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ORIGINAL ARTICLE

Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property

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Abstract Plants extract from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* was used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. Ag NPs were characterized by UV–vis spectrophotometer, X-ray diffractometer (XRD), atomic force microscope (AFM) and scanning electron microscope (SEM). The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV–vis spectrophotometer analysis. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using the Scherrer's equation. AFM showed the formation of silver nanoparticle with an average size of 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm corresponding to *O. tenuiflorum, S. cumini*, *C. sinensis, S. tricobatum* and *C. asiatica,* respectively. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate. Antimicrobial activity of the silver bio-nanoparticles was performed by well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae*. The highest antimicrobial activity of silver nanoparticles synthesized by *S. tricobatum*, *O. tenuiflorum* extracts was found against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively. The Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

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1. Introduction

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht et al., 2006). Recently, biosynthetic methods employing either biological microorganisms such as bacteria (Joerger et al., 2000) and fungus (Shankar et al., 2003a) or plants extract (Shankar et al., 2003b; Chandran et al., 2006; Gardea-Torresdey et al., 2002), have emerged as a simple and viable alternative to more complex chemical synthetic procedures to

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obtain nanomaterials. Different types of nanomaterials like copper, zinc, titanium (Retchkiman-Schabes et al., 2006), magnesium, gold (Gu et al., 2003), alginate (Ahmad et al., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms (Gong et al., 2007). Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities (Frattini et al., 2005). An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Synthesis of quasi spherical silver nanoparticles using purified apiin compound, extracted from henna leaf at ambient conditions (Kasthuri et al., 2009). Using green tea, *Camellia sinensis* extract as reducing and stabilizing agents produced gold nanoparticles and silver nanostructures in aqueous solution at ambient conditions (Nestor et al., 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Shankar et al., 2003b; Gardea-Torresdey et al., 2003). The reaction of aqueous $AgNO_3$ with an aqueous extract of leaves of a common ornamental geranium plant, *Pelargonium graveolens*, gave Ag NPs after 24 h (Shankar et al., 2003b). A vegetable, *Capsicum annum* L., was used to also synthesize Ag NPs (Li et al., 2007). In the present investigation, we report the easy synthesis of silver nanoparticles by an environmental friendly procedure involving the in situ reduction of Ag by *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* extracts and the evaluation of their antimicrobial activity against various human pathogenic bacteria.

2. Materials and methods

2.1. Selection and collection of plant material

Five different natural plants were selected for the silver nanoparticles synthesis. The leaves from *O. tenuiflorum* (Tulsi), *S. tricobatum* (Thudhuvalai), *S. cumini* (Naval), *C. asiatica* (Vallarai) and peel from *C. sinensis* (Orange) were collected. The leaves and peel were washed 2–3 times with de-ionized water.

2.2. Biosynthesis of silver nanoparticles

Silver nitrate, A.R. used in this study was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. 1.5 g of the leaves from *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and peels of *C. sinensis* were boiled in 100 ml of de-ionized water. 2.5 ml of ammonium solution was added to 5 ml of 1 mM $AgNO_3$ (solution, followed by addition of plants extract 1–10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water. For silver nanoparticles, the solution turned from yellowish to bright yellow and to dark brown. The Erlenmeyer flasks were incubated at 37 °C under agitation (200 rpm) for 24–48 h (Kasthuri et al., 2009).

2.3. Characterization of silver nanoparticles

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–540 nm using a UV–vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer). The de-ionized water was used as the blank. The samples from the maximum time point of production of silver nanoparticles were air-dried and allowed to characterize by Atomic Force Microscopy (Model-Nanosurf easyscan 2 AFM, made in Switzerland) for its detail size, morphology and agglomeration of silver. AFM Image was taken with silicon cantilevers with force constant 0.02–0.77 N/m, tip height 10–15 nm, contact mode. To check phase formation and purity, XRD patterns were recorded using powder X-ray diffractometer (Model-D8 Advance, made in BRUKER Germany). The samples from the maximum time point of production of silver nanoparticles were mounted on specimen stubs with double-sided adhesive tape and coated with gold in a sputter coater (HITACH, Model E-1010 Ion sputter) to avoid charging and examined under SEM (HITACH, Model S-3400N).

2.4. Antimicrobial activity by well diffusion method

The silver nanoparticles (Ag NPs) synthesized from *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. s*inensis were tested for their antimicrobial activity by well diffusion method against pathogenic organisms like *S. aureus*, *P. aeruginosa, K. pneumoniae* and *E. coli*. The pure cultures of organism were sub cultured on Muller–Hinton broth at 35 °C on rotary shaker at 200 rpm. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm have been made on Muller–Hinton agar plates using gel puncture. Using micropipette, 50 µl, 75 µl and 100 µl of the sample of nanoparticles solution were poured into wells on all plates. After incubation at 35 °C for 18 h, the different levels of zone of inhibition were measured.

3. Results and discussion

The detailed study on biosynthesis of silver nanoparticles by natural plants extract such as *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis* were employed and is reported in this work. The aqueous silver ions were reduced to silver nanoparticles when added to natural plant extract of *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis*. It was observed that the color of the solution turned from yellow to bright yellow and then to dark brown after 1, 24 and 48 h of the reaction, which indicated the formation of silver nanoparticles. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The UV–vis spectra showed maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract (Fig 1). The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag^+ ions took place extracellularly. It is reported earlier that absorbance at around 430 nm for silver is a characteristic of these nobel metal particles (Nestor et al., 2008).

In order to verify the results of the UV–vis spectral analysis, the samples of the silver ions exposed to the extracts of natural plants were examined by XRD. Fig 2 shows the XRD pattern for silver nanoparticles synthesized using natural plants ex-

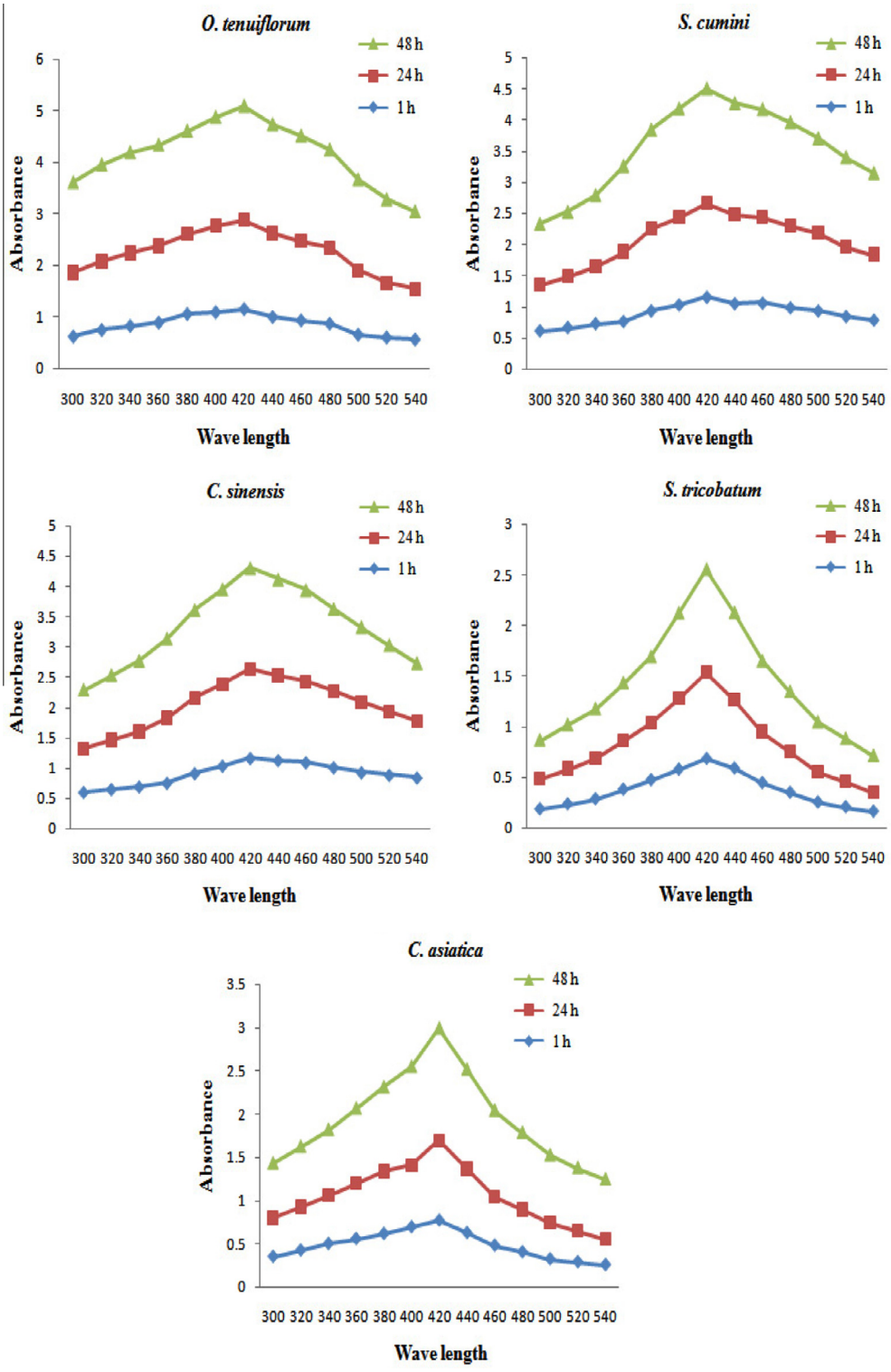

Figure 1 UV–vis spectra of silver nanoparticles synthesized using natural plant extracts.

tract. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using the following Scherrer's equation (Balaji et al., 2009):

$$D = \frac{K\lambda}{\beta^{1/2}\cos\theta}$$

The equation uses the reference peak width at angle θ, where λ is the X-ray wavelength (1.5418 Å), $\beta^{1/2}$ is the width of the XRD peak at half height and K is a shape factor. For natural plants extract synthesized silver nanoparticles the calculated average particle size of the silver was found to be 26 nm, 26 nm, 59 nm, 20 nm and 24 nm corresponding to *O. tenuiflorum, S. cumini*, *C. sinensis, S. tricobatum* and *C. asiatica* respectively.

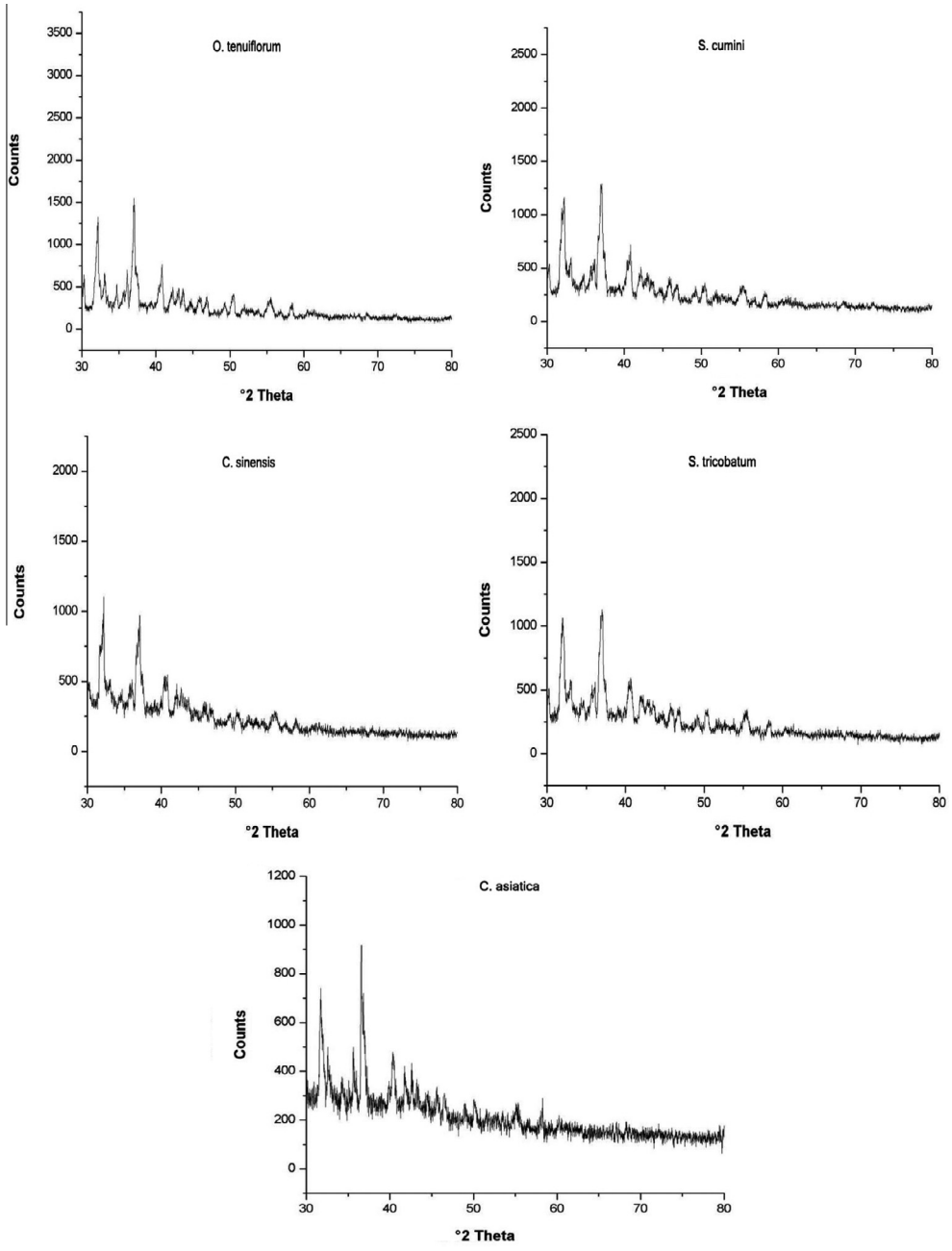

Figure 2 X-ray diffraction pattern of the silver nanoparticles were synthesized from natural plant extracts.

The silver nanoparticles were characterized by Atomic Force Microscopy (AFM) for its detail size and morphology of silver. The topographical images of irregular silver nanoparticles synthesized by natural plants extract are shown in Fig 3. The particle size of the silver nanoparticles was found to be 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm corresponding to *O. tenuiflorum, S. cumini*, *C. sinensis, S. tricobatum* and *C. asiatica* respectively. Fig 4 shows the scanning electron micrograph of the *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis* treated with 1 mM silver nitrate solution for 24 h. SEM determination of the brown color stable samples showed the formation of silver nanoparticles and well dispersed nanoparticles could be seen in the samples treated with silver nitrate.

Many researchers have reported the biosynthesis of nanoparticles with plants extract for biosynthesis reaction. Synthesis of quasi spherical silver nanoparticles using purified apiin compound, extracted from henna leaf at ambient conditions (Kasthuri et al., 2009). Using green tea, *C. sinensis* extract as reducing and stabilizing agents gold nanoparticles and silver nanostructures could be produced in aqueous solution at ambient conditions (Nestor et al., 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis (Torresdey et al., 2003; Shankar et al., 2003b, 2005). The reaction of aqueous $AgNO_3$ with an aqueous extract of leaves of a common ornamental geranium plant, *P. graveolens*, gave Ag NPs after 24 h. Biosynthesis of silver nanoparticles was also

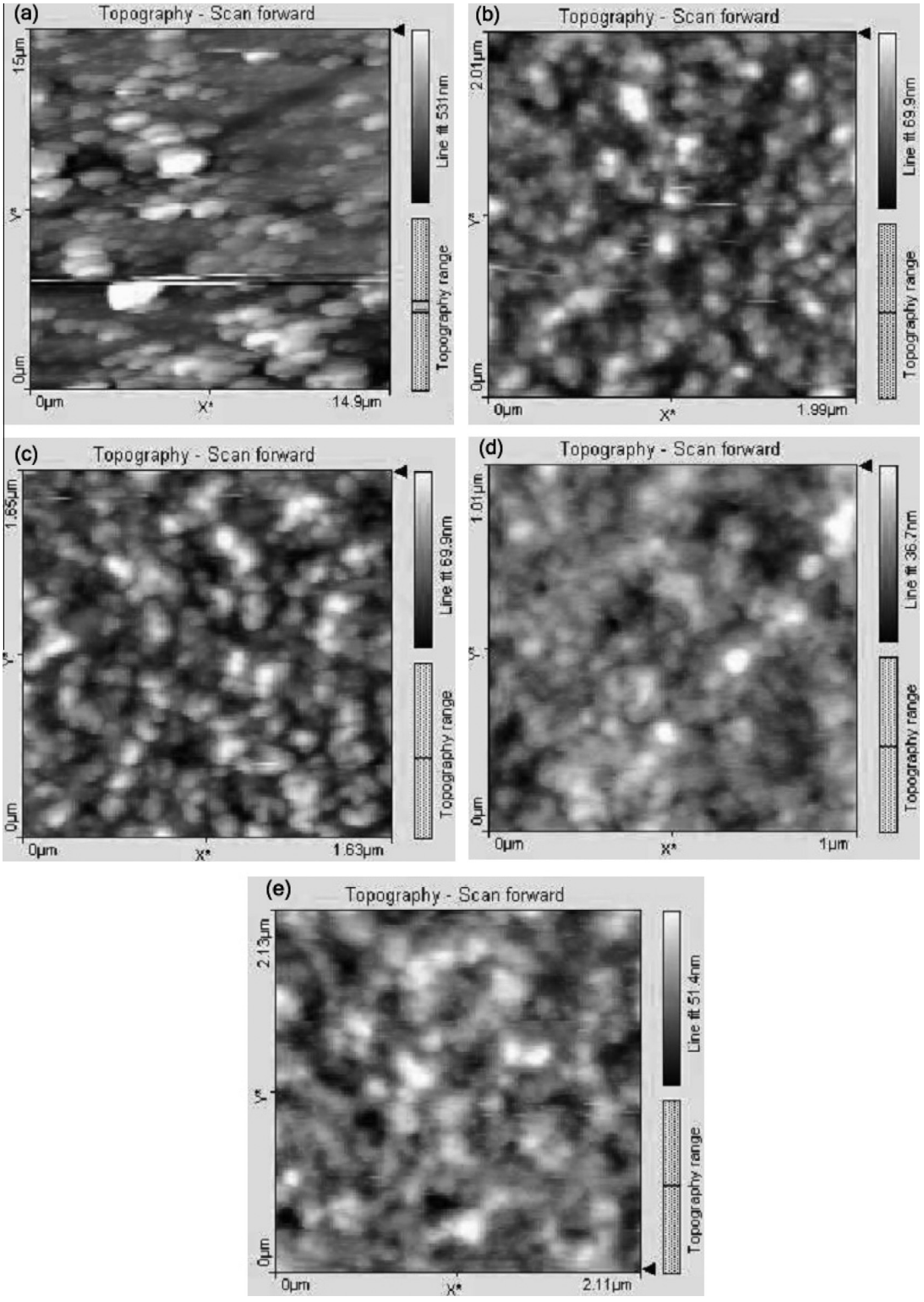

Figure 3 AFM images of the silver nanoparticles synthesized by natural plant extracts [(a) *O. tenuiflorum*, (b) *S. cumini*, (c) *C. sinensis*, (d) *S. tricobatum*, (e) *C. asiatica*].

conducted using *Cycas* leaf extract. *Cycas* belongs to the *Cycadaceos* family. It is a common gymnospermic plant and is a commercial source of sago. This plant is rich in flavonoids broadly belonging to the lass of phenolic compounds. The *Cycas* extract solution was treated with 20 ml of 0.25 M $AgNO_3$ solution and warmed on the steam bath for 20 min until the color of solution changes to brown. The size particle ranged from 2 to 6 nm and the average particle size comes out to be 3.29 ± 0.22 nm. The X-ray diffraction pattern obtained for silver nanoparticles synthesized by *Cycas* leaf broth

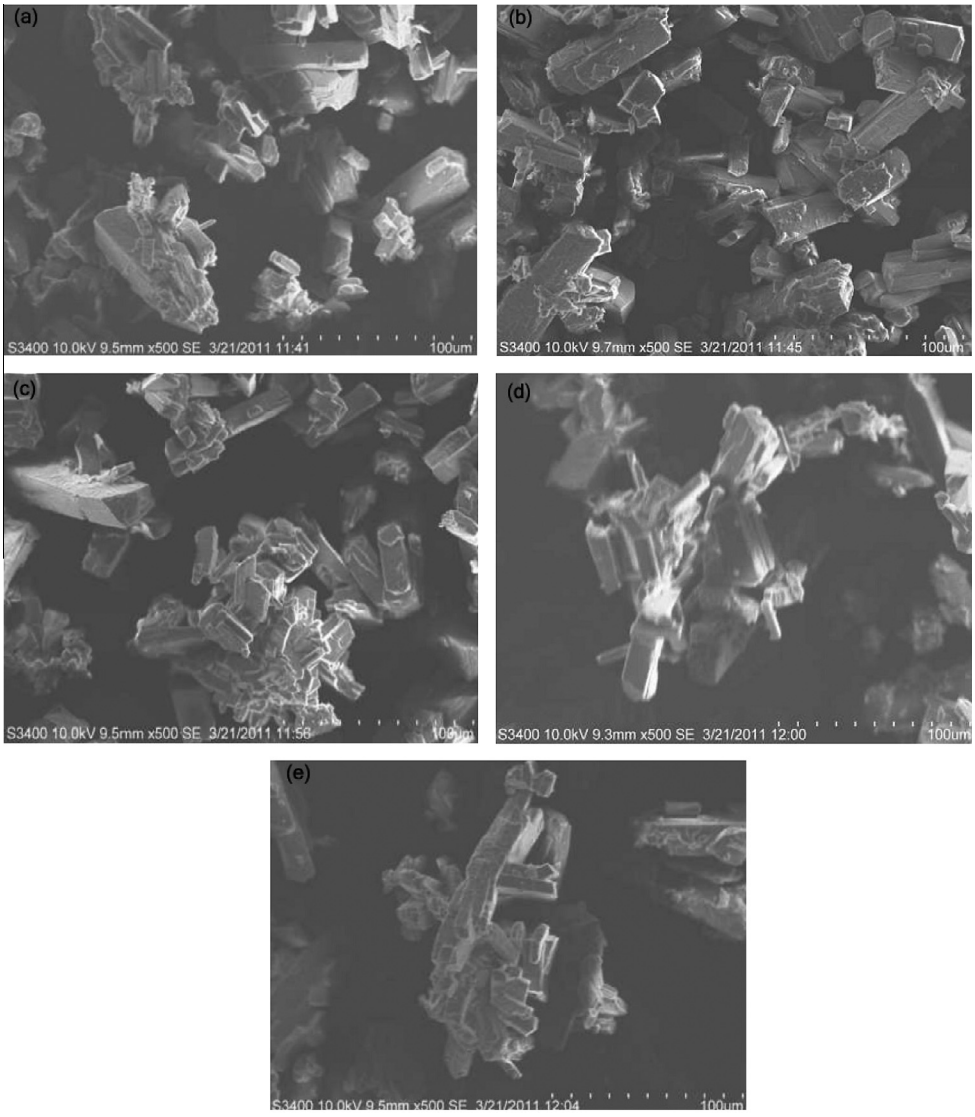

Figure 4 SEM images of the silver nanoparticles synthesized by natural plant extracts [(a) *O. tenuiflorum*, (b) *S. cumini*, (c) *C. sinensis*, (d) *S. tricobatum*, (e) *C. asiatica*].

Table 1 Zone of inhibition of silver nanoparticles synthesized by natural plant extracts against various pathogenic bacteria.

Silver nanoparticle samples	Zone of inhibition (mm) against pathogenic bacteria											
	Staphylococcus aureus			*Pseudomonas aeruginosa*			*Escherichia coli*			*Klebsiella pneumoniae*		
	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl
S1	12	19	25	15	17	20	20	25	30	15	17	19
S2	14	21	26	18	22	25	20	24	26	19	22	24
S4	17	21	27	13	15	18	13	15	17	12	14	16
S5	17	26	30	7	10	12	8	10	12	14	16	18
S6	20	21	26	11	13	15	15	19	21	15	17	20

Note: S1 – *Ocimum tenuiflorum*, S2 – *Syzygium cumini*, S4 – *Citrus sinensis*, S5 – *Solanum tricobatum*, S6 – *Centella asiatica.*

shows that the silver nanopaticles are crystalline in nature (Jha and Prasad, 2010).

The antimicrobial activity of silver nanoparticles synthesized by natural plants extract was investigated against various pathogenic organisms such as *S. aureus*, *P. aeruginosa, E. coli* and *K. pneumoniae* using well diffusion method. The diameter of inhibition zones (mm) around each well with silver nanoparticles solution is represented in Table 1. The silver nanoparticles synthesized by *S. tricobatum*, *O. tenuiflorum* extracts were found to have highest antimicrobial activity against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively and the lesser antimicrobial activity of silver nanoparticles synthesized by

S. tricobatum extract was found against *P. aeruginosa* (12 mm) and *E. coli* (12 mm). The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi and Salopek-Sondi, 2004; Morones et al., 2005).

4. Conclusion

The silver nanoparticles have been produced by *O. tenuiflorum, S. tricobatum, S. cumini, C. asiatica* and *C. sinensis* extracts, which is an economical, efficient and eco-friendly process. UV–vis spectrophotometer, XRD, AFM and SEM techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were formed in the antimicrobial screening test indicated, that the Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

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