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Biosynthesis of Silver nanoparticles using root extract of the medicinal plant *Justicia adhatoda*: Characterization, electrochemical behavior and applications

ABSTRACT

K. M. Ponvel*
T. Narayanaraja
J. Prabakaran

*Nanoscience, PG & Research
Department of Chemistry, V.O.
Chidambaram College,
Thoothukudi-628008, Tamil
Nadu, India.*

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A facile and green approach has been developed to synthesize silver nanoparticle (Ag-NPs). This was carried out by a biosynthetic route using *Justicia Adhatoda* root extract as reducing and stabilizing agent. The structure, composition, average particle size (~25 nm) and surface morphology of Ag-NPs were characterized by the X-ray diffraction, transmission electron microscope and atomic force microscope analyses. The possible functional groups in the plant extracts were identified by FT-IR analysis. Electrochemical property of the Ag-NPs was analysed by cyclic voltammetry that displayed an oxidation peak potential at $E_{pa} = 0.438$ V. Mechanism of the formation of Ag-NPs was proposed which showed that the phenolic compounds of the root extract respond for the reduction of silver ions to silver nanoparticles. Ag-NPs exhibit good antioxidant and antibacterial activities. This biosynthetic approach could open a path for environmentally friendly, simple, cost effective, alternate for conventional synthesis. This prevented hazardous chemicals and was useful for applications in medicine and large scale production of metallic nanoparticles.

Keywords: *Justicia Adhatoda; Plant extract; Silver nanoparticles; Biosynthesis; Antibacterial activity.*

INTRODUCTION

Silver nanoparticles (Ag-NPs) had become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, biosensing, imaging, drug delivery and medicine [1]. Silver and silver supported materials had been used as antimicrobial and antifungal agents for centuries [2-5], the recent resurgence in interest for this element particularly focused on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics. The advantage of Ag-NPs compared with bulk metal ions was the slow and regulated release of silver from nanoparticles thereby causing long lasting protection against microbes [5].

* Corresponding author:
K. M. Ponvel
*Nanoscience, PG & Research
Department of Chemistry,
V.O. Chidambaram College,
Thoothukudi -628008, Tamil
Nadu, India.*
Tel +91 9578072472
Fax +91 9578072472
Email km_ponvel@rediffmail.com

Different approaches had been developed for Ag-NPs synthesis such as chemical, electro and photochemical reduction, sonoelectrochemical, heat evaporation microwave assisted process, etc. [6-8]. However, significant increase of hazardous by-products [9], low material conversions and high energy requirements, led to difficulty in nanoparticles synthesis, which was a severe limitation for the application of Ag-NPs. Therefore, it was significant to synthesize Ag-NPs with environmental friendly methods.

Plant extract could reduce the metal ion, as it had been exploiting and reducing as a capping agent for the nanoparticles synthesis. Numerous works were reported on the application of plant materials such as *Artocarpus heterophyllus* Lam. Seed [10], *Lippia citriodora* [11], *Artemisia nilagirica* [12], *Ocimum sanctum* stems and roots [13], *Glycyrrhizaglabra* root [14], *Morindacitrifolia* root [15], *Delphinium denudatum* root [16], *Parthenium hysterophorus* root [17], *Coleus forskohli* root [18], and other extracts such as *Mango peel* [19], *Olea europaea* [20], *Abelmoschus esculentus* [21] in Ag-NPs synthesis due to their environmental, economical and antioxidant properties. It replaced the use of potentially hazardous chemicals.

Justicia Adhatoda is one of the most important, easily available, highly valuable ayurvedic medicinal plants used to treat cold, cough, asthma, bronchitis and tuberculosis [22]. These properties were tempted to use it in the synthesis of Ag-NPs [23].

The studies on the plant mediated biosynthesis of Ag-NPs using *Justicia Adhatoda* root extract as reducing and stabilizing agent, which were characterized using UV-vis spectroscopy, XRD, TEM, AFM, FTIR and cyclic voltammetry was reported. This work was also involved to evaluate antioxidant activity of AgNPs against 1,1-diphenyl-2-picryl-hydrazyl and antibacterial activity against bacteria.

EXPERIMENTAL

Preparation of Justicia Adhatoda root extract

Justicia Adhatoda root was properly washed, cut into fine pieces and dried. A 10 g of the root was boiled in 100 ml of distilled water at 80 °C

for 30 min. The root extract was cooled and filtered using Whatman No. 1 filter paper with pore size of 11 µm to obtain the pure root extract.

Synthesis of silver nanoparticles

A 10 ml of the root extract was added to 100 ml of 1 mM silver nitrate solution. This reaction mixture was incubated at 60 °C for 20 min. Silver nanoparticles were cooled to room temperature and collected by centrifugation.

Characterization

The formation of Ag-NPs was monitored by Jasco V-530 UV-Vis double beam spectrophotometer. X-ray diffraction measurement was carried out using a Panalytical X'Pert Powder X'Cellerator Diffractometer with Cu K α radiation, $\lambda = 1.54 \text{ \AA}$. The scanning was done between 10° and 80°. The size and shape of the Ag-NPs were characterized by Transmission Electron Microscopy (TEM) (Philips-CM200). The samples for TEM analysis were obtained by placing a drop of particle solution on a copper grid and allowing it to evaporate at room temperature. The shape and morphology of the Ag-NPs were studied using Atomic Force Microscope (AFM) using tapping mode. Sampling for AFM was done by coating method using a drop of aqueous solution of nanoparticles on a glass slide and scanned using Nanosurf Easysurf2. The functional groups of the Ag-NPs in the form of powder were analyzed using FTIR spectral measurements. The measurements were carried out on a Thermo Scientific Nicolet iS5 instrument in the diffuse reflectance mode at a resolution of 4 cm⁻¹ in KBr pellets. Root extract powder was used as controlling agent. Cyclic voltammetric measurements were made in distilled water using CHI 650C electrochemical workstation. A three electrode cell included reference Ag/AgCl, auxiliary Pt and working glassy carbon electrodes.

Determination of antioxidant activity

The free radical scavenging capacity of the plant extract and phytoconstituents capped Ag-NPs were determined by using DPPH assay [24]. DPPH (1, 1-diphenyl-2-picryl-hydrazyl) was a stable free radical and had been used as a model free radical compound to evaluate the effectiveness of antioxidants. A 1ml of 0.1 mM DPPH in ethanol was prepared. To that solution, plant extract or Ag-

NPs (varying concentrations from 50-250 $\mu\text{g/ml}$), 1 ml ethanol and 1 ml Tris HCl were added. The mixture was left for 30 min to measure the absorbance at 517 nm. The percentage of scavenging of DPPH free radical was measured using equation 1.

$$\% \text{ DPPH radical scavenging} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \quad (1)$$

Where A_{blank} was the absorbance of control reaction solution (containing all reagents except test compound), A_{sample} was the absorbance of the test compound.

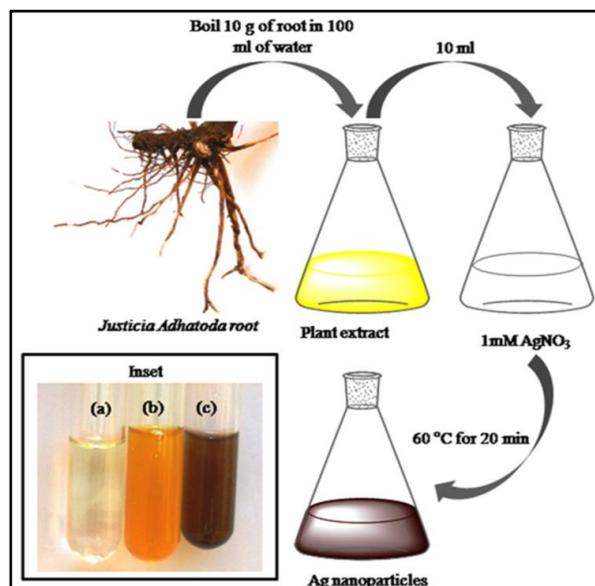
Antibacterial study

The biosynthesized Ag-NPs were used as antibacterial agents. The antibacterial activity was examined against bacterial cultures (*Salmonella paratyphi* and *Pseudomonas aeruginosa*,) using standard zone of inhibition (ZOI) microbiology assay. A 100 μl of microbial culture was spread on sterile nutrient agar plate. Sterile disc of 6 mm diameter was placed on the plate. The different discs were impregnated with 10 μl of Tetracycline as reference, water as control, root extract and Ag-NPs as samples. Then, the plates were sealed and incubated at 37 $^{\circ}\text{C}$ for 24 h. After the incubation period, the diameter of the inhibition zone around the plant extract or Ag-NPs saturated discs had been measured and was compared with the diameter of inhibition zone of commercial standard antibiotic discs.

RESULTS AND DISCUSSION

Synthesis

The reduction of aqueous solution of silver nitrate is one of the most widely used methods for the synthesis of Ag-NPs in colloidal form. From the [scheme 1](#), while silver nitrate solution (Inset a) was added to the yellow colour solution of *Justicia Adhatoda* root extract (Inset b), Ag-NPs were formed. The colourless solution turned brown (Inset C), indicating the reduction of silver ions. The reaction was completed within 20 min and centrifuged for several times at 10,000 rpm for 20 min.



Sche. 1. *Justicia Adhatoda* root extract reduces the silver ions and stabilizes the silver nanoparticles.

UV-Visible Spectroscopy analysis

UV-Vis spectroscopy is an important technique to determine the formation and stability of metal nanoparticles in aqueous solution. UV-Vis spectra of band peaks produced by root extract and Ag-NPs are shown in [Figure 1](#).

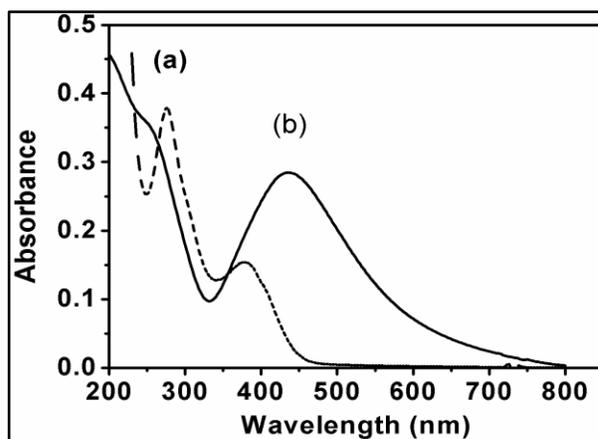


Fig. 1. UV- Vis. spectra of (a) *Justicia Adhatoda* root extract and (b) biosynthesized silver nanoparticles.

The electronic spectrum of the root extract showed a sharp band at 380 nm for $\pi\text{-}\pi^*$ transition and a peak at 265 nm for $\sigma\text{-}\pi^*$ transition ([Figure 1a](#)). The brown colour of Ag-NPs was

attributed to surface plasmon resonance. It occurred due to the interaction of electromagnetic radiation and the electron in the conduction band around the nanoparticles. The spectrum had been observed strongly in the range of 400-450 nm in visible region [25, 26]. In the present work, formation of the Ag-NPs was evidenced from the appearance of brown colour during peak observation at 430 nm (Figure 1b).

X-ray diffraction analysis

The XRD pattern of the AgNPs (Figure 2) showed diffraction peaks at $2\theta = 38.09, 44.27, 64.50$ and 77.50 which could be indexed to (111), (200), (220) and (311) planes of silver with a face-centred cubic (FCC) crystal structure. The well resolved and intense XRD pattern clearly showed that the silver nanoparticles are crystalline in nature. The diffraction pattern of the synthesized Ag-NPs was consistent with other literature reports [27-30]. This indicated that the major composition of the nanoparticles is of silver. The other additional unassigned peaks are also observed and this might be due to the formation of the crystalline metalloprotein that could be present in the plant extract [31]. Using, Debye-Scherrer equation, the average particle size of the Ag-NPs was calculated and found to be 25 nm.

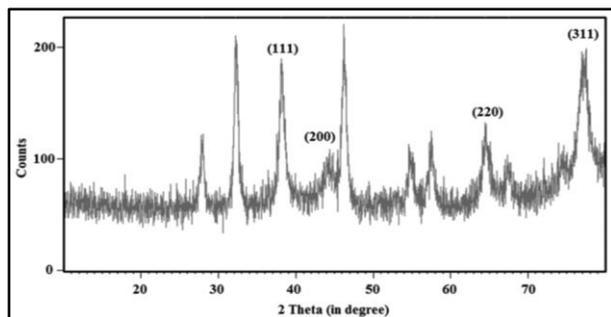


Fig. 2. XRD patterns of silver nanoparticles synthesized by *Justicia Adhatoda* root extract.

Transmission electron microscope and EDAX studies

The purpose of TEM in nanoscience and technology is significant to see the particles in nanoscale. Typical images of the sample observed under TEM are shown in Figure 3a-c. As is clear from the micrograph, formation of well defined spherical particles was observed. The size of the

nanoparticles was found to be 5 to 45 nm. The average size of the particles calculated from the image was found to be 25 nm. From the images (indicated by arrow), it could be confirmed that Phytoconstituents layer of *Justicia Adhatoda* plant stabilized the Ag-NPs, and the nanoparticles showed a very good dispersion inside the bio-reduced aqueous solution. The crystallinity of the Ag-NPs was detected by selected area diffraction (SAD) experiments. The appearance of discrete spots in the ring pattern revealed that the Ag-NPs are crystalline in nature (Figure 3d).

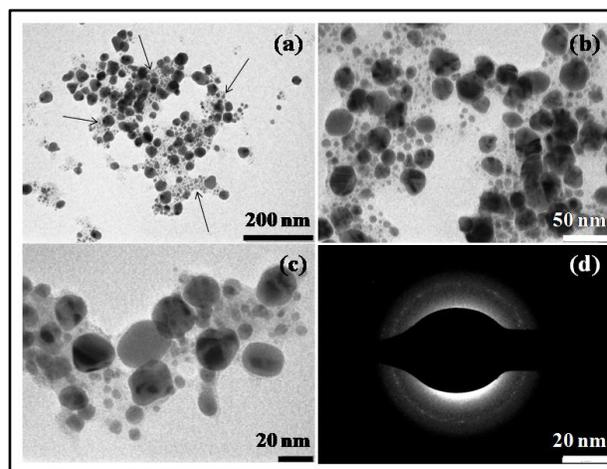


Fig. 3. TEM images with different scales (a) 200 nm; (b) 50 nm; (c) 20 nm; (d) SAD image of biosynthesized silver nanoparticles. The existence of a thin phytoconstituent layer surrounding the Ag aggregates is shown with arrows in the image.

EDAX analysis gives qualitative as well as the quantitative status of the elements involved in the formation of nanoparticles. The result of EDAX offered a clear idea about the elements present in the nanoparticles (Figure 4). The strong signal of the silver atoms was obtained at 3 keV and some peaks for C, O and Cu were also found. The emission energy at 3 keV indicated the reduction of silver ions to element of silver [32, 33]. The presence of oxygen peaks along with the silver signals confirmed that the plant constituents especially phenol through oxygen atom are essential for the stabilization of Ag-NPs. The C and Cu peak occurred from the TEM grid.

AFM studies

Atomic Force Microscopy was used to determine the topography of Ag-NPs. The surface

morphology of the nanoparticles was predicted through 2D image scanning in an area of 853×853 nm (Figure 5a). The corresponding 3D image has been shown in Figure 5b displaying spherical shape of nanoparticles with smooth surface, without any cracks.

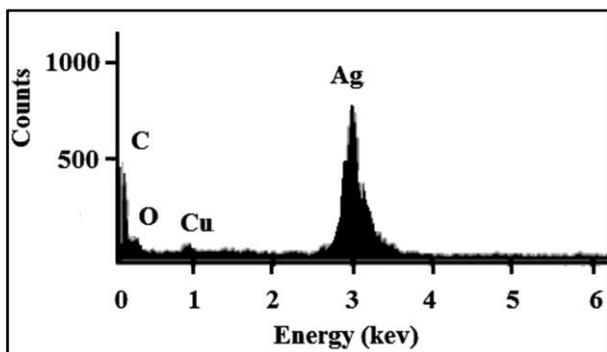


Fig. 4. EDAX pattern of biosynthesized silver nanoparticles.

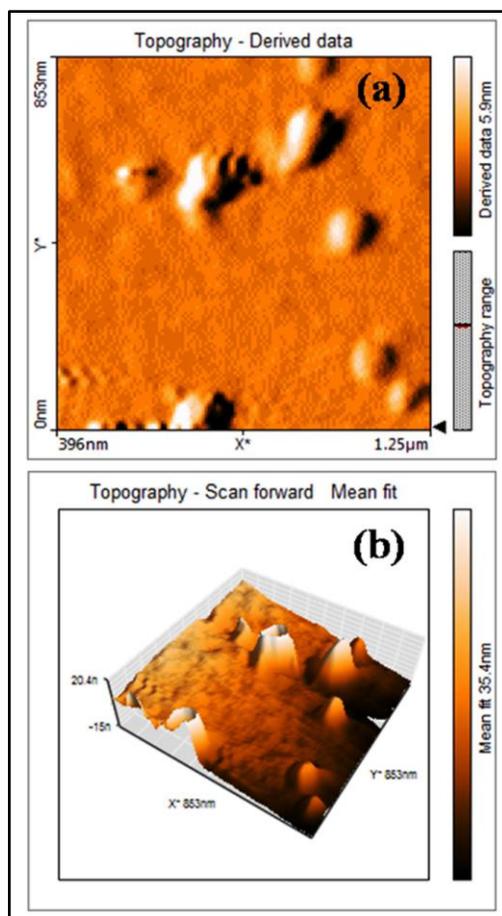


Fig. 5. AFM (a) 2D and (b) 3D images of silver nanoparticles synthesized by *Justicia Adhatoda* root extract.

FT-IR analysis

Fourier transform infrared analysis was performed to identify the possible biomolecules responsible for the reduction of the silver ions and capping of the Ag-NPs. FT-IR spectra of dried aqueous extract and synthesized Ag-NPs are shown in Figure 6. Presence of several absorption bands in the region of 3454 , 2922 , 2852 , 1614 , 1384 and 1078 cm^{-1} indicated the presence of active functional groups in the extract and in the Ag-NPs. The main phytoconstituents present in the *adhatoda* plant are vasicine, vasicinone, vasicol, vasicinol, vasicinolone, etc [34]. A characteristic band of $-\text{NH}$ and $-\text{OH}$ groups of root extract appeared at 3411 cm^{-1} , as shown in the IR spectra (Figure 6a). The observation of bands at 2922 and 2852 cm^{-1} are due to the aliphatic C-H stretching frequencies. The presence of carbonyl group and amine of the extract showed the stretching band at 1614 cm^{-1} . The IR band observed at 1384 cm^{-1} is ascribed to aromatic C-H group/heteroatom containing C-C group. The band at 1078 cm^{-1} indicated the presence of $-\text{C}-\text{O}-\text{C}$ stretching vibration. The absorption bands appeared in the IR spectrum of aqueous extract could also be seen in the IR spectra of phytocapped Ag-NPs (Figure 6b).

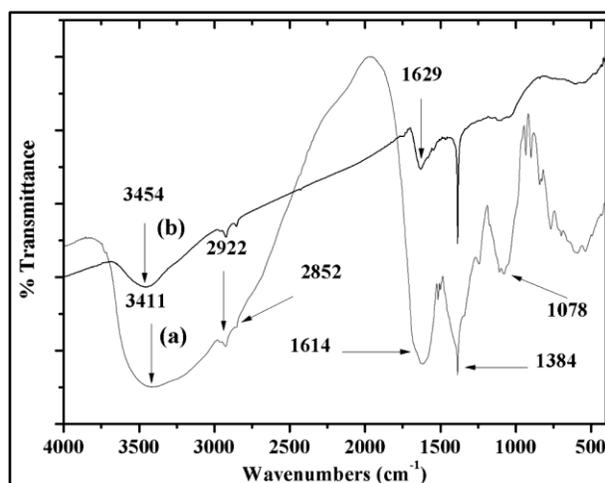


Fig. 6. FT-IR spectra of (a) aqueous root extract of *Justicia Adhatoda* and (b) phytoconstituents capped silver nanoparticles.

The peak of OH functional group shifted from 3411 to 3454 cm^{-1} indicating the involvement of OH group in the reduction of silver ions and the capping of Ag-NPs. The vibration band of carbonyl

group shifted from 1614 to 1629 cm^{-1} indicating the participation of carbonyl group of the carboxylic acid in the capping of Ag-NPs. Hence, it is confirmed that the phenolic group, carboxylic acid group, nitrogen group etc. are present in the root extract of *Justicia Adhatoda*. The phyto compounds were responsible for the reduction of silver ions and capping Ag-NPs.

Electrochemical behaviour

The cyclic voltammograms of the plant extract and the biosynthesized Ag-NPs were recorded from -0.1 to 0.1 V in aqueous solution at 50 mV s^{-1} on GC electrode. The plant extract did not show any redox peaks (Figure 7a) whereas Ag-NPs showed an irreversible oxidation peak (Figure 7b) at 0.438 V vs Ag/AgCl, and it can be deduced to the reaction,



Electrochemical behaviour for Ag-NPs was in agreement with previous literature [35]. The oxidation peak current intensity increased on higher scan rates (Figure 8) and the anodic peak current was found to be proportional to the square root of the scan rates indicating a diffusion controlled process, which was confirmed by the observed linear plot of square root of the scan rate vs anodic peak current [Figure 8 inset].

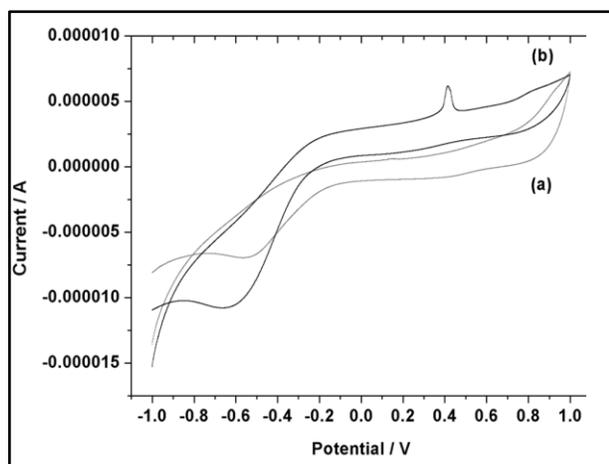


Fig. 7. Cyclic voltammogram of (a) *Justicia Adhatoda* root extract (1 mg / 1 ml); (b) biosynthesized silver nanoparticles (1 mg / 1 ml) in aqueous solution vs Ag/AgCl with TBAP as supporting electrolyte at 50 mVs^{-1} .

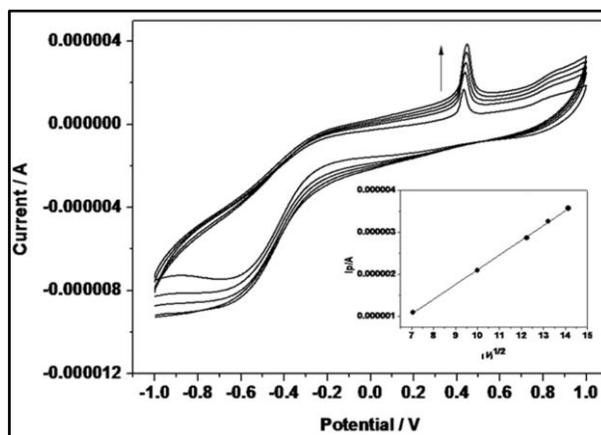


Fig. 8. Cyclic voltammogram of biosynthesized silver nanoparticles in aqueous solution vs Ag/AgCl with TBAP as supporting electrolyte at 50, 100, 125, 150 and 200 mVs^{-1} . Inset; plot of square root of scan rate versus current in A.

Mechanism of reduction of silver ions to Ag-NPs

The present study exhibits the mechanism involved in the reduction of silver nitrate to Ag-NPs by the phytochemical constituents of *Justicia Adhatoda*. Phenolic compounds were known to act as antioxidants [36, 37] not only because of their ability to donate hydrogen atoms or electrons but also because of their stable radical intermediates which prevent the oxidation of various food ingredients particularly fatty acids and oils. These antioxidative compounds delayed or inhibited the oxidation of molecules by inhibiting the initiation or propagation of oxidative chain reaction. The antioxidative activity of phenolic compounds is mainly due to their electrochemical property, which played an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [38]. Phenolic center of vasicol, vasicinolone and vasicinol readily form a phenoxy radical accounted for its potent antioxidant property. Thus it is possible that those phenolic compounds from *Justicia Adhatoda* root were oxidized by silver nitrate and reduced the silver ions to Ag-NPs (Figure 9). The mechanism was confirmed, supported and consistent with UV-Vis spectroscopy, TEM, XRD and FT-IR analysis.

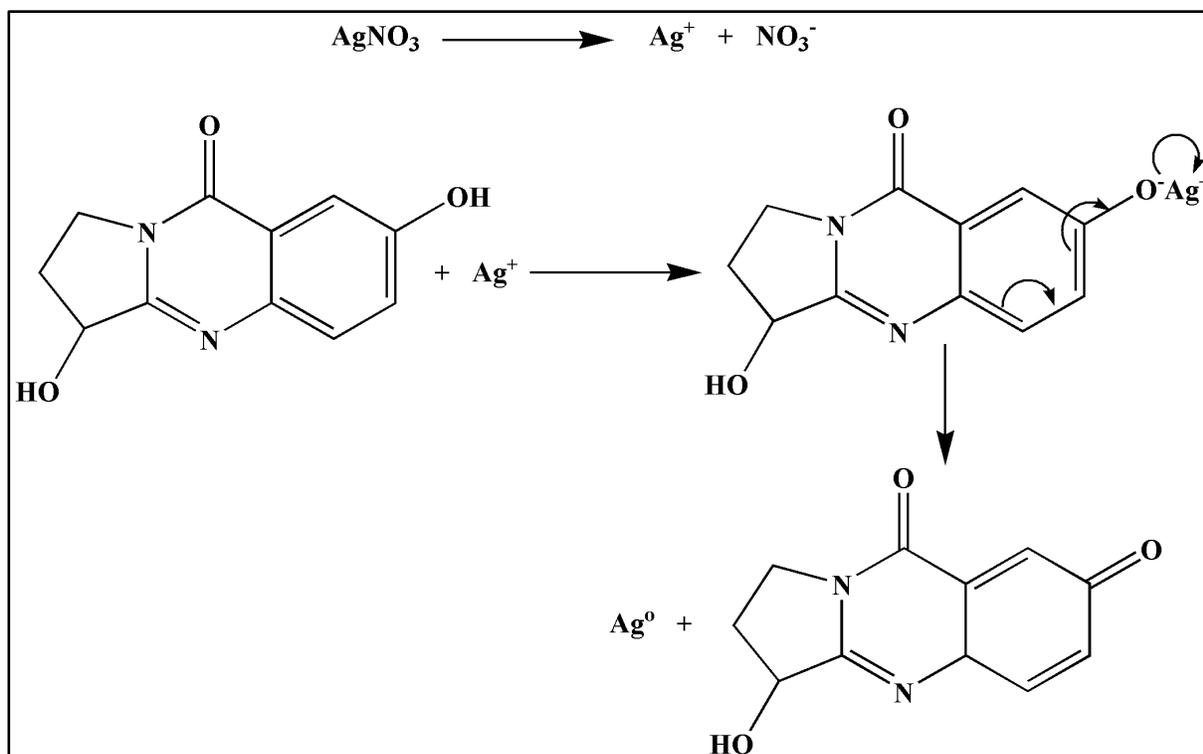


Fig. 9. Possible mechanism of reaction for the formation of silver nanoparticles by vasicinolone.

DPPH free radical scavenging activity

Further, the mechanism was also confirmed by the free radical scavenging activity. The free radical scavenging activity of DPPH resulted in a decrease in absorbance at 517 nm. As DPPH was considered as a lipophilic radical, which readily accepted electron from the antioxidant and converted its colour from violet to yellow, that was detected at 517 nm. Thus, DPPH was a stable free radical and accepted an electron or hydrogen radical. The absorbance of the DPPH solution (*i.e.* the blank) was 0.504. The antioxidant activities were measured in triplicate after 10 min and were given in Table 1. The decrease of absorption at 517 nm represented that the root extract and Ag-NPs had hydrogen donating ability or could scavenge free radicals. Free radical scavenging activity of the plant extract and the Ag-NPs on DPPH radicals were found to increase with increase in their concentrations. This is because, at higher concentration of the plant extract or Ag-NPs, numerous phenoxy compounds or Ag-NPs were available for scavenging the free radicals. Phytoconstituents capped Ag-NPs showed higher values of antioxidant activity than plant extract

alone as Ag-NPs were also able to provide an electron to the DPPH.

Table 1. Absorbance values from scavenging effect of test samples on DPPH at 517 nm.

Concentration (µg/ml)	% of free radical scavenging activity	
	Root extract	Silver nanoparticles
50	12.35	13.48
100	23.36	24.75
150	38.15	40.24
200	62.5	64.35
250	76.24	79.56

Antibacterial activity

The antibacterial activity of the Ag-NPs was tested against *Salmonella paratyphi* and *Pseudomonas aeruginosa* in nutrient agar plates by the agar diffusion method. The antibacterial effect

of Tetracycline against *Salmonella paratyphi* displayed 27mm inhibition zone (Figure 10a). The Ag-NPs with 10 μ L per disc against *Salmonella paratyphi* showed 15mm inhibition zone (Figure 10b) and at the same concentration, *Pseudomonas aeruginosa* showed 10mm inhibition zone (Figure 10c).

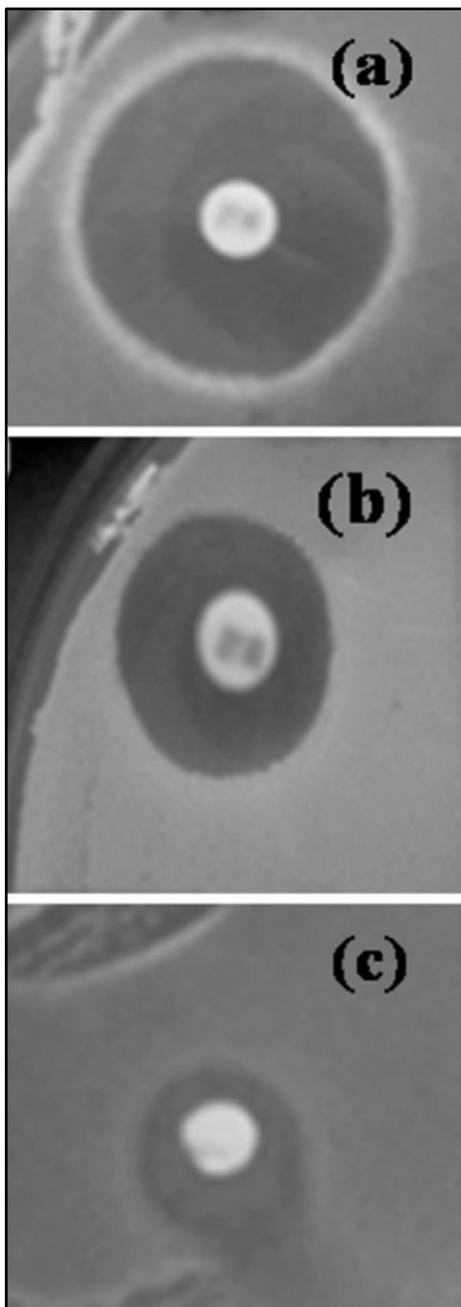


Fig. 10. Antibacterial activity of (a) Tetracycline against *Salmonella paratyphi*; (b) silver nanoparticles against *Salmonella paratyphi* and (c) silver nanoparticles against *Pseudomonas aeruginosa*.

No bacterial effect was found in the plant extract [23]. The zone inhibition showed that the Ag-NPs were attached with cytoplasmic membrane which killed the bacterial cell. Electrostatic attraction of Ag-NPs with the membrane caused the damage of bacterial cell membrane to the formation of pits on the surface and these structural changes took place due to cell expiration [39]. Mesosome cell organelles of the prokaryotic bacterial were present inside the plasma membrane. The Ag-NPs interacted with the bacterial cell membrane; bound with mesosome cell organelle reduced the mesosomal function. The Ag-NPs noticeably interacted with the membrane of bacterial and disordered the membrane integrity. The silver ions bound to sulphur, oxygen, and nitrogen of essential biological molecules also inhibited bacterial growth [40].

CONCLUSIONS

Ag-NPs with an average size of 25 nm were prepared. *Justicia Adhatoda* root extract performed as reducing as well as capping agent. The XRD, TEM, AFM and EDAX techniques confirmed the structure, composition, average particles size, and surface morphology of Ag-NPs. The phytoconstituents and their functional groups were authenticated by FT-IR analysis. The electrochemical study showed the oxidation peak of Ag-NPs is 0.438 V. Both root extract and Ag-NPs showed good antioxidant activity. Ag-NPs at concentration of 10 μ L showed the good antibacterial activity against *Pseudomonas aeruginosa* and *Salmonella paratyphi*. Biosynthesis of Ag-NPs had many advantages such as, rapid, facile, cost effective, eco-friendly, alternative for hazardous reducing agent and pledging for applications in medicine and large scale production of metallic nanoparticles. Further evaluation is needed so as to incorporate this *Justicia Adhatoda* mediated Ag-NPs for manufacturing drugs and also to vastly use it in food systems for its known beneficial effects.

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