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Phytosynthesis and Characterisation of Silver Nanoparticles Synthesised from Flower Extract of Roheda (*Tecomella undulata* G. Don)

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ABSTRACT

Green synthesis of silver nanoparticles was carried out using *Tecomella* undulat flower extract with 0.1mM silver nitrate solution. Within a few minutes colour change and variation of pH value could be observed. Synthesised nanoparticles were characterised through UV-Vis spectra, particle size analysis, zeta potential measurement, X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX) analysis and Atomic force microscopy (AFM). The UV-Vis absorption spectrum shows an absorption band near 450 nm, which is characteristic absorption peak of silver nanoparticle. The particle size distribution result showed two peaks at 5.23 nm (20.3 per cent) and 77.82 nm (79.7 per cent). XRD characterisation showed that these are highly crystalline silver nanoparticles having average size 12.5 nm. FTIR spectrum was recorded to identify the biomolecules involved in the synthesis process, which showed absorption band stretches at 2939.52 cm^{-1}, 2877.79 cm^{-1}, 2362.80 cm^{-1}, 1228.66 cm^{-1}, 1157.29 cm^{-1}, 1014.56 cm^{-1}, 831.32 $cm^{-1}$and 785.03 cm^{-1}. SEM image showed that particles were spherical in nature. The presence of Ag was confirmed by major peak of Ag in EDX spectrum. 2D and 3D images of silver nanoparticles were obtained by AFM and biogenic nanoparticles were measured in the size range between 20 nm - 70 nm. Various types of spectroscopic and microscopic characterisation indicated that these are stable silver nanoparticles synthesised from flower extract of *Tecomella undulata*.

Keywords: Phytosynthesis; *Tecomella* flower extract; Silver nanoparticles; Characterisation

1. INTRODUCTION

Silver nanoparticles (AgNPs) are particles of silver which are ranges between 1 nm to 100 nm. Nanostructure materials indicate unique physicochemical and biological, environmental, ecological, magnetic, catalytic activity and biological properties[1]. Recently its applications increased in medicine, agriculture, forestry and industry. AgNPs have high potential as commercial nanomaterials and an effective antimicrobial agent. Silver nanoparticles are prepared using different physical and chemical methods. But majority of these techniques are both expensive environmental hazardous[2]. Furthermore, synthesised nanoparticles may be unstable and tend to agglomerate rapidly and become useless unless capping agents are applied for stabilisation. Different shapes and sizes of nanoparticles are produced through UV irradiation[3], microwave irradiation[4], chemical reduction[5], electron irradiation[6], photochemical[7] and lithography methods[8]. However most of the methods involve more than one step, requirement of high energy, difficulty in purification and presence of hazardous chemicals[9].

Biogenic approaches of nanoparticle synthesis have received greater attention over physical and chemical synthesis, as it is non-toxic, non-hazardous, cost effective and eco-friendly[10,11]. Biosynthesis of silver nanoparticles has advantages like slower kinetics, better manipulation and control over crystal growth and having excellent stability[12]. Microbial mediated AgNPs synthesis is not industrially achievable as they need high maintenance of aseptic conditions and also time consuming. Therefore, the use of plant extract for synthesis of AgNPs is comparatively advantageous over other methods[13] due to slower kinetics and they offer better manipulation on control over crystal growth and their stabilisation. This has motivated an increase in research on the synthesis routes that also better control of size and shape for wide varieties of nanotechnological applications. Reports are very much scanty for the synthesis silver nanoparticles using flower extract. Recent reports indicated that marigold flower[14], *Mimusops elengi*[15] and *Lantana camara*[16] flower extracts have potential for the synthesis silver nanoparticles.

Tecomella undulata G. Don belongs to the family Bignoniaceae, locally known as Roheda in Rajasthan and is used in traditional medication system. It is widely distributed in Deserts of Rajasthan, India. In indigenous system of medicine, it is used against spleen, liver and abdominal complaints. The plant possesses many pharmacological activities such as anti-inflammatory, analgesic potential, antibacterial, antioxidant and hepatoprotective activity against thioacetamide induced hepatotoxicity. The phytochemical studies reported that the plant contains flavonoids, iridoidglucosides, alkaloids, saponins

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and it also contains anti-HIV agents[17]. Roheda flower extracted alkaloids are very useful pharmaceutical agents because of their biological activities such as antimicrobial, antioxidant, analgesic potential and anti-inflammatory activities[18].

In the present work, an attempt has been made to synthesize silver nanoparticles using aqueous flower extract of *Tecomella undulata*. We reported earlier that the leaf extract of *Tecomella undulat* has potential to synthesize highly stable monodisperse silver nanoparticles[19]. The flowers of *Tecomella* are generally wastage in the field. The flowers of *Tecomella* are easily available in desert area of Rajasthan. Flowers of this medicinally important and endemic Thar Desert plant of Rajasthan can be effectively used as a novel bio resource for the biosynthesis of silver nanoparticles.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh flowers of Roheda (*Tecomella undulata*) plants available in Defence Laboratory, Jodhpur campus (26°16'N 73°02'E), Jodhpur, India were collected in polythene packet.

Figure 1. Flowers of *Tecomella undulata.*

2.2 Preparation of Flower Extract

Fresh flowers thoroughly washed in tap water followed by Milli-Q water to remove any dust particles, dried with water absorbent paper and chopped into small pieces with scissor and placed in laboratory oven at 50 °C for three days. Then dried flowers pieces were blended in mixer grinder to fine powder and stored in air tight bottle for further use. 10 gm flower powder was mixed with 100 ml of sterile Milli-Q water in a 500 ml Erlenmeyer flask and placed in orbital shaker incubator at 60 °C with constant shaking at 120 rpm for 10 min. After cooling at room temperature and filtering through Whatman No.1 paper the aqueous flower extract was stored in refrigerator (4-7 °C) for further experiments. All the glass goods used were cleaned in chromic acid and autoclaved.

2.3 Synthesis of Silver Nanoparticles

The AR grade silver nitrate ($AgNO_3$) was procured from Merck and flower extract used for the reduction of Ag+ ions to Ag^0 at 60 °C in a constant stirring on magnetic stirrer fixed in laboratory incubator. In a typical experiment, 5 ml of aqueous

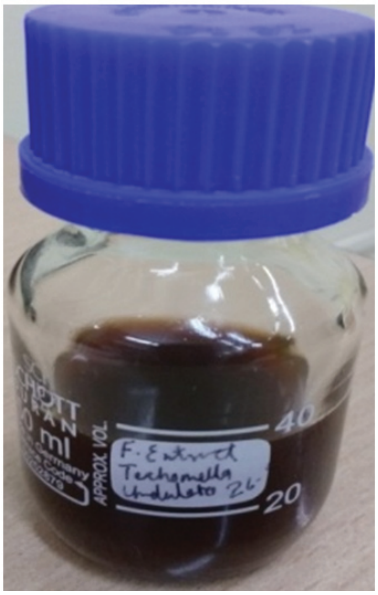


Figure 2. Aqueous extract of dried *Tecomella* flower.

flower extract was added to 500 ml of final concentration of 0.1 mM$AgNO_3$ dissolved in sterile Milli-Q water.

2.4 Characterisation of Silver Nanoparticles

2.4.1 pH Measurement

Silver nitrate aqueous solutions (0.1 mM) showed pH 6.03.Aqueous flower extract pH was 5.49. The changed pH of reaction mixtures were recorded using digital pH meter (Eutech Cybersacn pH 300), during synthesis of silver nanoparticles.

2.4.2 UV-Vis Spectroscopic Studies

The reduction in silver ion was monitored by measuring the UV-Vis spectroscopy of the reaction medium after diluting small aliquot of the reaction mixture 10 times diluted with Milli-Q water to avoid the error due to high optical density of reaction mixture at different intervals after 10 min. UV-Vis spectral analysis was done by using UV-Vis-NIR spectrophotometer (Ocean Optics, USA).

2.4.3 Particle Size Distribution and Zeta Potential Measurement

Particle size distribution and average size of silver nanoparticles was obtained through particle size analyser (Malvern zetasizer, Nano Z500, UK). The sample holder temperature was maintained at 25 °C. The measurements depend on the size particle core, the size of surface structure, particle concentration and the type of ion in the mixture. The zeta potential of the synthesised silver nanoparticles was determined in water as dispersant.

2.4.4 Scanning Electron Microscopy Analysis

Scanning electron microscopy (SEM) analysis was carried out using ZISS model machine. Thin film of the sample was prepared on carbon coated tape by adhering small amount of dried fine powder of the sample on the grid, the extra sample was removed with the help of blotting paper and the film on the SEM grid was allowed to dry by putting it under a mercury

lamp for 5 min. The SEM analysis was used to determine the surface structure of the reaction products during biosynthesis of silver nanoparticles.

2.4.5 Energy Dispersive X- ray Spectroscopic Analysis

EDX analysis was carried out to determine the chemical purity, elemental composition and stoichiometry of the synthesised silver nanoparticles.

2.4.6 Atomic Force Microscopy Analysis

The atomic force microscopy (AFM) is one of the foremost tools for imaging, measuring and manipulating material at nanoscale. It offers a capability of dimensional visualisation and both qualitative and quantitative information on many physical properties including size, morphology, surface texture and roughness. The liquid sample having silver nanoparticle was spread on mica sheet, dried at 35 °C incubator and scanned with semi contact mode with close loop 3 x 3 µm scanner (Solver Model, NTMDT, Russia).

2.4.7 XRD Analysis

The formation and quality of compounds were investigated by X-ray diffraction technique. For this purpose synthesised AgNPs were centrifuged (14000 rpm; at 8 °C) for 10 min, pellet was washed three times with ethanol and finally washed with sterile Milli-Q water for three cycles. The purified AgNPs precipitate was dried in oven at 60 °C and subjected to XRD analysis. The scanning was done in the region of 2θ from 20° to 80°.

2.4.8 FTIR Analysis

FTIR was used to identify the possible functional groups responsible for the reduction of Ag ion and capping of the bio-reduced silver nanoparticles. FTIR spectra were recorded using Shimadzu, Japan IR double beam spectrophotometer. FTIR analysis of the dried flower extract and flower extract mediated synthesised AgNPs powders were carried out through the potassium bromide (KBr) pellet method in 1:30 ratio (NPs:KBr) and spectra was recorded in transmittance mode operating at a resolution of 4 cm^{-1}. The peaks (stretching) obtained were plotted as % transmittance in Y axis and wave number (cm^{-1}) in X axis. The spectra were recorded in the wave number range of 500 – 4000cm^{-1} and analysed by subtracting the spectrum of pure KBr.

3. RESULTS

3.1 Colour Change

Incubation of reaction mixture in magnetic shaker at 60 °C, a change of colour from pale yellow to reddish brown and finally blackish was observed (Fig. 3) within few minutes. The change of colour indicates that the formation of silver nanoparticles. The intensity of colour gradually increased (Table 1) with increasing time.

Table 1. Colour change of reaction mixture during formation of biogenic silver nanoparticles

Reaction mixture	Before reduction	After reduction	Colour intensity	Time
0.1 mM$AgNO_3$	Transparent	White		
0.1 mM $AgNO_3$+flower extract	White pale yellow		+	0 min
0.1 mM $AgNO_3$ +flower extract	Pale yellow	Deep yellow	+ +	10 min
0.1 mM $AgNO_3$+flower extract	Deep yellow	Deep brown	+++	20 min
0.1 mM $AgNO_3$+flower extract	Brown	Blackish	++++	30 min

(a) (b) (c) (d) (e)

Figure 3. Colour change of the reaction mixture indicating the formation of silver nanoparticles: (a) 0.1 mM $AgNO_3$ solution (white colour), (b) 0.1 mM $AgNO_3$ solution + 5 ml flower extract (pale yellow colour), (c) After 10 min colour changed to deep yellowish colloidal solution, (d) After 20 min colour changed to deep brown, and (e) After 30 min colour changed to blackish colloidal solution indicating that formation of silver nanoparticles.

3.2 Variation in pH during Synthesis of Silver Nanoparticles

The pH of the reaction mixture was decreased from 6.48 to 5.5 in presence of *Tecomella* flower extract indicating that reduction of 0.1 mM $AgNO_3$ during formation of silver nanoparticles (Table 2). Reduction of pH during biogenic synthesis of silver nanoparticles was also reported in presence of jamun (*Syzygium cumini*) leaf extract[20].

Table 2. Reduction of pH during biosynthesis of silver nanoparticles

Solution	Before reduction (pH)	After reduction (pH)
Tecomella flower extract	5.49	
0.1mM$AgNO_3$	6.03	
Flower extract + 0.1mM$AgNO_3$	6.48	5.50

3.3 UV-Vis Spectral Analysis

Tecomella flower extract was able to synthesize the silver nanoparticles by the indication of suitable surface Plasmon resonance (SPR) with peaks near visible spectrum at 450 nm (Figure 4). The absorbance peak near 450 nm, can be attributed to the plasmonic peak of silver nanoparticles, formed in the reaction mixture.

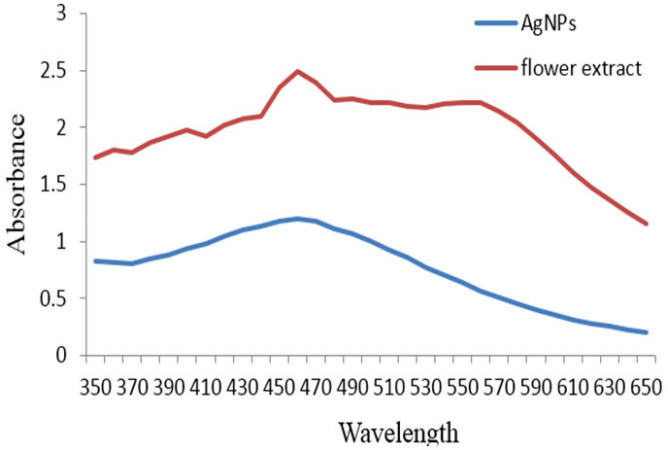


Figure 4. UV-Visible spectrum of biogenic silver nanoparticles.

3.4 Dynamic Light Scattering (DLS) Analysis

Figure 5 showed the DLS pattern of suspension of silver nanoparticles synthesised using *Tecomella* flower extract. The size distribution profile indicates that the size of these silver nanoparticles showed two peaks at 5.23 nm (20%) and 77.82 nm (80%). Polydisperse index (PDI) of silver nanoparticles suspension is 0.332 indicating that synthesised particles are more or less homogeneous.

3.5 Zeta Potential Measurement

Silver nanoparticles synthesised by green method are stable in nature even after three months storage at room temperature as it showed zeta potential – 22.2 mV shown in Fig. 6. The negative zeta potential value indicated that these are highly stable due to the capping of biomolecules present in the flower extract. Measurement of zeta potential is depends

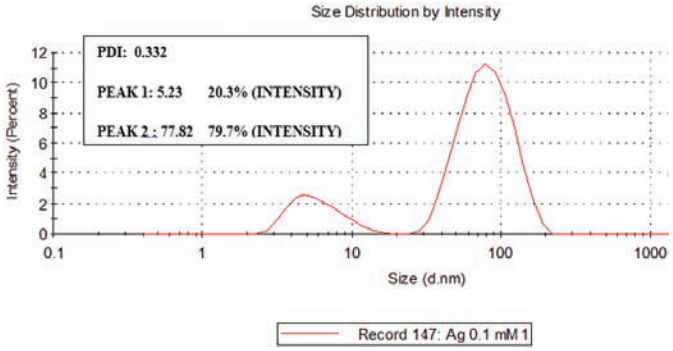


Figure 5. DLS pattern of biogenic silver nanoparticles.

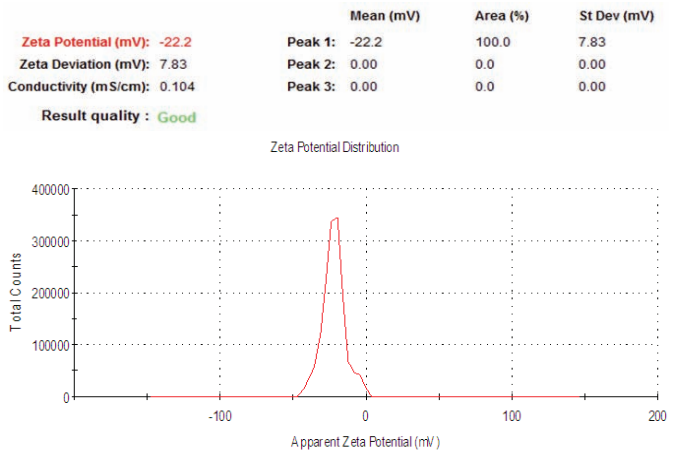


Figure 6. Zeta potential measurement.

on the movement of nanoparticles under influence of an applied electric field. The movement depends upon the surface charge and the local environment of the particle.

3.6 Scanning Electron Microscopy Analysis

SEM analysis showed the image of high density AgNPs synthesised by *Tecomella* flowers extract (Fig. 7). From the given SEM image, it is concluded that the green synthesised silver nanoparticles are spherical in shape and without agglomeration. Formation of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interaction between the biomolecules capping with Ag^0. The nanoparticles were not in direct contact, indicating stabilisation of nanoparticles by capping agent.

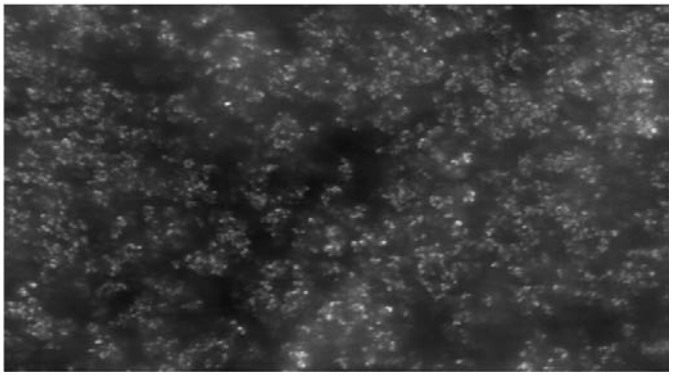


Figure 7. SEM image of biogenic silver nanoparticles.

3.7 Energy Dispersive Spectroscopic Analysis

The EDX analysis was helpful in identifying the elemental composition of the synthesised nanoparticles. The obtained spectrum confirmed the presence of AgNPs (Fig. 8) in which the vertical axis displayed the x-ray counts and the horizontal axis displayed the keV. The identified line for the energy emission for Ag was shown and the peak was matched with the spectrum of Ag and correctly identified. The strong signal of silver has been detected in EDX indicating the purity of synthesised silver nanoparticles and thus giving confidence that silver has been correctly identified. The other signals available in EDX may be coming from the bioactive molecules in the *Tecomella* flowers extract. Metallic silver nanocrystals generally showed typical optical absorption peak at 3 kev due to surface plasmon resonance. A sharp signal was observed at 3 kev also reported that distinctive peak for the absorption of crystalline nature of biogenic AgNPs[21].

3.8 Atomic Force Microscopy Analysis

To validate the surface morphology powder coated AFM images were taken in non- contact mode (Figs. 9(a) and 9(b)). Results showed variability in morphological features of *Tecomella* flower extract mediated silver nanoparticles and sizes varied from 20-70 nm. It is evident from the AFM images that particles are more or less homogeneous in size range and monodisperse nature.

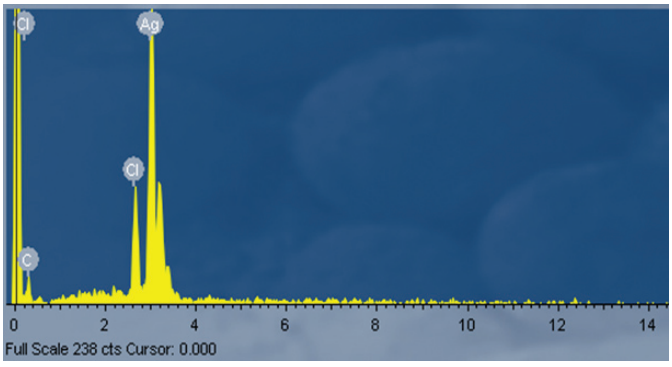


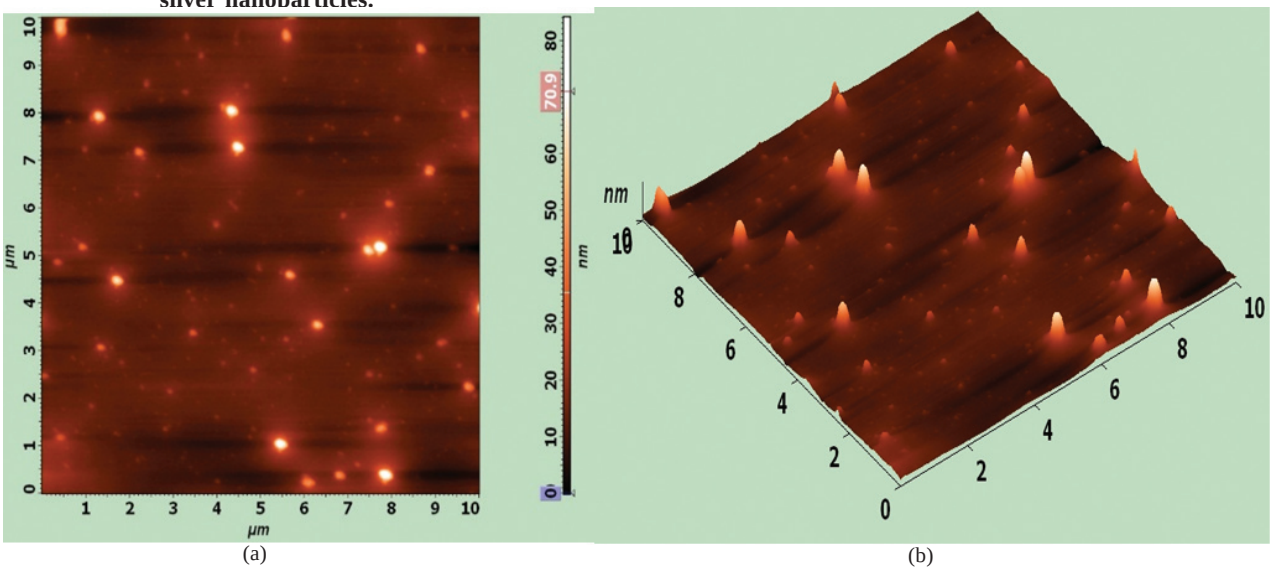
Figure 8. Energy dispersive X-ray spectrum of synthesised silver nanoparticles.

3.9 XRD Analysis

The XRD pattern was used to confirm the crystalline nature of the AgNPs. Figure 10 showed the XRD pattern of the synthesised nanoparticles and main peaks corresponding to 2θ values of 27.80°, 32.27°, 38.20°, 44.02°, 46.57°, 64.52°, and 77.42° that could be indexed to (98), (101), (111), (200), (200), (220), (311) planes respectively. Similar results were obtained in leaf *Clitoria ternatea* extracts mediated AgNPs synthesis, in which 2θ values were obtained at 28.07°, 32.50°, 38.33°, 44.54°, 46.50°, 57.71°, 64.75°, and 77.69°[22]. The average size of nanoparticles determined by XRD was 12.5 nm (using Debye-Scherrer equation).

3.10 FT-IR Analysis

FTIR gives the information about the functional groups present in the synthesised AgNPs for understanding their transformation from simple inorganic $AgNO_3$ to elemental Ag by the action of different phytochemicals present in the flower extracts. In order to determine the functional groups on *Tecomella* flower extract, FTIR analysis was performed. The FTIR spectrum of *Tecomella undulat* flower extract showed absorption band stretches at 3367-3315cm^{-1}, 2927.94cm^{-1}(C-H-Bond), 1647.21cm^{-1}, 1325.10cm^{-1},1236.37cm^{-1}, 1155.36cm^{-1} (C-O-Bond), 1018.41cm^{-1}(C-N-Bond), 916.19cm^{-1} (C-H-Bond), 759.95cm^{-1} (C-H-Bond) and other stretches (Figure 11). The FTIR spectrum of *Tecomella undulata* flower extract mediated silver nanoparticles showed (Fig. 12) absorption bands at 2939.52 cm^{-1}(C-H-Bond), 2877.79 cm^{-1}(C-H-Bond), 2362.8 cm^{-1}, 1157.29 cm^{-1} (C-O-Bond), 1014.56 cm^{-1}(C-N-Bond), 831.32 cm^{-1} (C-H-Bond), and 785.03 cm^{-1} (C-H-Bond).The characteristic of C-H bond, C-O bond and C-N bond stretching vibrations are common in both flower extract and flower extract mediated biogenic synthesised silver nanoparticles indicating that these biomolecules were involved in the reduction and capping of silver nanoparticles. C-H bond stretching mode found in alkane, C-O bond stretching mode of the carbonyl functional groups of esters and alcohols and C-N stretching bands are available in amine stretch of proteins and aminoacids present in flower extract of *Tecomella undulata*. This result



(a) (b)

Figure 9. (a) AFM 2D image of biogenicAgNPs and (b) Same field in 3D image.

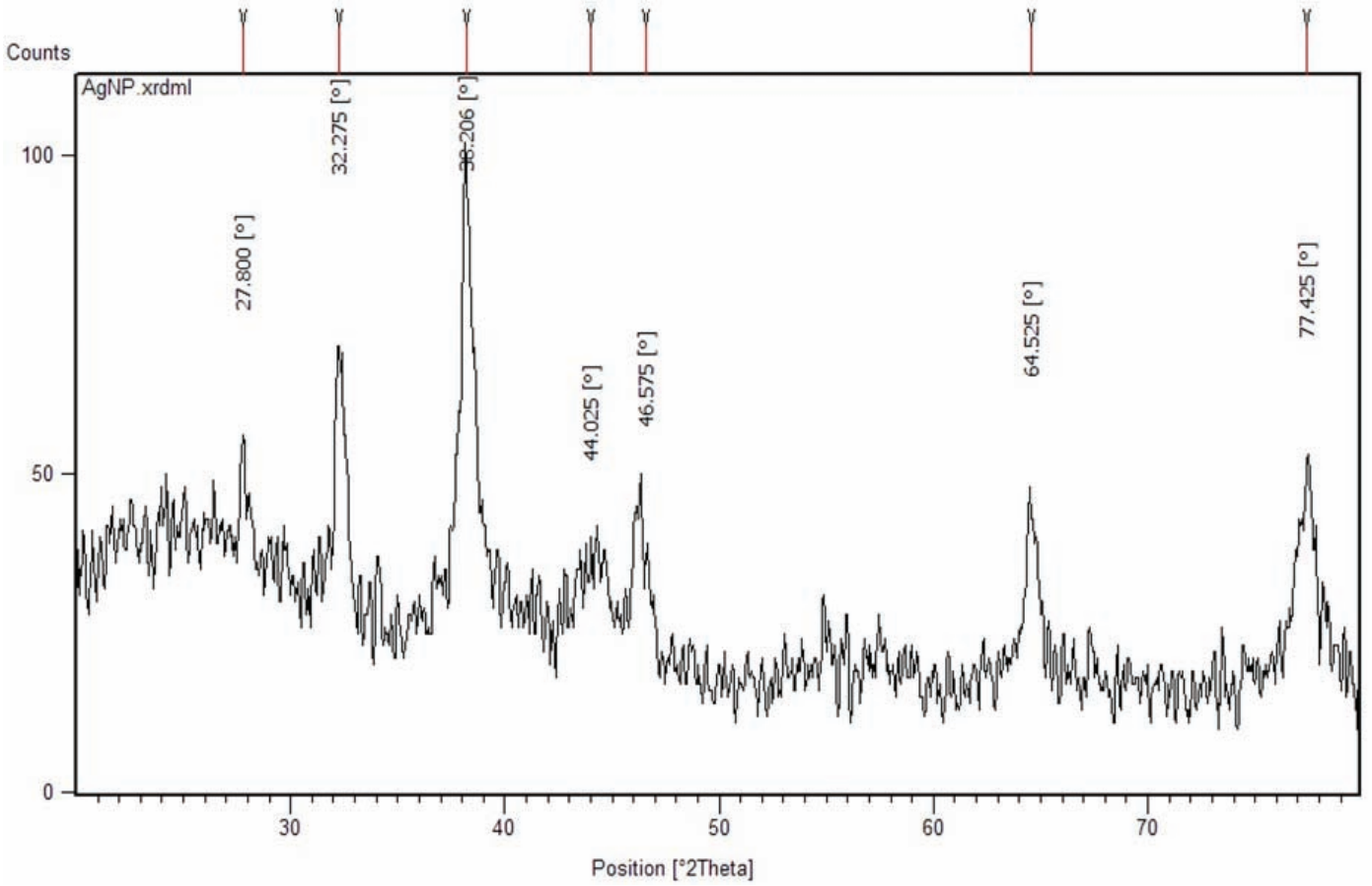


Figure 10. XRD pattern of biogenic (*Tecomella* flowers extract mediated) Ag-NPs.

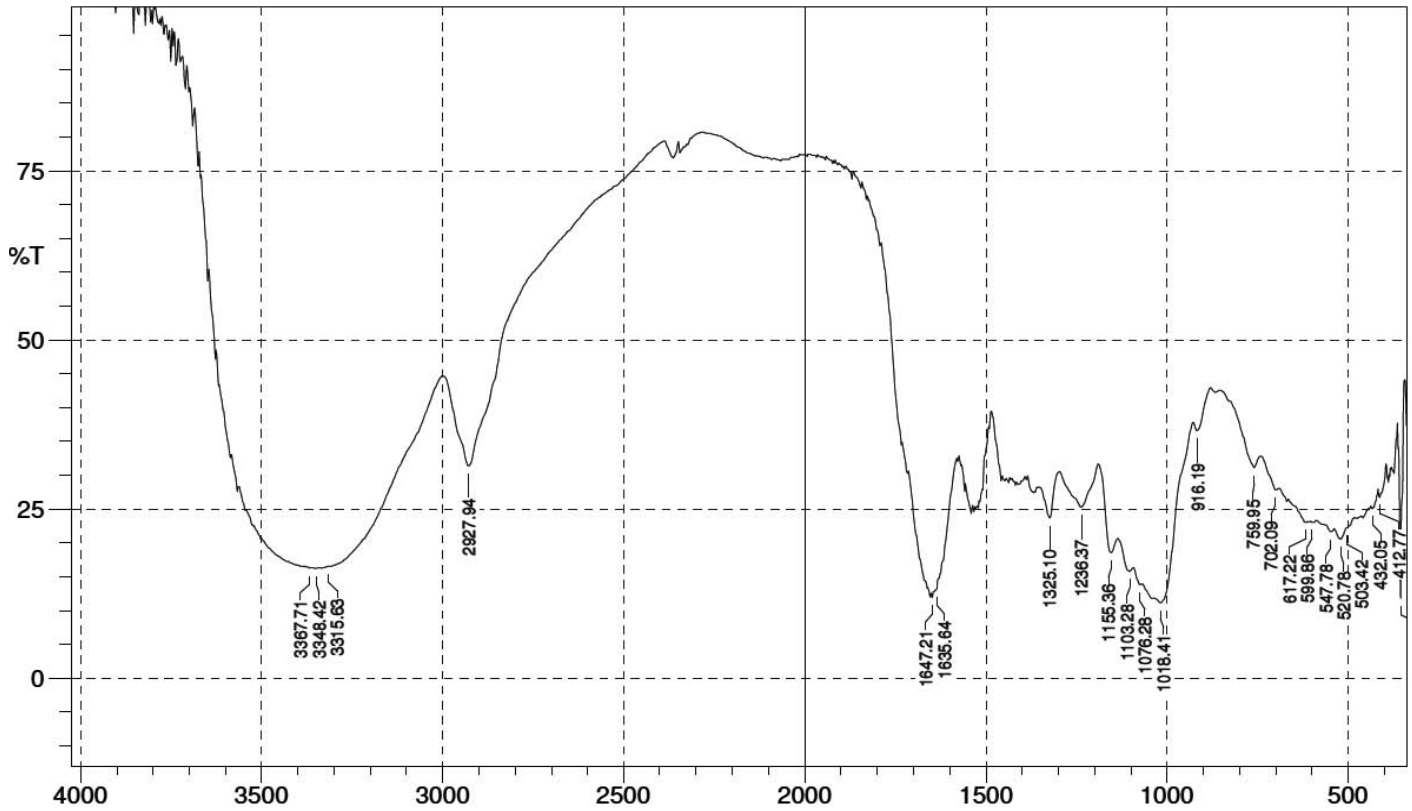


Figure 11. FT-IR spectrum of *Tecomella* flower extract.

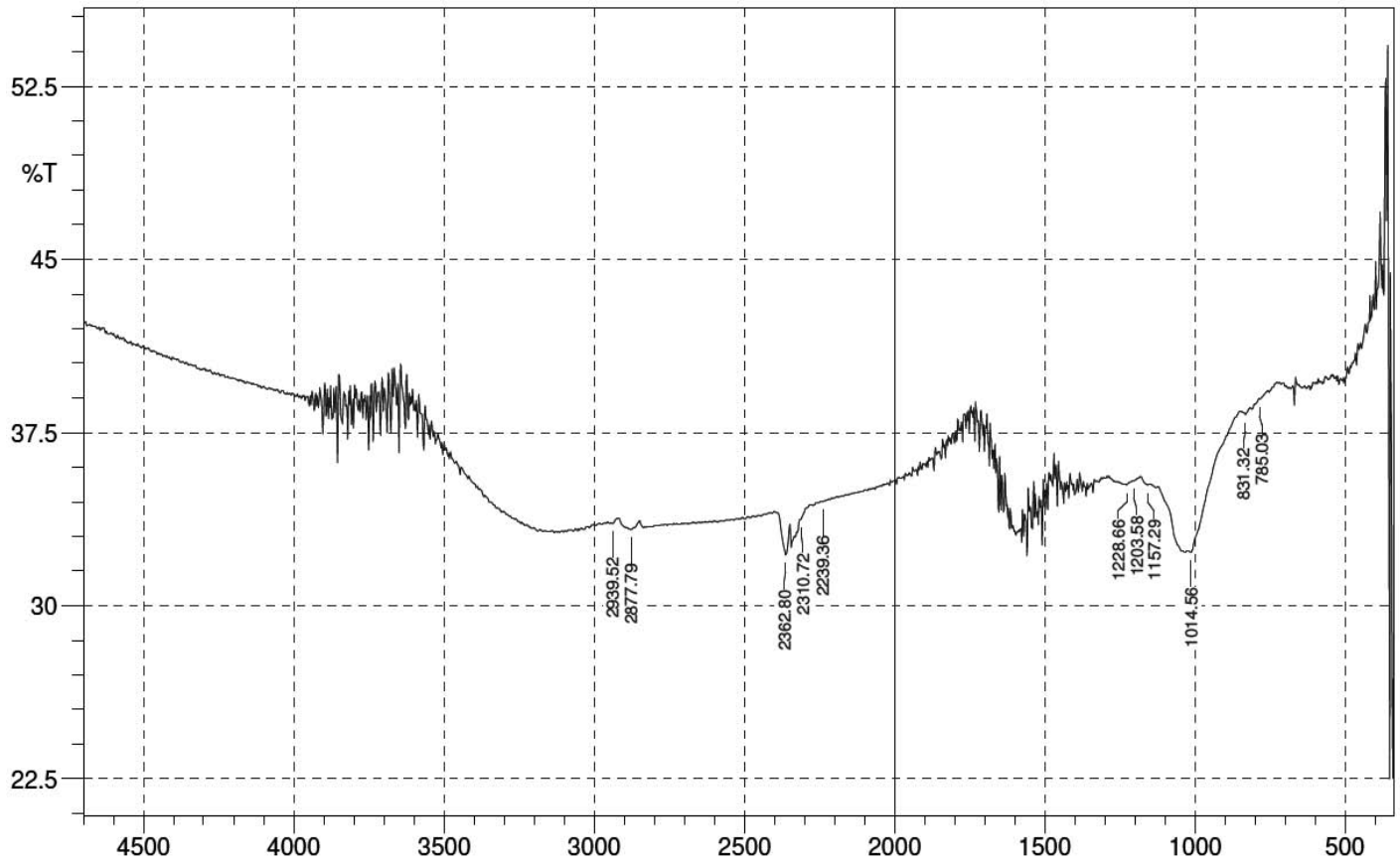


Figure 12. FT-IR spectrum of *Tecomella* flower extract mediated biogenic silver nanoparticles.

also correlates with already reported result stretches at 2933.29 cm^{-1}, 2852.45 cm^{-1}, 1233.71 cm^{-1}, 1149.92 cm^{-1}, 1023.67 cm^{-1}, 861.81 cm^{-1} and 757.59 cm^{-1}, plant mediated synthesis of AgNPs using dried stem powder of *Tinospora cordifolia*[23].

4. DISCUSSION

Different plant parts are used for the green synthesis of metal nanoparticles. Use of flower extract for synthesis of nanoparticles has an added advantage of environmental friendly. Flowers are normally thrown away into the environment, so use of flowers in biosynthesis of nanoparticles is a novel idea. To date, synthesis of AgNPs using flower extracts has scanty reports and synthesis of AgNPs with *Tecomella undulata* flower extract is reported for the first time.Silver nanoparticles have strong antimicrobial effects and are able to control and avoid plant diseases. Large particle size in DLS data in compare to AFM and XRD data could be due to hydrodynamic circumference and interaction in various forces in ionic condition. XRD data indicated that average particle size 12.5 nm smaller than AFM size range (20 nm - 70 nm) may be due to estimation of size of individual crystalline structure, whereas AFM estimate nanoparticles with capping of biomolecules. Earlier reports also supports our results indicated that particle size distribution in DLS data showed size range 10 nm - 150 nm, whereas SEM and TEM data showed 20 nm - 50 nm and XRD data showed average size 14 nm[24]. Various characterisation procedures indicated that the particle size of biogenic silver nanoparticles generally found in the order of DLS>AFM>XRD[25]. Silver nanoparticles in concentration of 0.5 ppm to 1000 ppm cause faster growth of plants and control pathogens[26]. Silver nanoparticles are beneficial of enhance seedling growth and germination[27] and act as growth stimulator[28]. Effects of silver nanoparticles on seedling growth parameters such as germination behaviour root/shoot length, fresh/dry weight were studied in different crop plants. Seed germination and plant growth parameters results indicated that AgNPs at their lower concentration promoted seed germination and early seedling growth in seedlings, however at higher concentration showed slight adverse effect. Defence Laboratory, Jodhpur is working in the area of arboriculture camouflage for the site specific implementation of long term camouflage scheme. In Thar Desert area of Rajasthan growing of tree is very much difficult task due to adverse climatic conditions and plants are generally slow growing. For the fast growth of tree saplings in nursery stage foliar spraying of different nanoparticles as growth stimulators may solve these problems. So attempts have made to synthesise silver nanoparticles through green technology using flower parts of local endemic plant species for the development of fast growing stress tolerant plant species.

5. CONCLUSION

Tecomella undulata flower extract showed great capability to synthesize AgNPs. UV absorption peak near 450 nm clearly

indicated the formation of AgNPs. XRD patterns confirmed the phase composition and nature of the synthesised nanoparticles. FTIR studies confirmed the bio-fabrication of the AgNPs by the action of different phytochemicals with its different functional groups present in the flower extract solution. EDX spectrum confirmed the presence of elemental signal of AgNPs as well as the significant presence of Ag without any impurities. Zeta potential indicated that AgNPs synthesised from *Tecomella* flower extract was stable even after three month storage. Thus, green synthesis of AgNPs using *T. undulata* flower extract is a cost-effective, safe, non-toxic, and an eco-friendly approach for mass production as well as having potential application in enhanced plant growth and development.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

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

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