E-ISSN: 2618-0618
P-ISSN: 2618-060X

NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(11): 169-173
Received: 27-08-2025
Accepted: 29-09-2025

Rajesh Kumar Meena
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Deepa H Dwivedi
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

ML Meena
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Aditiya Kumar Maurya
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Shivangi Mishra
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Corresponding Author:
Rajesh Kumar Meena
Department of Horticulture, School for Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

Green synthesis and characterization of silver nanoparticles using *Thevetia peruviana*: Optimization of temperature and reaction time

Rajesh Kumar Meena, Deepa H Dwivedi, ML Meena, Aditiya Kumar Maurya and Shivangi Mishra

DOI: https://www.doi.org/10.33545/2618060X.2025.v8.i11c.4161

Abstract
The present study explores the green synthesis of silver nanoparticles (Ag^+ NPs) using aqueous extract of stem of *Thevetia peruviana*, yellow Oleander, a plant rich in bioactive phytochemicals. Nanobiotechnology, a convergence of nanotechnology and biological sciences, enables an eco-friendly and cost-effective synthesis of nanoparticles with tailored physicochemical properties. In this investigation, various synthesis parameters, including temperature (30 °C, 50 °C, and 80 °C) and reaction time (20 to 60 minutes), were optimized to obtain a significant yield of Ag^+ NPs showing good stability. The biosynthesis of the Ag^+ NPs was confirmed by a visible color change from pale yellow to brown and further validated through UV-Visible spectroscopy, which exhibited a characteristic surface plasmon resonance (SPR) peak in 400-450 nm range. Scanning Electron Microscopy (SEM) revealed the morphological characteristics of the synthesized nanoparticles where size of the particles ranged from 0.5-1 micrometre. Optimal synthesis was obtained at 50°C temperature where reaction mixture was processed for 30 minutes, which facilitated efficient reduction and stabilization of Ag^+ ions. The presence of phytochemicals such as flavonoids, terpenoids, and alkaloids in the stem extract likely contributed to the dual role of reducing and capping agents. This study underscores the potential of *T. peruviana* as a sustainable bioreductant in the synthesis of silver nanoparticles, offering promising applications in biomedicine, catalysis, and material science under optimized synthesis conditions.

Keywords: *Thevetia peruviana*, Ag^+ NPs synthesis, yellow oleander, time & temperature

Introduction
Nanotechnology is a multidisciplinary field that involves the manipulation and control of materials at the nanoscale, typically ranging from 1 to 100 nanometres (nm). At this scale, materials often exhibit unique physicochemical, optical, electrical, and mechanical properties that differ significantly from their bulk counterparts due to increased surface area-to-volume ratios and quantum effects (Bhushan, 2017) [3]. One of the most important outcomes of nanotechnology is the development of nanoparticles (NPs), which are now widely applied in various sectors, (Khan *et al.*, 2019) [12].

In particular, nanobiotechnology, a fusion of nanotechnology and biology, has emerged as a transformative subfield. It focuses on the design, synthesis, and functionalization of nanoparticles using biological systems or inspired by biological principles (Nel *et al.*, 2006) [18]. Nanobiotechnology enables the controlled fabrication of nanoparticles with specific sizes, shapes, surface chemistries, and disparities, which are essential for targeted applications in areas such as drug delivery, diagnostics, tissue engineering, biosensors, and theranostics (Salata, 2004; Mitragotri *et al.*, 2014) [23, 14]. Due to its interdisciplinary nature and broad application range, nanobiotechnology is now considered a core area of modern material science, representing a new paradigm in the development of nanoscale devices and functional materials. It is attracting increasing global interest for its potential to revolutionize various fields, including medical care, cosmetics, food and feed, biomedical sciences, optics, chemical industries, electronics, space industries, catalysis, energy science, single-electron transistors, nonlinear optical devices, and photoelectrochemical applications (Rai *et al.*, 2008; Dykman & Khlebtsov, 2012) [22, 6].

However, while nanobiotechnology offers vast potential, it also brings significant challenges, particularly in terms of toxicity, environmental impact, long-term biocompatibility, and regulatory hurdles (Fadeel & Garcia-Bennett, 2010) [8]. As such, ongoing research is focused not only on improving the efficiency of NP synthesis but also on understanding their interactions with biological systems and ecosystems to ensure their safe and effective application.

The synthesis of silver nanoparticles (Ag^+ NPs) is highly influenced by reaction conditions, particularly temperature and time. These two parameters play a crucial role in determining the size, morphology, stability, and yield of the nanoparticles. Generally, at lower temperatures (e.g., 30, 50 and 80°C), the reduction of silver ions (Ag^+) occurs more slowly, leading to the formation of larger nanoparticles due to extended growth periods and limited nucleation. As the temperature increases to moderate levels (60 - 80 °C), the nucleation rate improves, resulting in smaller and more uniformly distributed nanoparticles. However, excessively high temperatures (above 90 °C) can lead to particle aggregation and instability due to increased kinetic energy, and in green synthesis methods, may even denature the biomolecules responsible for reduction and stabilization (Shameli *et al.*, 2010) [24]. Similarly, reaction time significantly affects nanoparticle characteristics. Short reaction times (5-15 minutes) often result in incomplete reduction of silver ions, producing low yields and poorly defined particles. Optimal reaction times, typically ranging from 30 to 60 minutes, enable complete reduction and the formation of stable, well-dispersed nanoparticles. However, extending the reaction beyond necessary durations (over 2 hours) can cause particle agglomeration or growth due to phenomena such as Ostwald ripening (Singh *et al.*, 2014) [26]. For instance, in a green synthesis using plant extracts such as *Aloe vera* or neem, studies have shown that increasing temperature from 30°C to 80°C while adjusting time from 15 to 60 minutes results in nanoparticles with smaller sizes and improved stability (Ahmed *et al.*, 2016; Iravani, 2011) [1, 9]. Thus, optimizing both temperature and time for the synthesis of silver nanoparticles is important in order to achieve desired physicochemical properties of the NPs, suitable for biomedical and industrial applications.

Materials and Methods

The present investigation was conducted in the Department of Horticulture, Babasaheb Bhimrao Ambedkar University, Lucknow - 226025, Uttar Pradesh, India.

Collection of Plant Material

Fresh stem pieces (15 g) of *Thevetia peruviana* were harvested from a mature, seven-year-old flowering tree located in the vicinity of the Central Library, Babasaheb Bhimrao Ambedkar University, Lucknow. The stem bark collected material was immediately transported to the laboratory for further processing.

Preparation of Leaf Extract

The collected stem (approximately 10 - 15 g) were thoroughly rinsed with 200 mL of double-distilled water (DH_2O) to eliminate surface impurities. The cleaned stem was then cut into small segments and subjected to boiling for 20 minutes over a heating mantle to obtain a crude aqueous extract. The resultant decoction was allowed to cool and subsequently filtered through Whatman No. 4 filter paper to remove any particulate matter. The clear filtrate was collected and stored for use in nanoparticle synthesis.

Biosynthesis of Silver Nanoparticles

To synthesize Ag^+ NPs, 5 mL of an aqueous silver nitrate (Ag NO_3) solution (1.0 mM) was mixed with 20 mL of the *Thevetia peruviana* stem extract prepared as above. The reaction mixture was stirred continuously using a magnetic stirrer. The processing was done at three temperatures and five different durations in order to optimise the two parameters. A visible color change in the solution indicated the initial formation of silver nanoparticles. Oluwaniyeyi *et al*, has also reported the efficacy of extract of *Thevetia peruviana* leaves in reduction and stability of Ag ions.

Standardisation of optimum temperature conditions for synthesis of Ag^+ NPs

In order to optimise the suitable temperature for the synthesis of the Ag NPs of desirable size and high yield three different temperatures (30, 50, and 80 ºC) were used for carrying out the synthesis. Each reaction mixture was observed for 0 to 30 minutes at each of the three temperatures under study for the preliminary colour change in the reaction mixture, indicating successful synthesis of NP.

Standardisation of time for processing sample

Once the temperature for the synthesis was standardised, the duration for processing the sample was optimised. The reaction mixture, heated to the temperature standardised above, was processed for five different durations (20,30,40,50 and 60 minutes) to optimise the ideal duration for the synthesis of silver nanoparticles.

Purification of silver nanoparticles

The nanoparticles synthesized at each of the three temperatures and five-time durations, were subjected to centrifugation at 8000 RPM for 10 minutes to facilitate separation of the nanoparticles from the reaction mixture. This process was repeated three times to ensure maximum purification. The final pellet, presumed to contain purified silver nanoparticles, was collected, washed and stored for subsequent characterization.

Characterization of silver nanoparticles

UV-Visible Spectroscopy

The formation and optical properties of silver nanoparticles (Ag^+ NPs) were investigated using UV-Visible spectroscopy. Metal nanoparticles, particularly those ranging in size from 2 to 100 nm, exhibit characteristic surface plasmon resonance (SPR) bands when exposed to light within the 300-1100 nm wavelength range (Zhou *et al.*, 2023) [28, 29]. The bio reduction of silver ions (Ag^+) to metallic silver (Ag^0) was monitored using a UV-Vis spectrophotometer (Model: LMSP-V320, Make: Labman Instruments). Spectral analysis was conducted over a wavelength range of 300-600 nm, with a resolution of 1 nm. The presence of a distinct SPR peak in the range of 400-450 nm served as confirmation of successful Ag^+ nanoparticle synthesis. In the current study, the SPR peak for *T. peruviana*-mediated silver nanoparticles (TSNPs) was recorded at approximately 460 nm, within the visible region of the electromagnetic spectrum. This absorption peak serves as a reliable spectroscopic signature confirming the successful synthesis of silver nanoparticles (Jenkins and Snyder, 1996; Bindhu and Umadevi, 2014) [11, 5].

Scanning Electron Microscopy (SEM)

The surface morphology and structural features of the biosynthesized silver nanoparticles were analysed using

Scanning Electron Microscopy (SEM) (Model: JSM 6490 LV, Make: JEOL, Japan). A thin layer of the dried nanoparticle powder was mounted on carbon-coated tape and air-dried under a mercury lamp for approximately five minutes prior to imaging.

Results and Discussion

Standardization of synthesis conditions for silver nanoparticles

Optimization of temperature for Ag^+ NPs synthesis

To determine the optimal thermal conditions for the biosynthesis of silver nanoparticles (Ag^+ NPs), the reaction was carried out at three different temperatures *viz.,* 30 °C, 50 °C, and 80 °C. The progression of the reaction was monitored over a time course ranging from 0 to 30 minutes for each of the reaction mixtures being processed at different temperatures described above. Visual observation of color change, indicative of nanoparticle formation (Elemike *et al.* 2014) [7], along with subsequent spectroscopic validation, was used to assess the extent of nanoparticle synthesis. Among the tested conditions, 50 °C was found to be the most effective temperature, facilitating rapid and stable nanoparticle formation. A change in the color of the reaction mixture from pale yellow to brown, was the first indication of formation of Ag^+ NPs, which is consistent with previous reports of biogenic silver nanoparticle synthesis (Shankar *et al.*, 2004, Elemike *et al.* 2014) [25, 7]. Spectroscopic analysis of the three reaction mixtures over a range of 300-600 nm shows the peak intensity of the surface plasmon absorption for TSNP in the optical region of the UV-Visible spectrum at 460 nm which is characteristic for formation of silver nanoparticles (Jenkins and Snyder 1996; Bindhu and Umadevi 2014) [11, 5]. Absorption intensity was observed to be highest at 50° centigrade as compared to the NPs synthesized at lower temperatures. In spectroscopic studies, the concentration of any substance is directly proportional to the absorbance recorded (Mohammadi *et al.*, 2018) [16]. This indicates the presence of a higher concentration of the reduced Ag° in the reaction mixture processed at 50 °C, and thus, it was identified as the optimum temperature for further Ag^+ NP synthesis protocols. Temperature plays a critical role in the rate of nucleation and growth of nanoparticles. In this study, synthesis at 50 °C was found to be optimal, as it facilitated rapid reduction of Ag^+ ions to Ag^0 and resulted in the formation of stable silver nanoparticles, as evidenced by the characteristic surface plasmon resonance (SPR) observed in the UV-Vis spectrum. At lower temperatures (30 °C), the reaction proceeded slowly, indicating a lower rate of Ag^+ bioreduction. Conversely, at higher temperatures (80 °C), while the reaction was faster, it likely led to agglomeration or instability due to excessive kinetic energy and rapid nucleation, which can adversely affect nanoparticle uniformity and morphology (Ahmed *et al.*, 2016; Singh *et al.*, 2018) [2, 26]. The SPR peak obtained via UV-Vis spectroscopy typically occurs between 400-450 nm for spherical silver nanoparticles, and its intensity and sharpness are reliable indicators of particle formation and stability (Song & Kim, 2009) [27].

Optimization of reaction time

To establish the ideal duration for the synthesis process at the temperature (50 °C) optimized above, the reaction was evaluated at five different time intervals, *viz.*, 20, 30, 40, 50, and 60 minutes. Narrow shaped surface plasmon resonance (SPR) bands are indicative of formation of spherical nanoparticles showing a homogeneous distribution (Oluwiyeyi *et al*,2016). Among the reaction mixtures under study for optimising duration of processing, 30 minutes was identified as the optimum reaction time, yielding a maximum yield of uniformly synthesized silver nanoparticles. Beyond this duration, no significant enhancement in nanoparticle yield or stability was observed, indicating the completion of the reaction. On the contrary flattened peaks were observed with increasing duration of processing (40, 50 and 60 minutes (Fig.2), possibly indicating that the nanoparticles formed have a tendency to agglomerate if processed for a longer duration. A bathochromic shift was also observed in these samples indicating a decrease in the average diameter of the Ag^+NPs.

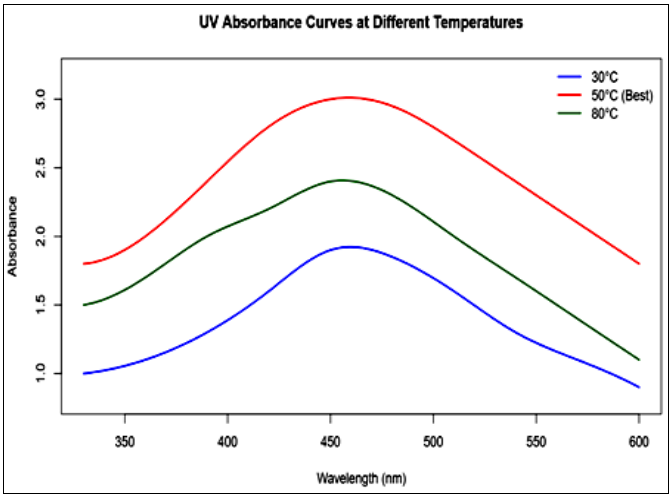

Fig 1: Optimization of temperature for Ag^+ NPs synthesis

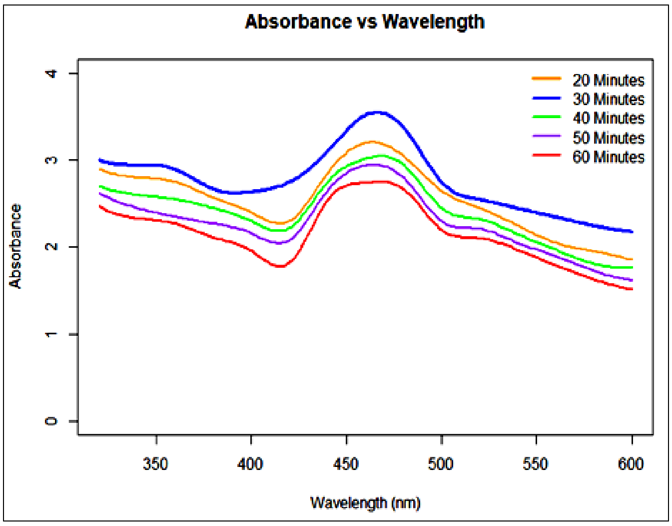

Fig 2: Optimization of reaction time

In terms of reaction time, 30 minutes was identified as the optimum duration for nanoparticle formation at the selected temperature of 50 °C. Reactions shorter than 30 minutes did not result in complete reduction of Ag^+ ions, while extending the reaction time beyond 30 minutes showed negligible improvement in nanoparticle yield or quality. This aligns with findings from previous studies, which suggest that prolonged reaction times do not significantly influence nanoparticle formation once the reduction process reaches completion (Nadagouda & Varma, 2008) [17]. On the contrary there are also reports where the concentration of the synthesised Ag^+NPs has increased with increase in the duration of reaction. However, this variation could be attributed to the use of *Thevetia* leaf extract as reducing agent while in the present study stem extract was used as the reductant.

Scanning electron microscopy (SEM)

The Ag^{+}NPs synthesized as per processing conditions (Temperature:50 °C; Time: 30 minutes) optimised above were subjected to scanning electron microscopy (SEM). Images of Ag^{+} NPs synthesized using *Thevetia peruviana* stem extracts were captured at magnifications of 5 µm and 1 µm (Fig 3 A and B). Morphological analysis indicates that the nanoparticles were predominantly spherical in shape although they exhibited a tendency to aggregate. These aggregates appear to possess a rough and crystalline surface texture. The SEM micrographs provide clear evidence of both discrete nanoparticles and aggregated structures, reflecting the high purity and structural integrity of the synthesized Ag^{+} NPs. Furthermore, the images highlight the remarkable potential of *T. peruviana* stem extract as a biological agent for the synthesis of silver nanoparticles. Similar morphological features were reported by Zahoor *et al.* (2023), supporting the reproducibility and consistency of the biosynthesis process.

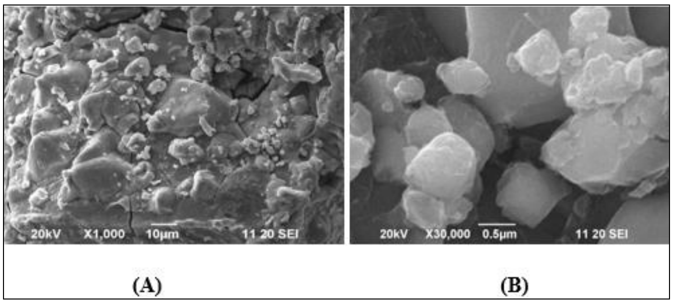

Fig 3: SEM images of Ag NPs showing their morphology at two different resolutions captured at (A) 1000X and (B) 30,000X magnification.

The synthesis of silver nanoparticles (Ag^{+} NPs) is highly influenced by reaction conditions, particularly temperature and time. These two parameters play a crucial role in determining the size, morphology, stability, and yield of the nanoparticles. Generally, at lower temperatures the reduction of silver ions (Ag^{+}) occurs more slowly, leading to the formation of larger nanoparticles due to extended growth periods and limited nucleation. However, excessively high temperatures can lead to particle aggregation and instability due to increased kinetic energy, and in green synthesis methods, may even denature the biomolecules responsible for reduction and stabilization (Shameli *et al.*, 2010) [24]. Short reaction times often result in incomplete reduction of silver ions, producing low yields and poorly defined particles. Optimal reaction times, typically ranging from 30 to 60 minutes, enable complete reduction and the formation of stable, well-dispersed nanoparticles. However, extending the reaction beyond necessary durations can cause particle agglomeration or growth due to phenomena such as Ostwald ripening (Singh *et al.*, 2014) [26].

Conclusion

The present study successfully demonstrated the green synthesis of Ag^{+} NPs using *Thevetia peruviana* stem extract as a natural reducing and stabilizing agent. Optimization of reaction conditions revealed that a temperature of 50 °C and a reaction time of 30 minutes were most effective in facilitating rapid, stable, and uniform nanoparticle formation. The characteristic colour change and the surface plasmon resonance (SPR) peak observed at approximately 460 nm in UV-Visible spectroscopy confirmed the successful bioreduction of Ag^{+} ions to metallic silver. Furthermore, scanning electron microscopy (SEM) analysis revealed that the synthesized nanoparticles were predominantly spherical and tended to form aggregates with rough, crystalline surfaces. These findings not only validate the efficacy of *T. peruviana* stem extract in nanoparticle synthesis but also underscore the potential of plant-mediated approaches as environmentally friendly and sustainable alternatives to conventional chemical methods. The presence of bioactive phytochemicals in the extract likely played a dual role in both reducing silver ions and stabilizing the resulting nanoparticles. This green synthesis method offers a cost-effective and scalable route for producing silver nanoparticles with desirable physicochemical properties, suitable for various biomedical and industrial applications. Future research should focus on detailed mechanistic studies, functional assessments, and biocompatibility analyses to further explore the application potential of these biosynthesized nanoparticles.

References

1. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plant extract-mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res. 2016;7(1):17-28.
2. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J Radiat Res Appl Sci. 2016;9(1):1-7.
3. Bhushan B. Nanotribology, nanomechanics and materials characterization. In: Springer Handbook of Nanotechnology. Berlin: Springer; 2017. p. 869-934.
4. Bindhu MR, Umadevi M. Synthesis of monodispersed silver nanoparticles using *Hibiscus cannabinus* leaf extract and its antimicrobial activity. Spectrochim Acta A Mol Biomol Spectrosc. 2013;101:184-190.
5. Bindhu MR, Umadevi M. Surface plasmon resonance optical sensor and antibacterial activities of biosynthesized silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc. 2014;121:596-604.
6. Dykman L, Khlebtsov N. Gold nanoparticles in biomedical applications: recent advances and perspectives. Chem Soc Rev. 2012;41(6):2256-2282.
7. Elemike EE, Onwudiwe DC, Fayemi OE, Ekennia AC, Ebenso EE, Tiedt LR. Biosynthesis, electrochemical, antimicrobial and antioxidant studies of silver nanoparticles mediated by Talinum triangulare aqueous leaf extract. J Clust Sci. 2017;28(1):309-330.
8. Garcia-Bennett AE, Everest-Dass A, Moroni I, Rastogi ID, Parker LM, Packer NH, *et al*. Influence of surface chemistry on the formation of a protein corona on nanodiamonds. J Mater Chem B. 2019;7(21):3383-3389.
9. Iravani S. Green synthesis of metal nanoparticles using plants. Green Chem. 2011;13(10):2638-2650.
10. 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.
11. Jenkins R, Snyder RL. Introduction to X-ray Powder Diffractometry. Vol. 138. New York: Wiley; 1996. p. 1-403.
12. Khan I, Saeed K, Khan I. Nanoparticles: properties, applications and toxicities. Arab J Chem. 2019;12(7):908-931.
13. Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol. 2019;84(2):151-157.
14. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals:

formulation and delivery strategies. Nat Rev Drug Discov. 2014;13(9):655-672.
15. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv. 2013;31(2):346-356.
16. Mohammadi A, Hashemi N, Ghassabzadeh M, Sharafi A, Yazdinezhad A, Danafar H. Green synthesis and toxicological evaluation of zinc oxide nanoparticles utilizing *Punica granatum* fruit peel extract: an eco-friendly approach. Sci Rep. 2025;15(1):20853.
17. Nadagouda MN, Varma RS. Green synthesis of silver and palladium nanoparticles at room temperature using coffee and tea extract. Green Chem. 2008;10(8):859-862.
18. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006;311(5761):622-627.
19. Odeja OO, Obi G, Ogwuche CE, Elemike EE, Oderinlo OO. Phytochemical screening, antioxidant and antimicrobial activities of *Senna occidentalis* (L.) leaves. 2014.
20. Oluwaniyi OO, Adegoke HI, Adesuji ET, Alabi AB, Bodede SO, Labulo AH, *et al*. Biosynthesis of silver nanoparticles using aqueous leaf extract of *Thevetia peruviana* Juss and its antimicrobial activities. Appl Nanosci. 2016;6(6):903-912.
21. Peter ME. Targeting of mRNAs by multiple miRNAs: the next step. Oncogene. 2010;29(15):2161-2164.
22. Rai M, Yadav A, Gade A. Current trends in phytosynthesis of metal nanoparticles. Crit Rev Biotechnol. 2008;28(4):277-284.
23. Salata OV. Applications of nanoparticles in biology and medicine. J Nanobiotechnol. 2004;2(1):3-10.
24. Shameli K, Ahmad MB, Yunus WMZW, Ibrahim NA, Rahman RA, Jokar M, *et al*. Silver/poly (lactic acid) nanocomposites: preparation, characterization, and antibacterial activity. Int J Nanomedicine. 2010;5:573-579.
25. 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(2):496-502.
26. Singh P, Kim YJ, Zhang D, Yang DC. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. 2018;34(7):588-599.
27. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng. 2009;32(1):79-84.
28. Zhou W, Xiao J, Li H, Chen Q, Wang T, Wang Q, *et al*. Soil organic matter content prediction using Vis-NIRS based on different wavelength optimization algorithms and inversion models. J Soils Sediments. 2023;23(6):2506-2517.
29. Zhou Y, Yang G, Mo L, Mu T, Tang Y. Recent advances in green synthesis of nanoparticles using plant extracts: mechanisms and applications. Mater Today Commun. 2023;35:106109.