNPs was confirmed using Millipore Spectroquant Pharo 300 spectrophotometer. The bio-reduction of Ag⁺ ions to form SNP was analyzed through the UV-visible absorption spectrophotometer (UV-1800) at a resolution of 2 nm between 300 nm to 700 nm. For the baseline adjustment, Millipore water was used as blank.

SEM and EDX analyses of the biosynthesized SNPs

SEM analysis was carried out on JEOL-JSM-5400, Japan, SEM operating at 30 kV to determine the surface morphology and shape of the fruit extract and the nanoparticle-mediated from it. Uniform films of each sample were spread on a carbon-coated copper grid by just dropping a very tiny quantity of the sample on the grid. Excess liquid was removed with the aid of a blotting paper, and then the prepared films on the SEM grid were dried by putting them under a mercury lamp for 5 minutes. After obtaining the SEM image, photomicrographs were taken at different magnifications; then, the SNP was passed through an EDX detector to determine the elemental composition of SNPs linked to the SEM machine (19).

XRD analysis of biosynthesized SNPs

The structures of SNP and fruit extracts were investigated by XRD instrument, X-ray diffraction using the Bruker –

D8 Advanced (Germany) instrument with Cu K α radiations at an angle of 2 θ . A wavelength (λ) of 0.15418 nm was used for these analyses. The sample holder was placed correctly in the XRD machine, and analysis was done through the inbuilt software. XRD patterns were recorded at the scan speed of 2° min⁻¹.

FTIR analysis

FTIR analysis was done to ascertain the likely biomolecules in the fruit extract using an FTIR spectrophotometer. The analysis was performed for both the biosynthesized SNPs and the fruit extract of *T. indica*. To separate the SNPs from organic compounds, the reaction mixture was centrifuged at 10 000 rpm for 10 minutes. The obtained pellets were freeze-dried and diluted with potassium bromide at a 1:100 ratio. The analysis was done by a software attached to the instrument (Nicolet iS10 FT-IR Spectrometer). The FTIR spectrum of samples was recorded; all measurements were taken in the 400–4000 cm range.

Antioxidant activity determination

DPPH radical scavenging activity

The fruit extract and biosynthesized SNPs were evaluated by DPPH method for their antioxidant potential (20). Tamarind SNP and fruit extract were prepared in varying concentrations (2, 4, 6, 8, and 10 μ g/mL), and each was thoroughly mixed with 3 mL of DPPH solution (0.1 mM). The mixture was incubated for 15 minutes. The reduction in the concentrations of DPPH was ascertained by measuring absorbance at 517 nm. Then, the reducing potential of Tamarind SNP was evaluated using gallic acid as the standard. The percentage inhibition of activity was calculated as:

$$[(A_o - A_e)/A_o] \times 100$$

[A_o = absorbance without extract; A_e = absorbance with extract].

Ferric reducing antioxidant power (FRAP) assay

The ability of the SNP and the methanolic fruit extract was assessed using the FRAP method (21). This method is based on the ability of the sample to reduce Fe⁺³ to Fe⁺² in the presence of TPTZ, resulting in the formation of a deep blue Fe⁺² – TPTZ complex. Briefly, 3 mL of FRAP solution was added to varying concentrations (2, 4, 6, 8, and 10 μ g/mL) of the SNP and the fruit extract in separate test tubes. The test tube containing the mixture was then incubated for 10 minutes at 37°C. The absorbance of each sample was measured at 593 nm. The results obtained were compared with standard and expressed in μ g/mL. The results obtained from DPPH analysis were expressed in IC₅₀.

Antibacterial potential of the biosynthesized SNPs

The antibacterial potential of the SNPs was performed

using the agar well diffusion technique on Muller Hinton Agar (MHA) medium. This assay was performed against some pathogens, including *Klebsiella pneumoniae*, *Kluyvera intestine*, *Proteus mirabilis*, *Staphylococcus epidermis*, *Shigella* spp, and *Escherichia coli*. All the tested bacteria were subcultured in nutrient broth (NB) at 37°C overnight to refresh the bacteria's metabolic activity. 100 μ L of the subculture bacteria suspension [0.5 McFarland's] was taken from each organism's test tube and dispensed on the surface of sterile MHA plates. The dispensed suspension was uniformly swabbed on the surface with a sterile cotton swab stick. Then, a sterile cork borer of 6 mm was used to make five wells on the inoculated plates. In the corresponding well, 20 μ L of SNP solutions with varying concentrations (100, 50, 25, 12.5 μ g) were dispensed. The fifth well was used for the control (plant extract). The antimicrobial activity of SNP was assessed using a control sample (plant extract). The plates were incubated for 24 hours at 37°C. The zones of inhibition around the well were measured on each plate after the incubation period and compared with the extract.

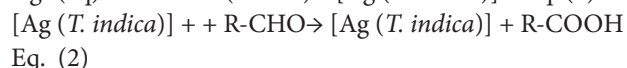
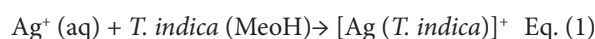
Statistical analysis

Experiments were carried out in triplicate. IBM SPSS Statistics 25 was used for the analysis. The results were reported in tables and figures as means and standard deviations. The inhibitory concentration of 50% (IC₅₀ value) was calculated by plotting the graph of the concentration (y-axis) against the percentage inhibition (x-axis) and the concentration using the linear equation. In determining the statistical significance of the differences between the SNP and the control sample means, the Welch 2-sample test was carried out at a 95% significance level ($P < 0.05$).

Results

The synthesis of SNPs, which occurred due to the reduction of Ag⁺ to Ag⁰, was confirmed through visual color changes (Figure 1).

After the reaction of silver ions (Ag⁺) with the extract of *T. indica* (Equation. 1), [Ag (*T. indica*)] + complex was formed. Ag⁺ reacted with various biomolecules present in the extract to produce [Ag (*T. indica*)] synthesized nanoparticle due to the reduction of silver ions through the oxidation of aldehyde to carboxylic acid groups (Equation 2).



The spectral analysis was done for further confirmation with the aid of UV-Vis spectroscopy. The UV-Vis spectrophotometer is equipment utilized for the detection of the surface plasmon resonance pattern of the metal

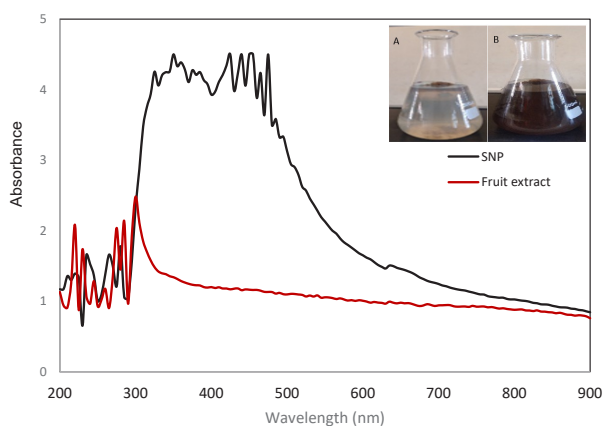


Figure 1. Spectroscopic studies: UV visible absorption spectra of the fruit extract and the synthesized silver nanoparticles from *Tamarindus indica* fruit. Insert pictures: Color changes of the solution (A) before (B) and after synthesis of SNPs from fruit the extract of *T. indica* fruit.

nanoparticles. For the methanolic fruit extract, the color changed from light brown to dark brown after synthesis. The synthesis of nanoparticles was confirmed due to the plasmon resonance peak, which was observed between the range of 320 to 480 (Figure 1) with the highest absorbance at a wavelength of approximately 480 nm, and this is in accordance with earlier reports (22,23).

XRD analysis

Figure 2A shows the XRD pattern of methanol extract. The distinct diffraction peaks at 32, 38, 47, 55, 64, and 77 at 2 theta degree correspond to 111, 102, 104, 220, 200, and 311 crystal planes for the extract, respectively. On the contrary, the XRD pattern of the silver nanoparticles in Figure 2B show different distinct diffraction peaks at 38, 44, 64.3, and 77.1 at 2 theta degree corresponding to 111, 200, 220, and 311 crystal planes of SNPs, respectively. These diffraction peaks can accurately be linked to the face-centered cubic (FCC) crystalline structure of silver nitrate used in terms of their peak positions and the relative intensity of their characteristic peaks as referenced in International Centre for Diffraction Data (ICDD). Other additional peaks observed could be prompted by the crystallization of bioorganic compounds on the nanoparticle's surface. These bioorganic compounds are in charge of reducing silver ions and stabilizing the synthesized nanoparticles. From the calculation, using Debye-Scherrer's equation: $D = \frac{0.94\lambda}{\beta \cos \theta}$, the crystallite size of the fruit extract is 1472 nm, while the synthesized nanoparticle is approximately 29 nm, which is closer to the size obtained from SEM analysis. This result collaborates with the findings of other researchers in previous studies (24,25).

FTIR analysis

Although FTIR analysis determined the bio-molecules

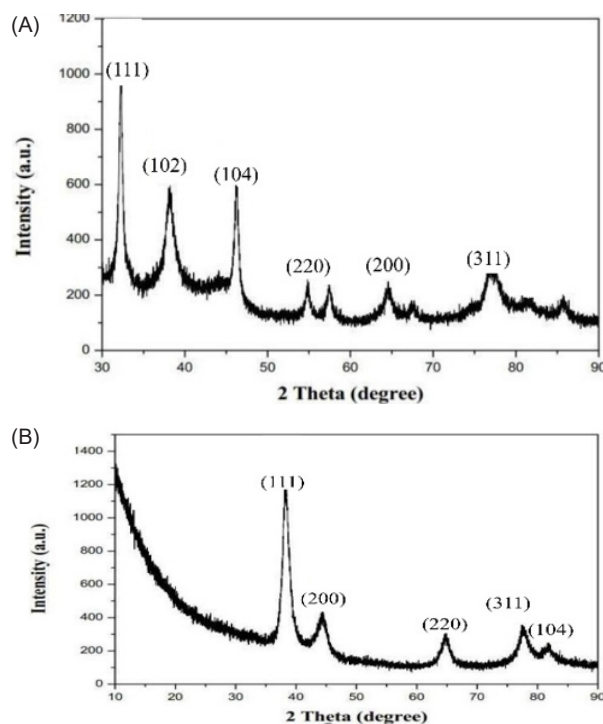


Figure 2. The X-ray diffraction pattern of the methanolic fruit extract (A) and biosynthesized silver nanoparticles (B) from methanol fruit extract of *Tamarindus indica*.

involved in stabilizing the freshly generated SNPs, varied functional groups contributing to the synthesis of SNPs was determined with different methods of vibration. In harmony with a previous study (26), the carbonyl groups of amino acids and peptides have a better potential to bind metals, while the proteins may build a coat around metal nanoparticles (26). The FTIR results of the fruit extract (Figure 3A) confirmed five predominant bands, including 3407 cm^{-1} , 2925.30 cm^{-1} , 1602.00 cm^{-1} , 1377.00 cm^{-1} , and 1029 cm^{-1} , corresponding to OH stretch of alcohol and phenols, C-H stretch of alkene, carbonyl N-H stretch of primary amines induced by vibrations in protein amide linkages, C-H alkane groups, and C-N vibration of aliphatic amide, respectively. The second spectrum (Figure 3B) depicts the absorbance bands of the biosynthesized SNPs and specifies the probable functional groups present. Eight prominent bands were at 3778.31 cm^{-1} , 3376.00 cm^{-1} , 2918.00 cm^{-1} , 2853.01 cm^{-1} , 2360 cm^{-1} , 1595 cm^{-1} , 1369 cm^{-1} , 1032 cm^{-1} , and 307 cm^{-1} . The alcohol OH at 3778.31 cm^{-1} peak represented stretching vibration, the NH amide bond stretching had vibrations in primary and secondary amines of amino acids, the peptides and proteins had wavelengths of 3376.00 cm^{-1} , 2918.00 cm^{-1} , and 2853.01 cm^{-1} correspond to the aliphatic C-H stretching of alkanes hydrocarbon chains; 2360 cm^{-1} illustrates to C:N, nitriles, the narrow bend at 1595 cm^{-1} is due to the presence of carbonyl C=O compounds such as ketones. The band illustrated by peaks 1369 cm^{-1} and 1032

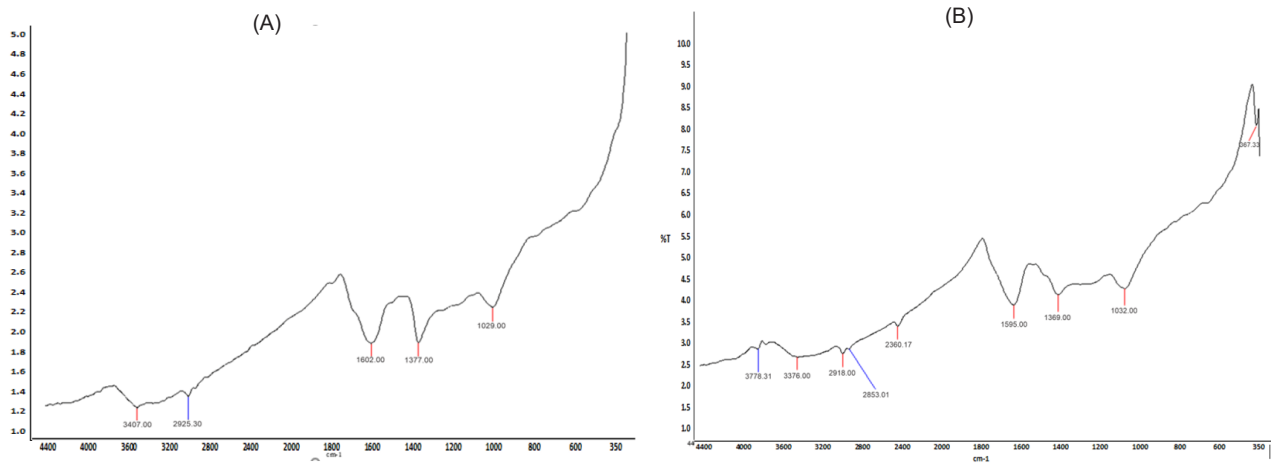


Figure 3. Fourier transform infrared spectroscopy of *Tamarindus indica* fruit extract (a) and biosynthesized silver nanoparticles (b).

cm^{-1} are attributed to the vibration of the CO band, and 307 cm^{-1} is the C–Br stretch of alkyl halides.

SEM results

Electron microscopy studies of the synthesized SNPs

The shape and structure of the biosynthesized SNPs were investigated through SEM-EDX analysis. The SEM images displayed that the morphology of the synthesized SNPs was polydisperse, nearly spherical structures in the range of 18 nm to 50 nm with agglomeration (Figure 4B), while the size of the plant extract was quite large with an average size of 1560 nm as displayed in Figure 4a. The particle

size was determined using an advanced software named “ImageJ”, and the results were almost comparable to XRD. With the EDX analysis, which was performed with a detector coupled to the SEM, the elemental composition of the green synthesized SNP was recognized (Figure 5B). It shows the presence of silver (Ag) at 10.6 keV and other elements including oxygen (O), carbon (C), iron (Fe), potassium (K), calcium (Ca), silicon (Si), aluminum (Al), and manganese (Mg). Several elements were observed in the fruit extract (Figure 5A). This report corroborates the findings of Jayaprakash et al (27) on the studies of silver nanoparticles mediated from plants extracts.

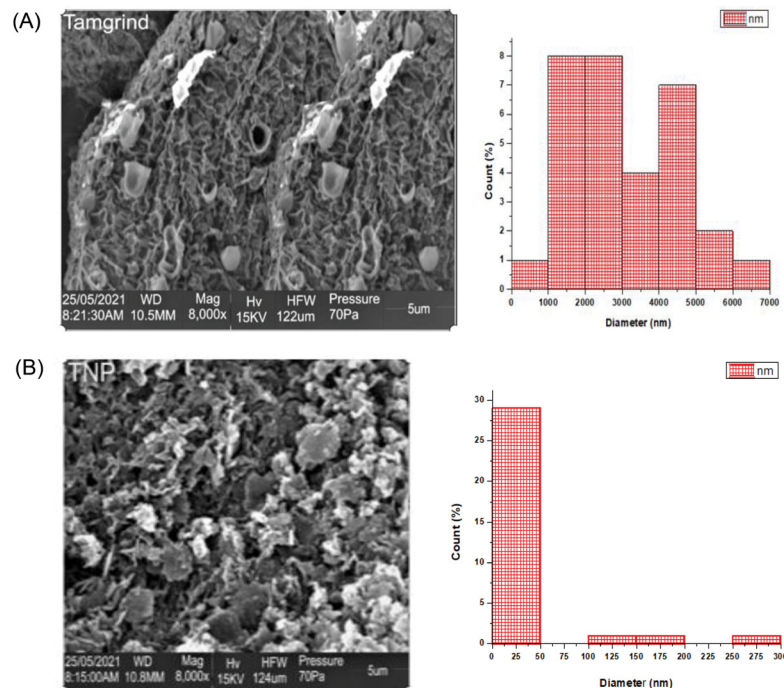


Figure 4. The X-ray diffraction pattern of the methanolic fruit extract (A) and biosynthesized silver nanoparticles (B) from methanol fruit extract of *Tamarindus indica*.

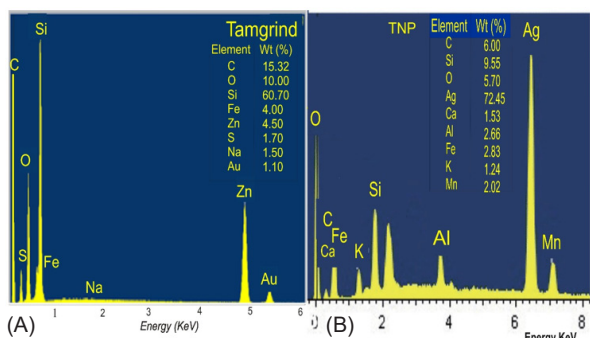


Figure 5. Energy-dispersive X-ray spectroscopy of methanol fruit extract (A) and biosynthesized silver nanoparticles (B) from the fruit of *Tamarindus indica*.

Antioxidant activities of SNP

Antioxidant activities

The analysis of a sole antioxidant property makes it hard to reveal the actual antioxidant capacity of samples; therefore, two techniques, DPPH and FRAP, were selected to assess the antioxidant capacity of the green SNPs. The activity of the plant extract was steadily lower than the SNP and the standard reference. Moreover, the antioxidant activities of the SNP and the fruit extract using DPPH are shown as IC_{50} values compared with the standard (gallic acid). The IC_{50} of SNP and the methanol extract were 9.91 $\mu\text{g}/\text{mL}$ and 13.21 $\mu\text{g}/\text{mL}$, respectively. The IC_{50} value of 9.91 $\mu\text{g}/\text{mL}$ demonstrated by the SNP indicated its potent scavenging ability. Low IC_{50} values

correspond to high radical scavenging potential (25). A similar trend was observed in the FRAP assay (Figure 6B); the SNP displayed good free radical scavenging property with a dose-dependent inhibition activity. An increase in the concentration of SNP showed a significant scavenging power. SNP showed a significant ferric reducing power than the gallic acid used as a standard reference. The reducing potential of a sample is linked to its ability to transfer electrons and thus may serve as a vital pointer to its probable antioxidant activity (28). The reducing ability of the SNP improved with the increasing concentration of the sample. The activity of fruit extract was concentration-dependent, and its free radical scavenging potential was due to the presence of some phytochemicals such as tannins, alkaloids, and flavonoids. Our findings are consistent with previous findings (29) demonstrating the DPPH scavenging potentials of *T. indica* methanolic extract.

Discussion

The current study describes the synthesis of SNPs from *T. indica* fruit pulp extract. The visible color change observed was an initial confirmation of SNPs synthesis, which is a result of the reduction of silver ions by the different bioactive compounds present in the fruit extract. Further confirmation was observed by the UV-Vis analysis (Figure 1). The absorbance peak was recorded around 480 nm, in the SNP absorbance range.

The crystallinity state of the synthesized SNPs

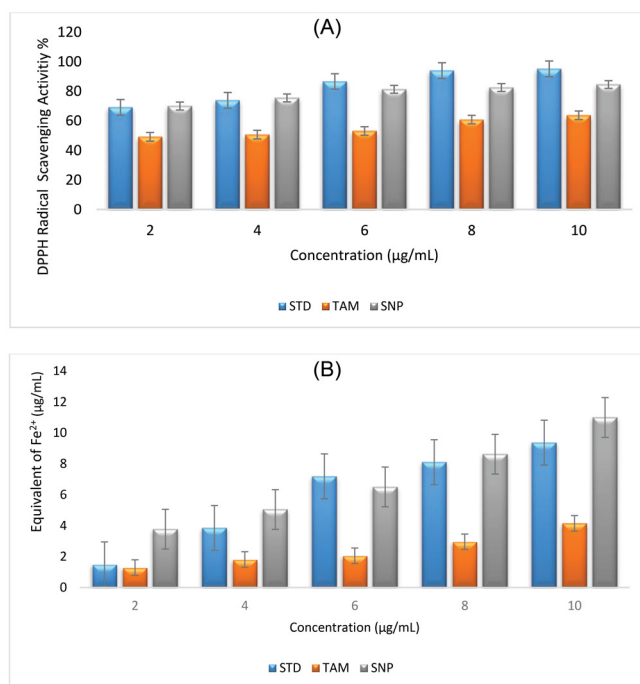


Figure 6. (A) The mean DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (%). There is a significant difference ($P < 0.05$) in the average scavenging potentials of the samples tested. (B) Ferric reducing potential activity of the SNP. There is a significant difference ($P < 0.05$) in the average reducing power of the samples tested. STD, Gallic acid; TAM, Tamarind fruit extract; SNP, Silver nanoparticle.

was confirmed by XRD (Figure 2). The SEM results demonstrated the variable size of the SNPs between the sizes of 15 to 28 nm (Figure 4). The FTIR results revealed the presence of multiple functional groups on the surface of bioactive compounds (Figure 3). These functional groups are responsible for capping SNPs and keeping them stable at the nanoscale; other researchers have confirmed this finding (30,31). Literature has established that the presence of biomolecules such as the carbonyl group of proteins adsorbed strongly to metals on the surface of SNPs is a critical influence in the green synthesis of nanoparticles (32). Moreover, the presence of biomolecules in the fruit extract suggests it has a robust ability to bind metal, signifying the development of a layer covering SNP and acting as a capping and stabilizing agent.

The DPPH and FRAP results confirmed that SNPs have strong antioxidant potential. The SNPs' antioxidant activities were dose-dependent, as shown in Figures 6a & 6b, and the values were significant at ($P < 0.05$). The DPPH results confirmed the radical scavenging potential of SNPs compared to gallic acid used as the standard. The SNP has 84% scavenging activity, while the standard has 95% potential. The biosynthesized SNPs exhibited a maximum reducing capability of 92.5% at 10 $\mu\text{g/mL}$, higher than gallic acid (90.5%). Furthermore, the IC_{50} value exhibited by the SNP was significant when compared to the extract. The findings strongly support SNPs as natural antioxidants for balanced healthy living due to their power to lessen the impact of various oxidative stressors linked with degenerative diseases. Substances with significant antioxidant activities are suitable for preventing the pathogenicity of disease in the human body (33). Investigation of antioxidant ability is mandatory for SNPs before their in vivo usage. This result shows that the antioxidant activity of SNP from *T. indica* is a good source of antioxidant agents. This report aligns with earlier reports that SNP mediated from plant extract was an excellent antioxidant source (34,35).

Moreover, the fast development of resistance in pathogenic bacterial strains has impacted healthcare systems globally (36). As a result, the potential benefits of SNPs on many prevalent human pathogenic bacteria might aid in the development of novel potent antimicrobial therapies against the resistant strains of bacteria. In this study, the preliminary screening of antimicrobial activities of the SNP from *T. indica* fruit was evaluated using the well diffusion method, while the plant extract was used as a control. The results in Figure 7 depict the visual proof of antibacterial activity of SNP mediated from *T. indica* fruit pulp. SNP exhibited significant antibacterial potential to all the tested pathogens at 50 $\mu\text{g/mL}$, however, with a varying zone of inhibition (Figure 8). The methanol extracts of *T. indica* fruit used as control had no inhibitory effects on any tested strains; this could be linked to the large size of

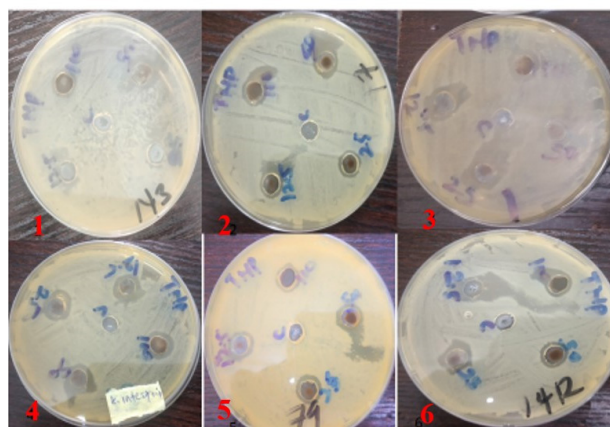


Figure 7. Antibacterial activities of silver nanoparticles (SNPs). Plate 1: *Proteus mirabilis*, plate 2: *Shigella* spp, plate 3: *Escherichia coli*, plate 4: *Kluyvera intestine*, plate 5: *Salmonella* spp, and plate 6: *Staphylococcus epidermis*.

the biomolecules, thus preventing the penetration into the bacteria cell wall. The maximum antibacterial activity was seen against *Proteus mirabilis*, *Shigella* spp, and *Kluyvera intestine* with a zone of 20 mm, followed by *Staphylococcus epidermis* and *E. coli* with both having 18 mm as the zone of inhibition.

For all the different concentrations used, all the tested strains were significantly inhibited except for *Proteus mirabilis*, which was only significantly inhibited at 50 $\mu\text{g/mL}$. Similar reports have been documented on *Proteus mirabilis* when assessed with green synthesized SNPs (37). The observed antibacterial activity is in accordance with earlier reports on green synthesized nanoparticles (24,25,38). The difference observed in antibacterial activity was caused by bacterial susceptibility to SNPs. Many reports have focused on the antibacterial potential of biogenic SNPs; however, their mechanisms of action are not well known. Different possible mechanisms of action of SNPs on bacterial cells have been proposed in many works in the literature. The most likely mechanism of action on bacterial cells is the suspension of SNPs, which causes Ag ions to be released and intermingled with sulfur-containing proteins in bacteria's cell walls, modifying the roles of proteins (36). The possibility of interacting with Sulphur compounds was confirmed by (39) with the aid of equipment such as X-ray energy dispersive spectrometer and scanning tunneling electron microscopy; it was confirmed that SNPs were seen both on the surface of the cell membrane and within the bacteria. This raises the possibility of the SNPs being infiltrated within the bacterial cells. Another suggested mechanism is that SNP may cling to the surface of the cell membrane and disrupt permeability and respiration-related power functions. Based on the large surface area possessed by nanoparticles, they easily bind to the bacteria for interaction. Tiny particles with larger surface areas

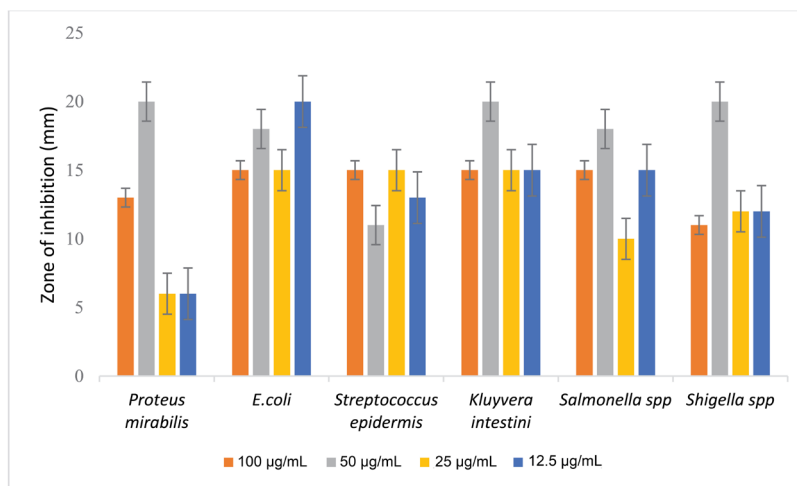


Figure 8. Inhibition zones of bacterium isolates produced by green synthesized silver nanoparticle from *Tamarindus indica* fruit pulp and their standard deviation.

will have more excellent antibacterial properties than bulk materials (40). It is also postulated that due to their adhesion ability, SNPs invade the bacterial cell wall, alter the structure of the membrane, and subsequently cause cell death. The antibacterial activity of SNPs is suspected to be influenced by their small size and large surface area. They effectively interact with the bacterial cell and can access nuclear materials (25). Another possible mode of action of SNPs is the generation of free radicals. When SNPs encounter bacteria, electron spin resonance spectroscopy shows that free radicals are produced. These free radicals cause pores in the cell membrane, alter its structure, and eventually cause cell death (41). Our findings clearly show that fruit from *T. indica* can be used to synthesize SNPs with therapeutic potential, which is a significant step in developing nanomaterials.

Conclusion

In this study, methanol extract from *T. indica* fruit pulp was successfully used for the biological synthesis of SNPs. The synthesis was confirmed via many analyses such as UV-vis spectrum analysis, XRD, FTIR, and SEM-EDX. The average size of the nanoparticles was between 18 to 35nm, and they were nearly spherical. The biosynthesized SNPs were found to exhibit good scavenging potential, and they possessed powerful antibacterial activities on all the tested pathogens. Based on the outcome of this study, the production of antibiotics for the treatment of diseases and the removal of free radicals might be considered from this fruit. Further study is recommended to ascertain the proper dosage and cytotoxicity level before applying these SNPs *in vivo*.

Authors' contributions

FKO and OOO designed and conducted the laboratory experiment, FKO wrote the first draft, OOO and CR

supervised the work and corrected all drafts. OOO contributed to the conception of protocol and corrected the final manuscript. FKO submitted the manuscript for publication. All authors read and confirmed the final version for publication.

Conflict of interests

The authors declared they do not have any conflict of interest.

Ethical considerations

This study does not involve the use of animal or human models. Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been ultimately observed by the authors.

Funding/Support

No funding was received for this work.

References

- Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z. An introduction to nanotechnology. In: Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z, Atarod M, eds. Interface Science and Technology. Vol 28. Elsevier; 2019. p. 1-27. doi: 10.1016/b978-0-12-813586-0.00001-8.
- Khan SA. Metal nanoparticles toxicity: role of physicochemical aspects. In: Shah MR, Imran M, Ullah S, eds. Metal Nanoparticles for Drug Delivery and Diagnostic Applications. Elsevier; 2020. p. 1-11. doi: 10.1016/b978-0-12-816960-5.00001-x.
- Singh N, Paknikar KM, Rajwade J. RNA-sequencing reveals a multitude of effects of silver nanoparticles on *Pseudomonas aeruginosa* biofilms. Environ Sci Nano. 2019;6(6):1812-28. doi: 10.1039/c8en01286e.
- Haase A, Manton A, Graf P, Plendl J, Thuenemann AF, Meier W, et al. A novel type of silver nanoparticles and their advantages in toxicity testing in cell culture systems. Arch

- Toxicol. 2012;86(7):1089-98. doi: 10.1007/s00204-012-0836-0.
5. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. *Nanomedicine*. 2009;5(4):452-6. doi: 10.1016/j.nano.2009.01.012.
 6. Kuppusamy P, Yusoff MM, Maniam GP, Govindan N. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications - an updated report. *Saudi Pharm J*. 2016;24(4):473-84. doi: 10.1016/j.jsps.2014.11.013.
 7. Das RK, Pachapur VL, Lonappan L, Naghdi M, Pulicharla R, Maiti S, et al. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. *Nanotechnol Environ Eng*. 2017;2(1):18. doi: 10.1007/s41204-017-0029-4.
 8. Mane-Gavade SJ, Nikam GH, Dhabbe RS, Sabale SR, Tamhankar BV, Mulik GN. Green synthesis of silver nanoparticles by using carambola fruit extract and their antibacterial activity. *Adv Nat Sci Nanosci Nanotechnol*. 2015;6(4):045015. doi: 10.1088/2043-6262/6/4/045015.
 9. Hossain MM, Polash SA, Takikawa M, Shubhra RD, Saha T, Islam Z, et al. Investigation of the antibacterial activity and *in vivo* cytotoxicity of biogenic silver nanoparticles as potent therapeutics. *Front Bioeng Biotechnol*. 2019;7:239. doi: 10.3389/fbioe.2019.00239.
 10. Vankar PS, Shukla D. Biosynthesis of silver nanoparticles using lemon leaves extract and its application for antimicrobial finish on fabric. *Appl Nanosci*. 2012;2(2):163-8. doi: 10.1007/s13204-011-0051-y.
 11. Kumar B, Smita K, Cumbal L, Debut A. Green synthesis of silver nanoparticles using Andean blackberry fruit extract. *Saudi J Biol Sci*. 2017;24(1):45-50. doi: 10.1016/j.sjbs.2015.09.006.
 12. Saidu FK, Mathew A, Parveen A, Valiyathra V, Thomas GV. Novel green synthesis of silver nanoparticles using clammy cherry (*Cordia obliqua* Willd) fruit extract and investigation on its catalytic and antimicrobial properties. *SN Appl Sci*. 2019;1(11):1368. doi: 10.1007/s42452-019-1302-x.
 13. Roy K, Biswas S, Banerjee PC. 'Green' synthesis of silver nanoparticles by using grape (*Vitis vinifera*) fruit extract: characterization of the particles and study of antibacterial activity. *Res J Pharm Biol Chem Sci*. 2013;4(1):1271-8.
 14. Sandesh P, Velu V, Singh RP. Antioxidant activities of tamarind (*Tamarindus indica*) seed coat extracts using *in vitro* and *in vivo* models. *J Food Sci Technol*. 2014;51(9):1965-73. doi: 10.1007/s13197-013-1210-9.
 15. Zohrameena S, Mujahid M, Bagga P, Khalid M, Noorul H, Nesar A, et al. Medicinal uses & pharmacological activity of *Tamarindus indica*. *World J Pharm Res*. 2017;5(2):121-33.
 16. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. *J Pharm Bioallied Sci*. 2020;12(1):1-10. doi: 10.4103/jpbs.JPBS_175_19.
 17. Patra JK, Baek KH. Biosynthesis of silver nanoparticles using aqueous extract of silky hairs of corn and investigation of its antibacterial and anticandidal synergistic activity and antioxidant potential. *IET Nanobiotechnol*. 2016;10(5):326-33. doi: 10.1049/iet-nbt.2015.0102.
 18. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci*. 2014;9(6):385-406.
 19. Patra JK, Das G, Kumar A, Ansari A, Kim H, Shin HS. Photo-mediated biosynthesis of silver nanoparticles using the non-edible accrescent fruiting calyx of *Physalis peruviana* L. fruits and investigation of its radical scavenging potential and cytotoxicity activities. *J Photochem Photobiol B*. 2018;188:116-25. doi: 10.1016/j.jphotobiol.2018.08.004.
 20. Rout S, Rath B, Bhattamisra SK, Rath I, Kumar A. Antioxidant and anti-inflammatory activities of methanol and aqueous extracts of *Sargassum wightii*. *J Herbmed Pharmacol*. 2021;11(1):75-82. doi: 10.34172/jhp.2022.08.
 21. Du HY, Li HM, Xu GD, Xiong JH, Wang WJ, Chen WP, et al. *Lilium casa blanca* petals mediated silver nanoparticles with antioxidant and surface enhanced Raman scattering activities. *Food Biosci*. 2020;38:100792. doi: 10.1016/j.fbio.2020.100792.
 22. Eustis S. Gold and Silver Nanoparticles: Characterization of Their Interesting Optical Properties and the Mechanism of Their Photochemical Formation [dissertation]. Georgia Institute of Technology; 2006.
 23. Chandran S, Ravichandran V, Chandran S, Chemmanda J, Chandarshekar B. Biosynthesis of PVA encapsulated silver nanoparticles. *J Appl Res Technol*. 2016;14(5):319-24. doi: 10.1016/j.jart.2016.07.001.
 24. Wang L, Wu Y, Xie J, Wu S, Wu Z. Characterization, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from *Psidium guajava* L. leaf aqueous extracts. *Mater Sci Eng C Mater Biol Appl*. 2018;86:1-8. doi: 10.1016/j.msec.2018.01.003.
 25. Ravichandran V, Vasanthi S, Shalini S, Shah SAA, Tripathy M, Paliwal N. Green synthesis, characterization, antibacterial, antioxidant and photocatalytic activity of *Parkia speciosa* leaves extract mediated silver nanoparticles. *Results Phys*. 2019;15:102565. doi: 10.1016/j.rinp.2019.102565.
 26. Khandel P, Yadav RK, Soni DK, Kanwar L, Shahi SK. Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. *J Nanostructure Chem*. 2018;8(3):217-54. doi: 10.1007/s40097-018-0267-4.
 27. Jayaprakash N, Vijaya JJ, Kaviyarasu K, Kombaiah K, Kennedy LJ, Ramalingam RJ, et al. Green synthesis of Ag nanoparticles using tamarind fruit extract for the antibacterial studies. *J Photochem Photobiol B*. 2017;169:178-85. doi: 10.1016/j.jphotobiol.2017.03.013.
 28. Akintola AO, Kehinde BD, Ayoola PB, Adewoyin AG, Adedosu OT, Ajayi JE, et al. Antioxidant properties of silver nanoparticles biosynthesized from methanolic leaf extract of *Blighia sapida*. *IOP Conf Ser Mater Sci Eng*. 2020;805(1):012004. doi: 10.1088/1757-899x/805/1/012004.
 29. Bressiani PA, De Lima GRF, Düsman E, Tonin LTD. Cytotoxic and antioxidant activities of *Tamarindus indica* pulp extract from Brazil. *J Food Meas Charact*. 2021;15(3):2743-9. doi: 10.1007/s11694-021-00855-4.
 30. Aslam M, Abdullah AZ, Rafatullah M. Recent development in the green synthesis of titanium dioxide nanoparticles using plant-based biomolecules for environmental and antimicrobial applications. *J Ind Eng Chem*. 2021;98:1-16. doi: 10.1016/j.jiec.2021.04.010.
 31. Suryavanshi A, Kumar S, Kain D, Arya A, Vandana. *In vitro* antidiabetic, antioxidant activities and chemical composition of *Ajuga parviflora* Benth. shoot. *J Herbmed Pharmacol*. 2022;11(1):131-9. doi: 10.34172/jhp.2022.15.

32. Annamalai J, Nallamuthu T. Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. *Appl Nanosci*. 2016;6(2):259-65. doi: 10.1007/s13204-015-0426-6.
33. Abdelaziz S, Hassan WH, Elhassanny AE, Al-Yousef HM, Elsayed MA, Adel R. Ultra performance liquid chromatography-tandem mass spectrometric analysis of ethyl acetate fraction from Saudi *Lavandula coronopifolia* Poir and evaluation of its cytotoxic and antioxidant activities. *J Herbm Pharm*. 2020;9(3):268-76. doi: 10.34172/jhp.2020.34.
34. Kharat SN, Mendhulkar VD. "Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Elephantopus scaber* leaf extract". *Mater Sci Eng C Mater Biol Appl*. 2016;62:719-24. doi: 10.1016/j.msec.2016.02.024.
35. Ruttkay-Nedecký B, Dočekalová M, Hosnedlová B, Uhlířová D, Staňková M, Kepinska M, et al. Antioxidant activity of silver nanoparticles prepared by green synthesis. In: *Proceedings of 10th Nanomaterials International Conference 2018 (NANOCON 2018): Research and Application, 17-19 October 2018, Brno, Czech Republic*. Ostrava: Tanger; 2018. p. 392-6.
36. Hussain A, Mehmood A, Murtaza G, Ahmad KS, Ulfat A, Khan MF, et al. Environmentally benevolent synthesis and characterization of silver nanoparticles using *Olea ferruginea* Royle for antibacterial and antioxidant activities. *Green Process Synth*. 2020;9(1):451-61. doi: 10.1515/gps-2020-0047.
37. Parveen A, Yalagatti MS, Abbaraju V, Deshpande R. Emphasized mechanistic antimicrobial study of biofunctionalized silver nanoparticles on model *Proteus mirabilis*. *J Drug Deliv*. 2018;2018:3850139. doi: 10.1155/2018/3850139.
38. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. *Nanotechnology*. 2005;16(10):2346. doi: 10.1088/0957-4484/16/10/059.
39. Salayová A, Bedlovičová Z, Daneu N, Baláž M, Lukáčová Bujňáková Z, Balážová L, et al. Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: morphology and antibacterial efficacy. *Nanomaterials (Basel)*. 2021;11(4):1005. doi: 10.3390/nano11041005.
40. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, et al. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. *J Phys Chem B*. 2006;110(33):16248-53. doi: 10.1021/jp063826h.
41. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine*. 2007;3(1):95-101. doi: 10.1016/j.nano.2006.12.001.