In vitro synthesis of antimicrobial silver nanoparticles by mangroves, saltmarshes and plants of coastal origin

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Abstract: A total of 13 leaf extracts of coastal plants were used for the synthesis of silver nanoparticles and to test the antimicrobial potential of nanoparticles. The highest amount of nanoparticles was obtained by Sesuvium portulacastrum followed by Prosopis chilensis, Hibiscus rosasinensis and Clerodendrum inerme. The synthesised nanoparticles were spherical with variable size ranging from 5 to 20 nm, as evident by transmission electron microscopy. The synthesis of silver nanoparticles was confirmed with X-ray diffraction spectrum. Fourier transform infrared spectroscopy identified the presence of amide I, II and III, aromatic rings, geminal methyls and ether linkages indicating the presence of biomolecules responsible for stabilisation of the silver nanoparticles. Protein bands with molecular weight in a range of 35 to 100 kDa were detected. The nanoparticles inhibited clinical strains of bacteria and fungi with more distinct antifungal activity, which was enhanced when polyvinyl alcohol was added as a stabilising agent.

Keywords: antimicrobial activity; coastal plants; mangroves; saltmarsh; silver nanoparticles.


Biographical notes: Kandasamy Kathiresan and his team have been working in the field of coastal ecology and bio-prospecting of coastal products for the last three decades. His team has established the techniques for development of high value products from the mangroves and other coastal entities. His recent focus is on rapid synthesis of silver nanoparticles.

Nabeel M. Alikunhi has been working in the field of coastal ecology and bio-prospecting of coastal products along with his supervisor, Professor Kathiresan. His recent focus is on rapid synthesis of silver nanoparticles through economically viable approaches.

Asmathunisha Nabikhan is a young researcher, who has initiated her research career on nanoparticles synthesis.
1 Introduction

Nanoparticles play a significant role in the field of biology and medicine. Among the nanoparticles, silver nanoparticle has drawn considerable attention owing to its several important and diverse properties like catalysis, magnetic and optical polarisability (Shiraishi and Toshima, 2000), electrical conductivity (Tripathy et al., 2010), antimicrobial activity (Sharverdi et al., 2007) and surface enhanced Raman scattering (Efrima and Zeiri, 2009). Many methods, such as chemical reduction, polyol process and radiolytic method (Li et al., 2003; Kim et al., 2004; Muniz-Miranda, 2004) are employed for the synthesis of silver nanoparticles. With the development of new chemical or physical methods, the level of environmental contaminations is also increasing. Hence, there is a growing need to develop environmentally benign nanoparticle synthesis which does not use toxic chemicals in the synthetic protocols and not liberate hazardous byproducts for its commercial applications. In this regard, synthesis of nanoparticles using microbes (Kathiresan et al., 2009) and plants provides advantageous over chemical and physical methods as it is a cost effective and environment friendly method which does not require high pressure, energy, temperature and toxic chemicals (Nabikhan et al., 2010). Use of plant extracts for the synthesis of nanoparticles could also be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining microbial cell cultures. However, such attempts are restricted to a few terrestrial plants, and not the plants of coastal origin expect a few (Nabikhan et al., 2010). To fill the knowledge gap, the present study was made to test the potential of coastal plants for the reduction of silver ions to silver nanoparticles, besides analysing XRD, FTIR, TEM and antimicrobial aspects of the silver nanoparticles.

2 Materials and methods

2.1 Chemicals

All analytical reagents and media components were purchased from Hi-Media (Mumbai, India) and Sigma chemicals (St. Louis, USA).

2.2 Collection of plant materials

In the present study 13 plants of coastal origin including five mangroves, two saltmarshes and six other plants were collected from Parangipettai (Lat. 11° 29'N; Long 79° 46'E) in the south east coast of India. The plants were identified as Rhizophora apiculata Blume, R. mucronata Poir., R. annamalayana Kathir. (Rhizophoraceae) Acanthus ilicifolius L. (Acanthaceae), Cissus quadrangularis L. (Vitaceae), Clerodendrum inerme Gaertn. (Lamiaceae), Excoecaria agallocha L. (Euphorbiaceae), Hibiscus rosasinensis L. (Malvaceae), Lawsonia inermis L. (Lythraceae), Suaeda maritima (L.) Dumort (Chenopodiaceae), Sesuvium portulacastrum L. (Aizoaceae), Prosopis chilensis L. (Fabaceae) and Thespesia populnea (L.) Sol.ex Correa (Malvaceae).
2.3 Synthesis of silver nanoparticle

Leaf extract was prepared from the 13 plants separately. Fresh leaves weighing 20 g were finely cut into small bits, washed thoroughly with distilled water, extracted in 100 mL of boiling distilled water for 5 min. For reduction of Ag⁺ ions, 5 mL of leaf broth was added to 45 mL of 10⁻³ M aqueous AgNO₃ solution as the substrate and kept under dark. The reduction of Ag⁺ ions was monitored by visual observation and also by measuring absorbance of the reaction mixture at regular intervals (0 min., 10 min., 20 min., 30 min., 40 min., 50 min., 60 min., 2 h, 3 h, 4 h, 6 h, 24 h and 48 h. The absorbance was measured at a resolution of 420 nm, as this was found to be the absorption maximum while measuring the optical density at different wave lengths from 400 to 700 nm at the different durations of incubation (data not shown) using UV-visible spectrophotometer (Elico, Chennai).

2.4 Characterisation of silver nanoparticles

The X-ray diffraction (XRD) measurement of silver nanoparticles synthesised by leaf extracts of four selected plants – Sesuvium portulacastrum, Prosopis chilensis, Hibiscus rosasinensis and Clerodendrum inerme – after 24 h of incubation was carried out using Cu-Kα radiation source in powder diffractometer (PANalyticalX’per PRO model X-ray diffractometer), on films of the solutions drop coated onto glass substrates on the instrument operating at a voltage of 50 kV and a current of 30 mA. For Fourier transform infrared spectroscopy (FTIR) measurement, the following method was adopted. The silver nanoparticles synthesised by leaf extracts of four selected plants – Sesuvium portulacastrum, Prosopis chilensis, Hibiscus rosasinensis and Clerodendrum inerme – after 24 h of reaction with the leaf extract, centrifuged at 10,000 rpm for 15 min, and the pellet was redispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the silver nanoparticles. The purified pellets were then dried and the powder was subjected to FTIR spectroscopy measurement (Paragon 500, Perkin Elmer – RX1 spectrophotometer) in the diffuse reflectance mode at a resolution of 4 cm⁻¹ in KBr pellets. After 24 h of incubation, the reaction mixture was used to form a film of silver nanoparticles produced by leaf extracts of four selected plants – Sesuvium portulacastrum, Prosopis chilensis, Hibiscus rosasinensis and Clerodendrum inerme on carbon coated copper transmission electron microscopic (TEM) grids (Electron microscopy sciences, Hatfield, PA 19440) and analysed under a TEM (JOEL, JEM 100 SX) at a voltage of 120 kV.

2.5 Partial purification and PAGE analysis

After 24 h of incubation, the reaction mixture was precipitated by using solid ammonium sulphate to 80% saturation. The pellet obtained after centrifugation was dissolved in 0.05 M phosphate buffer (pH 8.0). The concentrated protein thus obtained was dialysed overnight against 0.05 M phosphate buffer (pH 8.0) to remove salt. Samples were analysed on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to check the purity and to determine the molecular weight of the purified protein by comparing with protein standard having molecular weight ranging from 10 to 250 kDa.
2.6 Stabilisation of silver nanoparticles

The nanocomposites consisting of silver nanoparticle and polyvinyl alcohol (PVA) were prepared by following the method of Lili et al. (2007) with slight modification. In this, 0.8 g PVA was dissolved in 100 ml distilled water at 80°C by vigorously stirring to form homogeneous solution. To this, 20 mL of silver nanoparticles prepared using leaf extract was added with PVA solution. This solution was then allowed to stir in a flask for about 10 min. and then the solution was purged with nitrogen. To this solution containing PVA and nanoparticles, a fresh aqueous sodium borohydride solution with the concentration of $5 \times 10^{-3}$ M was introduced drop by drop, then stirred for 15 min under inert atmosphere at room temperature of 25 ± 2°C. This nanocomposite of PVA and silver nanoparticles was compared to silver nanoparticles for their antimicrobial activity.

2.7 Antimicrobial activities by disc-diffusion method

The antibacterial assay was done by disc diffusion assay method (Casida, 1986). In this method, 50 μl of silver nanoparticle synthesised by leaf extract of four selected plants – *Sesuvium portulacastrum*, *Prosopis chilensis*, *Hibiscus rosasinensis* and *Clerodendrum inerme*, was mixed separately in 1 ml of distilled water and applied to sterile paper discs of 5 mm diameter (Hi media, India). Similarly 50 μl nanocomposite of silver nanoparticle and PVA prepared from leaf extract was mixed in 1 ml of distilled water and applied to sterile paper disc. The discs were then placed on Mueller Hinton Agar swabbed with clinical strains of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Micrococcus luteus* and *Klebsiella pneumoniae*) and fungi (*Alternaria alternata*, *Penicillium italicum*, *Fusarium equisetii* and *Candida albicans*), at a concentration of $10^6$ bacteria/ml for bacteria and $10^3$ spore/ml for fungi. The plates were incubated at 37°C for overnight. The zone of inhibition was measured in millimetres after the 24 h of incubation.

2.8 Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan’s (1957) multiple range test to understand the variation in antimicrobial activity against test pathogens. Results are expressed as mean ± standard deviation from three replicates in each group.

3 Results

3.1 Screening of plants for the synthesis of silver nanoparticles

There was no change in colour in the plant extracts without addition of silver nitrate. Leaf extracts initiated change in colour within ten minutes when they were added with silver nitrate. The colour of the leaf extracts increased up to 24 h of incubation and there was no significant change afterwards (Figure 1). The synthesis of silver nanoparticle was well pronounced with four plants in a decreasing order: *Sesuvium portulacastrum > Prosopis chilensis > Hibiscus rosasinensis > Clerodendrum inerme* (Figure 1).
3.2 Characterisation of silver nanoparticles

The synthesised nanoparticles were characterised by using XRD, TEM analysis and FTIR spectra. The XRD pattern of silver nanoparticles produced by using plants (*S. portulacastrum*, *P. chilensis*, *H. rosasinensis* and *C. inerme*) are shown in Figure 2. The XRD exhibited intense peaks in the whole spectrum of $2\theta$ value ranging from 20 to 80 and this pattern is similar to the Bragg’s reflection of silver nanocrystals. The shape and size of silver nanoparticles was analysed after 24 h of incubation by using TEM is depicted in Figure 3. In general, the nanoparticles were in spherical shape with varying size ranged from 5 to 20 nm. Most of the nanoparticles were aggregates with only a few of them were scattered, as observed under TEM. FTIR spectra of silver nanoparticle produced by four plants are shown in the Figure 4 with prominent peaks for *S. portulacastrum* at 2,229, 1,761, 1,695, 1,507, 1,241 and 734 cm$^{-1}$, *P. chilensis* at 2,817, 2,512, 2,391, 2,011, 1,772, 1,692, 1,565, 1,213, 653 and 332 cm$^{-1}$, *H. rosasinensis* at 2,352, 1,935, 1,713, 1,615, 1,351, 1,035 and 654 cm$^{-1}$, and for *C. inerme* at 2,793, 2,435, 2,051, 1,787, 1,577, 1,405, 1,257, 1,055, 795 and 251 cm$^{-1}$. The peaks corresponding to amide I, II and III, aromatic rings, geminalmethyls and ether linkages were found commonly present in the nanoparticles synthesised by the plant species.
In vitro synthesis of antimicrobial silver nanoparticles

Figure 2  XRD patterns of silver nanoparticles synthesised by using plants of coastal origin, (a) Sesuvium portulacastrum (b) Prosopis chilensis (c) Hibiscus rosasinensis (d) Clerodendrum inerme

Figure 3  TEM micrographs of silver nanoparticles synthesised by using plants of coastal origin, (a) Sesuvium portulacastrum (b) Prosopis chilensis (c) Hibiscus rosasinensis (d) Clerodendrum inerme

Note: Scale bar: 50 nm
Figure 3  TEM micrographs of silver nanoparticles synthesised by using plants of coastal origin, (a) *Sesuvium portulacastrum* (b) *Prosopis chilensis* (c) *Hibiscus rosasinensis* (d) *Clerodendrum inerme* (continued)

Note: Scale bar: 50 nm

Figure 4  FTIR spectra of silver nanoparticles synthesised by using plants of coastal origin, (a) *Sesuvium portulacastrum* (b) *Prosopis chilensis* (c) *Hibiscus rosasinensis* (d) *Clerodendrum inerme*
In vitro synthesis of antimicrobial silver nanoparticles

Figure 4 FTIR spectra of silver nanoparticles synthesised by using plants of coastal origin, (a) Sesuvium portulacastrum (b) Prosopis chilensis (c) Hibiscus rosasinensis (d) Clerodendrum inerme (continued)

3.3 Partial purification of protein

The molecular weight estimated by SDS-PAGE of the protein revealed the presence of multiple proteins in the leaf extracts treated with silver ions (Figure 5). There were two prominent bands for *P. chilensis* (85, 70 kDa), *H. rosasinensis* (100, 70 kDa), *C. inerme* (85, 35 kDa) and for *S. portulacastrum* (75, 45 kDa).
Antimicrobial activities of synthesised silver nanoparticles with and/or without PVA are shown in the Tables 1 and 2. The nanoparticle with PVA showed higher antimicrobial activities than that without PVA against all the tested microbes. In general, antibacterial activity was more pronounced than antifungal activity. The inhibition varied significantly between test microbes as well as plant extracts ($P < .05$). The highest inhibition zone of 30 mm diameter was formed against *Pseudomonas aeruginosa* by nanoparticles derived from *P. chilensis* added with PVA, and the lowest of 8 mm was recorded against *Micrococcus luteu* by nanoparticles synthesised from *H. rosasinensis* added without PVA.
In vitro synthesis of antimicrobial silver nanoparticles

Table 1  Antibacterial activity of silver nanoparticles by plants of coastal origin, with and/or without PVA against clinical bacterial strains of PA-Pseudomonas aeruginosa, KP-Klebsiella pneumoniae, SA-Staphylococcus aureus, LM-Listeria monocytogenes, ML-Micrococcus luteus

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Diameter of the inhibition zone in (mm)</th>
<th>PA</th>
<th>KP</th>
<th>SA</th>
<th>LM</th>
<th>ML</th>
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<td>+PVA</td>
<td>20 ± 2.4(^{a1})</td>
<td>20 ± 2.1(^{a1})</td>
<td>22 ± 2.5(^{a1})</td>
<td>18 ± 1.9(^{a1})</td>
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<td></td>
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<td>15 ± 1.7(^{b1})</td>
<td>18 ± 2.1(^{b1})</td>
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<td>17 ± 1.9(^{b1})</td>
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<tr>
<td>S. portulacastrum</td>
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<tr>
<td></td>
<td></td>
<td>+PVA</td>
<td>18 ± 1.9(^{a1})</td>
<td>12±1.3(^{b2})</td>
<td>28 ± 3.2(^{b3})</td>
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<tr>
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<td>-PVA</td>
<td>23 ± 2.5(^{a2})</td>
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<td>15 ± 1.6(^{a3})</td>
<td>08 ± 1.3(^{b3})</td>
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<tr>
<td>H. rosasinensis</td>
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<td>+PVA</td>
<td>09 ± 1.2(^{a1})</td>
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<td>11 ± 1.3(^{a1})</td>
<td>20 ± 2.2(^{a1})</td>
<td>15 ± 1.6(^{b1})</td>
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<td>C. inerme</td>
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<td></td>
<td>+PVA</td>
<td>12 ± 1.4(^{b1})</td>
<td>15 ± 1.6(^{b1})</td>
<td>13 ± 1.2(^{c1})</td>
<td>20 ± 2.2(^{a2})</td>
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<tr>
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<td>-PVA</td>
<td>13 ± 1.6(^{a1})</td>
<td>12 ± 1.4(^{c1})</td>
<td>14 ± 1.6(^{a1})</td>
<td>13 ± 1.5(^{a1})</td>
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<tr>
<td>P. chilensis</td>
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<td>18 ± 2.0(^{a2})</td>
<td>20 ± 2.2(^{a2})</td>
<td>19 ± 2.0(^{a2})</td>
</tr>
<tr>
<td></td>
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<td>-PVA</td>
<td>25 ± 2.7(^{a1})</td>
<td>17 ± 1.9(^{a2})</td>
<td>19 ± 1.7(^{a2})</td>
<td>18 ± 1.7(^{a2})</td>
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Notes: Values are mean of three replicates ± standard deviation. Values sharing a different superscript varies significantly between each other (alphabetical superscript refers to the variation between the rows were as the numerical superscript refers to the variation between the columns).

Table 2  Antifungal activity of silver nanoparticles produced by plants of coastal origin, with and/or without PVA against clinical fungal strains of AA-Alternaria alternata, CA-Candida albicans, PI-Penicillium italicum, FE-Fusarium equisetii, and PVA-poly vinyl alcohol

<table>
<thead>
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<th>Plant species</th>
<th>Diameter of the inhibition zone in (mm)</th>
<th>AA</th>
<th>CA</th>
<th>PI</th>
<th>FE</th>
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<td>11 ± 1.3(^{a1})</td>
<td>18 ± 2.1(^{a1})</td>
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<tr>
<td>S. portulacastrum</td>
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<td>+PVA</td>
<td>15 ± 1.7(^{a1})</td>
<td>14 ± 1.6(^{a1})</td>
<td>15 ± 1.8(^{a1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-PVA</td>
<td>13 ± 1.6(^{a1})</td>
<td>12 ± 1.4(^{a1})</td>
<td>14 ± 1.6(^{a1})</td>
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<td>H. rosasinensis</td>
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<td></td>
<td></td>
<td>+PVA</td>
<td>15 ± 1.4(^{a1})</td>
<td>12 ± 1.5(^{a1})</td>
<td>10 ± 1.3(^{b2})</td>
</tr>
<tr>
<td></td>
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<td>11 ± 1.3(^{a1})</td>
<td>06 ± 0.9(^{b1})</td>
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<td>C. inerme</td>
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<td>+PVA</td>
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<td>18 ± 1.7(^{a2})</td>
<td>09 ± 1.1(^{b3})</td>
</tr>
<tr>
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<td>-PVA</td>
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<td>16 ± 1.5(^{a1})</td>
<td>08 ± 1.1(^{a1})</td>
</tr>
</tbody>
</table>

Notes: Values are mean of three replicates ± standard deviation. Values sharing a different superscript varies significantly between each other (alphabetical superscript refers to the variation between the rows were as the numerical superscript refers to the variation between the columns).

4 Discussions

The plants of terrestrial origin are known to synthesise silver nanoparticles: Azadirachta indica (Neem) (Shankar et al., 2004), Medicago sativa (Alfalfa) (Gardea-Torresdey et al., 2003), Aloe vera (Chandran et al., 2006), Emblica officinalis (Amla, Indian Gooseberry) (Ankamwar et al., 2005), Parthenium hysterophorus (Parashar et al., 2009), Diopyros
kaki (Song et al., 2010) and also in marine plant, Sesuvium portulacastrum (Nabikhan et al., 2010). The present work proved for the first time that plants of coastal origin could also be able to reduce silver ions and produce silver nanoparticles. Of the 13 plants screened, the synthesis of antimicrobial silver nanoparticles was highest by the saltmarsh species, Sesuvium portulacastrum followed by Prosopis chilensis, Hibiscus rosasinensis and Clerodendrum inerme (Figure 1). None of these plants has already been reported for the synthesis of silver nanoparticles, except Sesuvium portulacastrum. Tissue culture-derived callus of this plant is efficient in silver nanoparticles synthesis over leaves (Nabikhan et al., 2010). The plant extracts were found to synthesise the nanoparticles extracellularly and this offers a great advantage over an intracellular process of synthesis from the application point of view. Since the nanoparticles formed inside the biomass would have required additional step of processing for release of the nanoparticles from the biomass by ultrasound treatment or by reaction with suitable detergents. The nanoparticle synthesis varied with plant species and duration of incubation (Figure 1). The synthesis of silver nanoparticles was noted by visual observation that the reaction mixture consisted of leaf extract and AgNO₃ showed change in colour to yellowish brown with intensity increasing during the period of incubation. Control without silver ion did not show any change in colour when incubated in the same condition. The appearance of the brown colour was an indication of formation of silver nanoparticles in the reaction mixture and the brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Ahmad et al., 2003). The colour of the leaf extracts changed to intense brown after 24 h of incubation. The solution remained as hydrosol and there was no precipitation even after 72 h of incubation. This indicated that the particles were well dispersed in the solution and there was not much aggregation. Monodispersity is an important characteristic of the nanoparticles and it is reportedly very good for silver nanoparticles (Ahmad et al., 2003). In order to understand the exact nature of the silver nanoparticles formed by plants of coastal origin, the XRD-spectrum was analysed (Figure 3). This clearly shows that, the silver nanoparticles formed by the plants are crystalline in nature. The intense peaks observed in the spectrum are in agreement to the Braggs’s reflection corresponding to the (111), (200), (220), (311), and (222) sets of lattice planes of silver nanocrystals (Lu et al., 2003; Bhainsa and D’Souza, 2006). Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallisation of the capping agents was ruled out due to the process of centrifugation and redispersion of the pellet after nanoparticle formation as part of purification process. Therefore XRD results also suggest that crystallisation of the bio-organic phase occurs on the surface of the silver nanoparticles (Shankar et al., 2004). The silver nanoparticles synthesised by plants of coastal origin revealed that the nanoparticles were of variable shape, mostly spherical in nature with varying size which ranged from 5 to 20 nm (Figure 4). The terrestrial plants are also known to produce the silver nanoparticles of different sizes: 50 to 100 nm by Azadirachta indica, 25 to 85 nm by Anina sativa, 15.2 nm by Aloe vera and 15 to 20 nm by Emblica officinalis (Mohanpuria et al., 2008). Thus, the plants of coastal origin could produce silver nanoparticles of comparatively smaller size. Particle size of the nanoparticles is one of the important aspects in nanotechnology, as the decrease in particle size will increase the efficiency of the nanoparticles for application (Bürgi and Pradeep, 2006).

FTIR measurements were carried out to identify the possible biomolecules that are responsible for capping and efficient stabilisation of the silver nanoparticles synthesised
by plants of coastal origin. The prominent peaks corresponding to amide I, II and III, aromatic rings, geminalmethyls and ether linkages were present commonly in all the leaf extracts with nanoparticles. The adsorption on the surface of metal nanoparticles is a characteristic of flavanones and terpenoids (Shankar et al., 2004), which may be able possibly by interaction through carbonyl groups or \( \pi \)-electrons in the absence of other strong ligating agents in sufficient concentration (Shankar et al., 2004). It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehydic groups to carboxylic acids (Shankar et al., 2004). The presence of the peaks in the amide I and II regions characteristic of proteins/enzymes that have been found to be responsible for the reduction of metal ions for synthesis of metal nanoparticles (Ahmad et al., 2003; Mukherjee et al., 2001a, 2001b). It is well known that proteins can bind to silver nanoparticle through either free amine groups or cysteine residues in the proteins (Gole et al., 2001). There are earlier reports on NADH dependent reductases (Ahmad et al., 2003; Anilkumar et al., 2007) and polysaccharides (Huang and Yang, 2004) as factors involved in biosynthesis and stabilisation of the nanoparticles, respectively. The presence of polyphenolic compounds in the coastal plants might be responsible for the formation of silver nanoparticles for the reason that the plants are generally rich in polyphenols like tannic acids (Kathiresan and VeeraRavi, 1990) and these plant-derived compounds are efficient reducing agent in the synthesis of silver nanoparticles (Sivaraman et al., 2009). Thus, several factors may determine the nanoparticle synthesis by plants of coastal origin and however, the exact mechanism is yet to be elucidated.

Silver is well known as one of the most universal antimicrobial substances (Tokumaru et al., 1974; Oloffs et al., 1994; Oka et al., 1994). Silver ion and silver-based compounds are highly toxic to microorganisms (Slawson et al., 1992), showing strong biocidal effect against microbial species (Spadaro et al., 1974). However, silver ions or salts have only limited usefulness as antimicrobial agents for several reasons: interfering effects of salts and the antimicrobial mechanism of continuous release of enough concentration of silver ion from the metal form. In contrast, these kinds of limitation can be overcome using silver nanoparticles as these are highly reactive species because of large surface area. The silver nanoparticles produced by using microbes and/or plant extracts are known to exhibit potent antimicrobial activity (Jain and Pradeep, 2005; Son et al., 2004; Pal et al., 2007; Lok et al., 2005; Sondi and Sondi, 2004). A similar observation was made here with the silver nanoparticles produced by plants of coastal origin to have antimicrobial activity against the clinical strains of bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes, Micrococcus luteu and Klebsiella pneumoniae) and fungi (Alternaria alternata, Candida albicans, Penicillium italicum and Fusarium equisetii) (Tables 1 and 2). The antimicrobial activity in terms of inhibition zone significantly varied with test microbes and plant species. This differential activity of silver nanoparticles can be attributed to their differential sizes and shape. The antimicrobial activity increases with decreasing size of the silver nanoparticles (Baker et al., 2005). The present work recorded that the antibacterial activity was more pronounced than antifungal activity. This can be attributed to the fact that silver at low concentrations does not enter the fungal cells, but it is adsorbed onto the bacterial surface just as silver tends to adsorb to other surfaces, thus silver ions immobilise dehydrogenation because respiration occurs across the cell membrane in bacteria rather than across the mitochondrial membrane as in eukaryotic cells of fungi (Liau et al., 1997). The present study proved that the antimicrobial activity of silver nanoparticles could be enhanced by PVA (Tables 1 and 2). PVA is frequently used as a particle stabiliser in chemical
syntheses of metal colloids, since it prevents agglomeration and precipitation of the particles (Porel et al., 2005; Mbhele et al., 2003). If the metal colloids coagulate their antibacterial activities are poor and this problem can be overcome by encapsulating metal nanoparticles with polymer materials (Mbhele et al., 2003). PVA is known makes the silver nanoparticles smaller in size, more uniform in shape, and narrower in size distribution (Silva et al., 2008). It is concluded from the present study that, the coastal plants have the enormous potential to synthesise the silver nanoparticles and it could be used as an effective antimicrobial agents.

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References


In vitro synthesis of antimicrobial silver nanoparticles


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