

Biosynthesis and Characterization of Silver Nanoparticles using *Ziziphus mauritiana* Leaf Extract

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In the area of Nano technological research, green synthesis of Nanoparticles (NPs) has pulled in a ton of interest in light of the fact that the green-synthesized Ag NPs show more prominent antimicrobial and inhibitory qualities, in perspective on which they could be utilized in various applications in the areas of medical and drug delivery. It might be the most appropriate option for the conventional techniques that are commonly conflicting and exert dangerous impacts on the earth. In this research, green synthesis of silver NPs using *Ziziphus mauritiana* leaf extract was directed. The incorporated Ag NPs were contemplated using UV-V is spectrophotometry, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) techniques. The consequences of UV-Vis spectroscopy showed plasma resonance peaks around 413 nm, proving the existence of Ag NPs. The result of SEM demonstrated NPs to be spherical and in the 4–96 nm range. The practical gatherings topping the NPs and the organic compounds engaged with the decrease procedure of biosynthesis and stabilization of silver NPs were studied with FTIR and were observed to be phenols, alcohols, primary amines, and alkenes. The XRD pattern demonstrated the FCC structure of AgNO₃ and average particle was seen to be 12.0 nm.

Keywords: Silver nanoparticles, SEM, FTIR, X-ray diffraction, Plasma Resonance.

Nanotechnology has turned into a prominent zone of the revolutionary interdisciplinary research that manages structure, synthesis, and manipulation of nano sized particles. It has an immense potential for use in various applications such as drug gene delivery, biomedical sciences,

and mechanics (Shanmuganathan *et al.*, 2018). At present, research on nanoparticles (NPs) isn't identified with applications yet in addition to the synthesis method (Ghiassi *et al.*, 2018). Chemical and physical syntheses of NPs yield byproducts that are dangerous and unsafe to environment;

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these methods are not efficient (Gavade *et al.*, 2015, Mancuso *et al.*, 2020). In this sense, a lot of premium has been created by the term “green engineering science” that incorporates a wide scope of potential applications such as an ecologically friendly producing procedure that lessens squander items and pollution (Bankar *et al.*, 2010, Begum *et al.*, 2018). Keeping this in view, green synthesis increases an immense interest, which includes the utilization of ecofriendly compatible materials such as bacteria, fungi, and plants (Ahmad *et al.*, 2010). In this manner, a part of nanotechnology, green synthesis of NPs, is very rapidly developing scientific arena that fills in as a critical strategy for growing clear, safe, and eco-accommodating methodology for the synthesis metal NPs (Yallappa *et al.*, 2013; Huang *et al.*, 2004). Benefits of green synthesis of NPs over conventional methods include being economical and easy to regulate. Besides it produces less wastage, is an energy-efficient procedure, has decreased rates of fewer accidents, yields safe products, is competitive, and contributes to healthier workplaces and communities (Kumar *et al.*, 2016; Wang *et al.*, 1991). Nature has structured various strategies for the synthesis of nano- and micro-sized inorganic materials. They help being developed of a novel and uninvestigated area zone of research dependent on the green synthesis of NPs (Mandalet *et al.*, 2018; Tripathy *et al.*, 2010). Numerous works have been distributed on the green synthesis of NPs utilizing microorganisms such as fungi, bacteria, and plants, because of their diminishing qualities that lead to the decrease of metal compounds to respective NPs (Mittal *et al.*, 2017). Among many NP biosynthesis methods, microbe-mediated synthesis is not thought to be feasible on industrial scale as it requires high aseptic conditions and a considerable maintenance (Sinha *et al.*, 2015). Accordingly, plant extract uses for this reason is conceivably valuable over microbes because of the simplicity of the less biohazard and opportunity from expand procedure of keeping up cell cultures (Banerjee *et al.*, 2014, Pambuk *et al.*, 2019). In such a way, plant extracts provide a better platform for synthesis of NPs as it is devoid of harmful chemicals and yields natural capping agents (Obaid *et al.*, 2015; Graf *et al.*, 2003; Sasikala *et al.*, 2015). Among the different important NPs, silver NPs are becoming a quite significant product as they have gained huge

interest because of their novel characteristics like chemical stability, excellent conductivity, catalytic activity, and antifungal, antibacterial, and antiviral properties (Parmar *et al.*, 2011). On account of their good anti-inflammatory activities, silver NPs can be consolidated into various materials, for example, cryogenic superconducting materials and composite fibers, into cosmetics, and into electronic parts. They exhibit extensive biocidal action against microorganisms by disrupting their unicellular membrane (Sutradhar and Saha, 2015; Malhotra *et al.*, 2013). As a consequence, it has huge potential in biomedical applications such as topical creams, antiseptic sprays, fabrics, and wound dressings. A few investigations have been led on the utilization of plant concentrates to blend silver NPs. In these works, phytochemicals acquired from leaf extracts of *Solidago altissima* (Kumar *et al.*, 2016), *Acalypha indica* (Krishnaraj *et al.*, 2010; Sorbiun *et al.*, 2018), *Murraya koenigii* (Christensen *et al.*, 2011, Al-Quwaie *et al.*, 2020), *Xanthium strumarium* L. (Mittal *et al.*, 2017), seed extricate of *Acacia farnesiana* (Yallappa *et al.*, 2013), *Ocimum sanctum* (Ahmad *et al.*, 2010), root extracts of *Trianthema decandra* (Geethalakshmi and Sarada, 2012), *Macrotyloma uniflorum* (Vidhu *et al.*, 2011), fruit extricate of *Musa paradisiaca* peels (Bankar *et al.*, 2010), *Carica papaya* (Jain *et al.*, 2009), and stem extracts of *O. sanctum* (Ahmad *et al.*, 2010) to fill in as diminishing or/and topping specialists reaction with silver nitrate (AgNO_3) as precursor have been studied. In any case, plausibility of plants to be utilized as organic materials for synthesizing NPs has not been totally considered.

Among the members of the family Rhamnaceae, plants of genus *Ziziphus* have been utilized for a long time due to their medicinal and nutritive properties (Sutradhar and Saha, 2015, Khatak *et al.*, 2020). They are common plants that are mostly available throughout the world. There are about 40 species available in genus *Ziziphus*. Among them, *Z. mauritiana* is the one that mostly grows in dry places and has abundant amount of starch, sugar, carbohydrate, mucilage, proteins, and vitamins (Parmar *et al.*, 2011; Dahiru *et al.*, 2006). The dried ripe fruit of this plant is a mild laxative and fruits are used for treating depression, diabetes, and ulcers (Lopez *et al.*, 2018). It is utilized as a medicine in fevers and the

leaves of this plant are useful in liver problem and asthma. Also, the powder of its leaves is applied on wounds. It is appeared to have antioxidant, antimicrobial, antitumor, and anticancer activities, consequently demonstrated to be one among the most encouraging plants to be utilized for phytomedicinal applications (Gavade *et al.*, 2015; Parmar *et al.*, 2011). In this investigation, *Z. mauritiana* was used as plant source for creation of silver NPs. Further, integrated NPs were portrayed by various techniques.

METHODOLOGY

Preparation of plant extract

Every single fine chemicals, solvents, and media used in this investigation were acquired from Merck (Mumbai, India) and HiMedia (Mumbai, Maharashtra, India) and of AR grade. Every one of the arrangements were made in sterile Milli-Q water. Commonly, a plant-extract-mediated biosynthesis of NPs incorporates blending the watery plant separate with a fluid solution of the suitable metal salt. Here, *Z. mauritiana* leaf concentrate was utilized to get ready silver NPs thinking about simplicity of availability, cost-effectiveness, and medicinal properties. Fresh leaves were collected from Hussain Sagar Road, Hyderabad district, Telangana, India, in the month of January. They were surface-cleaned by tap water followed by double-distilled water to remove contaminated organic contents and other debris. Additionally, they were parched at room temperature and turned into a fine powder utilizing an electric blender. About 10 g of the powder was overflowed with 150 mL double-distilled water for 30 min at 70-80 °C and was hatched overnight. The extract was chilled off and sifted with Whatman Filter Paper Number 1. The filtrate was centrifuged at 13000 rpm for 3 minutes and the supernatant was utilized for the further explores.

Biosynthesis of Silver NPs

Initially, 500 mL, 1 mM (0.08 g silver nitrate was disintegrated in 500 mL distilled water to prepare 1 mM solution of AgNO₃) solution of silver nitrate was set up in an Erlenmeyer flask. Thereafter, 50 mL aqueous extract of *Z. mauritiana* leaves was mixed with 500 mL AgNO₃ solution. The solution mixture was heated on a mantle

at 70-80 °C for 30 min. To maintain a strategic distance from photo-activation, the solution was brooded in a dim chamber at room temperature overnight. Decrease of Ag⁰ from Ag⁺ was affirmed by the adjustment in color of the solution, from colorless to brown. After medium-term incubation, the mixture was centrifuged at 12000 rpm for 4 minutes. Supernatant was isolated and the pellets were washed two times with twofold refined water. Further, particles were secluded by presenting to centrifugation again at 12000 rpm for 3 min. The washed pellets were collected in a watch glass and left to dry in a hot-air oven at 30-42 °C. After totally drying out, the AgNPs, which seemed dim dark colored, were scratched utilizing a surgical tool and put away in a cool dry spot.

Characterization of Silver NPs

Fundamentally, the silver NPs synthesized from the *Z. mauritiana* leaf extract were portrayed by UV-Vis spectral analysis, which was completed by using a UV-Vis spectrophotometer (UV-1800 Model; Shimadzu, Japan) with goals of 1 nm somewhere in the scope of 300 and 600 nm with de-ionized water as clear. At that point, 1 mL sample was lay hold of test tube and in this way dissected at room temperature. Dynamic light dispersing (Spectroscatter 201) was done to choose the ordinary size of blended silver NPs. Fourier-transform infrared spectroscopy (FTIR) was utilized to distinguish the conceivable biomolecules present that are in charge of keeping up the dependability, capping, and formation of silver NPs in the *Z. mauritiana* aqueous leaf extract. It likewise decided the potential physiochemical connections among the components of the extract. The estimations were taken for the synthesized silver NPs after 24 h incubation using an FTIR spectrophotometer (8400S; Shimadzu) with a wavelength scope of 4000–500 cm⁻¹ and resolution of 4/ cm⁻¹. The examples were joined into KBr pellets to gain the spectra. The got outcomes were looked at for computing shift in functional peaks of critical value. The morphology of the synthesized silver NPs was contemplated by a SEM (S-3700N; Hitachi, Japan). The crystalline reality of the synthesized silver NPs was investigated using an X-ray diffractometer (XRD700 Model; Shimadzu) with K-beta filter, using monochromatic Cu K α radiation of wavelength 1.5418 Å. The X-ray

generator was worked at 30 mA and 40 KV, and the checking mode was nonstop with scanning range (2θ) from 4° to 90° .

RESULTS AND DISCUSSION

Visual Monitoring and UV-Vis Spectroscopy

It is notable that silver NPs display a solid retention band in the obvious region. In this analysis, expansion of plant extract of *Z. mauritiana* to aqueous solution of silver nitrate prompted the adjustment in the color of the blend from yellowish to rosy dark colored (reddish brown). The imaged properties of silver NPs were examined by UV-Vis assimilation spectroscopy. The Plasmon resonance reverberation band distinguished at 400-430 nm is practically like that revealed by Obaid *et al.* in 2015, and is delineated in Figure 1. The slight varieties in the estimations of absorbance imply that there are adjustment in particle size (Tripathy *et al.*, 2010). The reddish brown color showed up because of the excitation of the surface plasmon resonance (SPR). Silver NPs having absorbance esteems in the visible scope of 410-448 nm have been accounted for somewhere else (Banerjee *et al.*, 2014; Tripathy *et al.*, 2010). The examined tests displayed a strong and steady noticeable ingestion spire in the scope of 413 nm (Figure 2) because of excitation of SPR. The results are near the reports introduced in literature demonstrating absorbance crest at 413 nm for silver NPs combined

by *Cochlospermum religiosum* extricate (Sasikala *et al.*, 2015) and by *Pithophorae dogonia* extract (Sinha *et al.*, 2015).

X-ray Diffraction Analysis

So as to check the aftereffects of the UV-V is spectral analysis, the examples of the synthesized silver NPs were analyzed by X-ray diffraction (XRD). Figure 2 outlines the XRD pattern for silver NPs. The mean molecule distance across of silver NPs was resolved from the XRD pattern as indicated by the line girth of the plane and refraction peak using the Debye-Scherrer equation. The diffractogram comprised of four distinct reflections at 38.01° (1/ 1/ 1), 44.35° (2/ 0/ 0), 64.39° (2/2/ 0), and 77.37° (3/ 1/ 1). Consequently, the XRD result affirmed the crystalline nature of the sample. The diffraction peaks of the sample corresponded to Bragg's reflections of typical face-centered cubic (FCC) structure of silver NPs. The XRD pattern additionally demonstrated a peak

Table 1. Values of diffraction peaks in the range of 2θ and FWHM

Sl. No.	2θ (deg)	FWHM (deg)
1	31.673 (8)	0.10 (3)
2	38.014 (14)	0.563 (16)
3	44.35 (4)	1.15 (4)
4	64.39 (2)	0.47 (4)
5	77.377 (15)	0.44 (4)

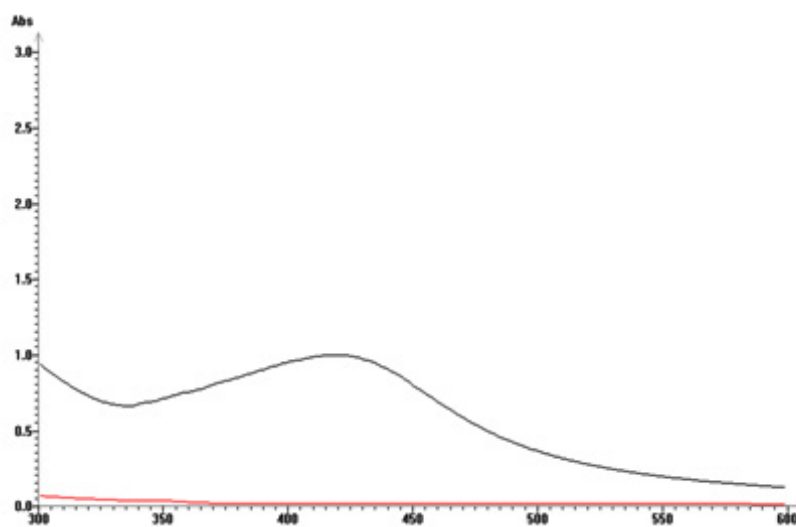


Fig. 1. Surface plasmon resonance peak for biosynthesized silver nanoparticles

at 31.67° . This might be because of the presence of other organic compounds of the leaf extract or crystalline impurities present on the surface of silver NPs. For example, a few investigations describing green synthesis of silver NPs using plant extracts by XRD analysis have likewise revealed the presence of comparable additional peaks in the XRD pattern of silver NPs. From the Debye-Scherrer equation, the average normal size of silver NPs was seen as 12.0 nm.

Scanning Electron Microscope Studies

Figure 3 demonstrates the images of SEM synthesized silver NPs, which uncovers that a large portion of the silver NPs are predominately round with smooth surface, and the particles are in the scope of 4-96 nm. Additionally, the examination utilizing SEM showed that the totals of silver NPs were very much scattered in a balanced out structure as they were in indirect contact with

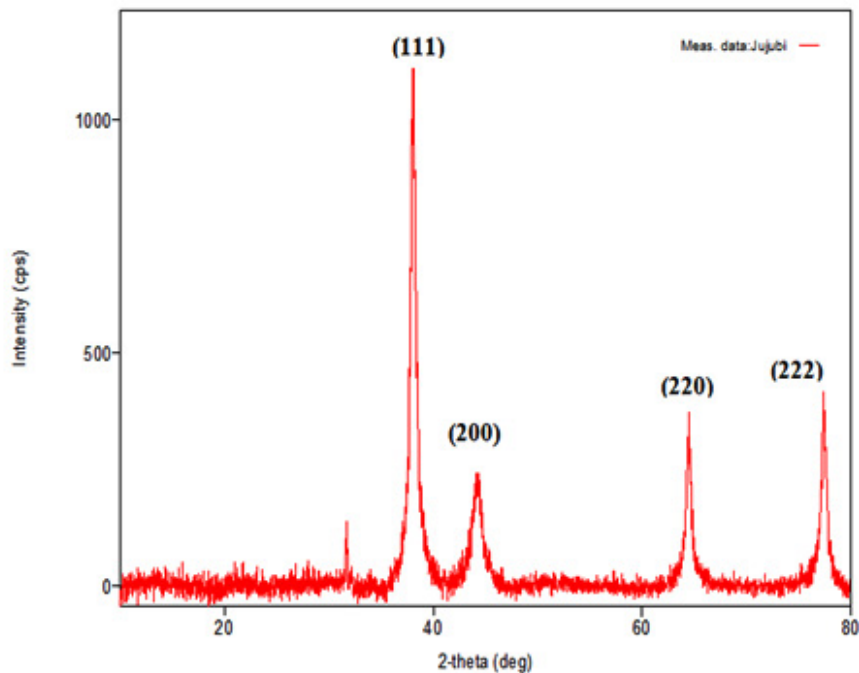


Fig. 2. XRD pattern of silver NPs synthesized using *Z. mauritiana*

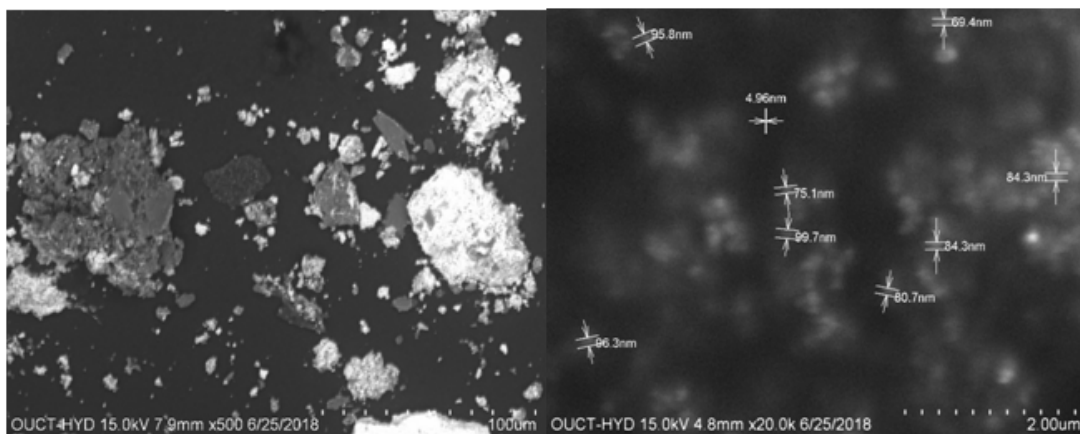


Fig. 3. Scanning electron microscopy image indicating the size of the biosynthesized silver nanoparticle

each other because of plant-capping agents. Using software, ImageJ, it was discovered that the normal particle size was about 13.25 nm. The acquired particle size was practically similar to that gotten from the XRD.

FTIR Spectroscopy Studies

The results of the synthesized NPs from FTIR were looked at for figuring the move in functional peaks of critical value. The spectrum is spoken to in Figure 4. The FTIR spectrum studies of the *Z.mauritiana* leaf extract shows various bands, which uncovers a perplexing nature of the leaf extract. An aggregate of 41 pinnacles were watched, among which 3 sharp peaks were seen

at 3417.98, 3444.98, and 1629.90 cm⁻¹ relating to the O-H stretch of the alcohol, N-H stretch of amides, ArO-H H-bonded phenol compound, and Ar-CH=CHR compound of alkenes, as appeared in Figure 4. Likewise, the outcomes were analyzed using IRPal2.0 software. Peak value and the functional groups present in leaf extracts of *Z. mauritiana* obtained by FTIR studies are shown in Table 2. They demonstrate the presence of natural mixes containing carbon and oxygen, along these lines proposing that silver may be topped by organic components of plant extract, stabilizing them and further upgrading their antimicrobial activity. The shifting of bands at 3444.12 and 1629.90 implies

Table 2. FTIR peak value and its functional groups present in leaf extracts of *Z. mauritiana*

Peak	Range	Bond	Compound	Structure
3444	3600-3400	O–H stretch	Alcohols	RCH ₂ OH
	3600-3400	O–H stretch	Alcohols	R ₂ CHOH
	3600-3400	O–H stretch	Alcohols	R ₃ COH
	3445-3435	NH- stretch	Amides	RCONHR
	3500-3200	ArO–H H-bonded	Phenols	ArO–H bonded
3417	3600-3400	O – H stretch	Alcohols	RCH ₂ OH
	3600-3400	O – H stretch	Alcohols	R ₂ CHOH
	3600-3400	O – H stretch	Alcohols	R ₃ COH
	3500-3200	ArO–H H-bonded	Phenols	ArO–H bonded
1629	1640-1600	NH out of plane	Amides	RCONH ₂
	1630-1620	Ar–CH=CHR	Alkenes	Ar–CH=CHR
	1700-1615	C=N	–	C=N

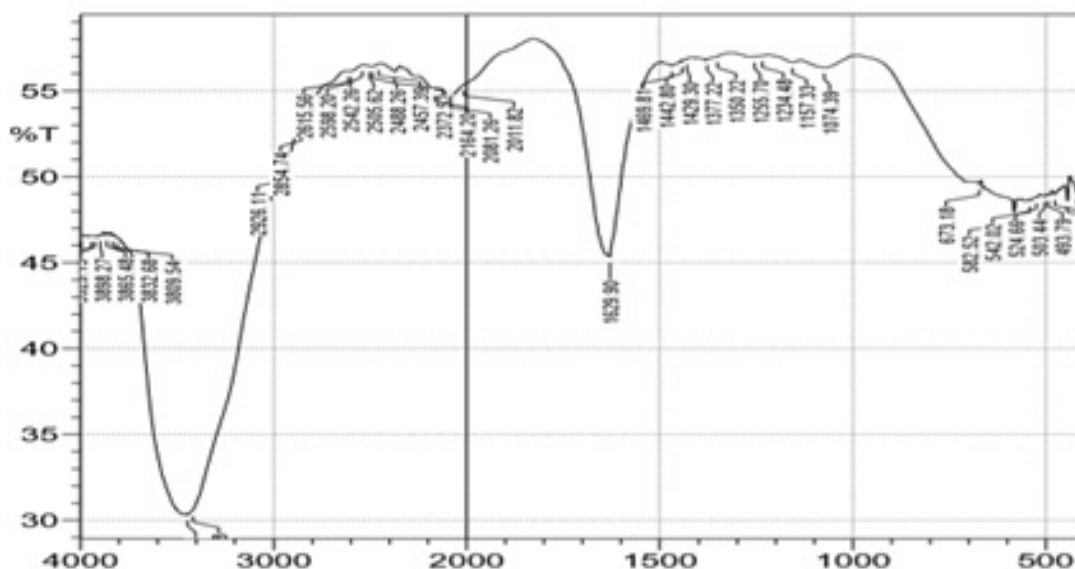


Fig. 4. FTIR pattern of AgNPs synthesized using *Z. mauritiana*

the presence of organic components such as phenols, alcohols, alkenes, and primarily primary amines of the plant associated with the decrease procedure for arrangement of silver NPs. Carboxyl groups, a middle formation of phenolic gatherings, proteins, and carbohydrates of *Z. mauritiana* leaf extract are associated with the decrease forms for synthesis of silver NPs. Silver NPs are built up as antimicrobial agents, while the presence of plant bioorganic capping material on the silver NPs gives them improved antibacterial movement and potential to be utilized as antioxidant agents.

CONCLUSION

The improvement of cost-efficient and eco-friendly methods for synthesis of nanomaterials still stays a scientific logical challenge. In this study, *Z. mauritiana* plant leaf extract was effectively used as a diminishing and balancing out agent for consistent and quick synthesis of silver NPs. Silver NPs were synthesized by a clean, nontoxic, low-cost, and eco-friendly method. The green blend of silver NPs was performed by mixing *Z. mauritiana* leaf extract with 1 mM AgNO₃ at 70-80 °C for 30 minutes. The spectroscopic portrayals from UV-Vis, FTIR, SEM, and XRD bolster the development and dependability of the biosynthesized silver NPs. The results of UV-Vis qualitative analysis confirmed the existence of silver as a result of the actual peak in the 400–430 nm region. The SEM analysis affirmed spherical and uniform silver NPs with broadness varying from 4 to 96 nm. The normal crystallite size determined from Scherrer equation was 12 nm. This is a simple, proficient, and quick technique for green synthesis of silver NPs that can be utilized in various biomedical and biotechnological applications. Such sort of studies, for instance, production of NPs using plant extracts, which are used for mediating the NPs for rapid single-step protocol, can beat a several environmental issues and can give another dimension to green synthesis of silver NPs.

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Conflict of Interest

Authors assure to disclose there is no conflict of interest including honorarium, grants, membership, employment, ownership of stock or non financial interest.

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