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Green synthesis and antimicrobial activity of silver nanoparticles onto cotton fabric: An amenable option for textile industries

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ABSTRACT

Biosynthesis, characterizations and applications of nanoparticles have become an important branch of nanotechnology nowadays. In this paper, green synthesis of silver nanoparticles (AgNPs) using the alcoholic extract of *Clerodendron infortunatum* as a reducing and stabilizing agent, has been discussed. This biosynthetic method is simple, cost-effective and reproducible. Formation of AgNPs was established by X-ray diffraction, scanning and transmission electron microscopy, UV-visible spectroscopy techniques. The phytochemicals responsible for nano-transformation were principally phenolics, tannins and organic acids present in the leaves. Further, AgNPs were used for antibacterial treatment of cotton fabrics which was tested by antibacterial activity assessment of textile material by agar diffusion method against *Staphylococcus aureus*. The colloidal AgNPs have been soaked onto cotton fabrics and studied for their effective antibacterial activity toward *Staphylococcus aureus* which showed remarkable antibacterial activity.Copyright © 2016 VBRI Press.

Keywords: Biosynthesis; nano-silver; bionanotechnology; microstructure; plant system.

Introduction

Recent times have witnessed an up surge in plant or plant parts (including fruits) mediated synthesis of variety of nanoparticles as they are safe to handle, economical and bestow a genuinely green option for nanosynthesis [1-21]. Clerodendron infortunatum, commonly known as 'Bhant', is very common undershrub in India. It belongs to the family Verbenaceae (Lamiaceae), and bears widely proven spectrum of medicinal activities. This plant is quite rich in its characteristic medicinal terpenoid-clerodin, along with other significant metabolites. It is a commonly used medicinal plant since ages. Leaves are used as a substitute for Chiretta. Leaves and roots are employed externally for skin diseases and alopecia and are prescribed in headache too. In homoeopathy, the fresh leaves are employed for colic due to worms, diarrhea associated with nausea, chronic fever with loss of appetite and in enlargement of liver and spleen with indigestion and constipation. The alcoholic extract of the whole plant showed anti-protozoal activity against Entamoeba histolytica. It also exhibited hypoglycaemic activity in albino rats. The leaves exhibit antifungal activity [22]. The fresh juice of leaves from this plant has effectively been used against malaria especially among children. Root system of this plant is very effective against muscular sprains, cramps and rheumatism. Old literatures also suggest its antipyretic property [23]. This plant is widely available all through the year and

unfortunately enough, this has invited very little attention of phytochemists and nanotechnologists for applications.

Staphylococcus aureus is a bacterium which is frequently found in the human respiratory tract and on the skin. It is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease associated strains often promote infections by producing potent protein based toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic S. aureus (e.g. MRSA) is a worldwide problem in clinical medicine.

Moreover, plants with reported medicinal/antibacterial properties add further advantage in the sense that in one hand they negotiate the synthetic cues while the unused metabolites adds further to their anti-microbial/medicinal properties. Recently, several efforts have been made on synthesis of different nanoparticles and/or nanocomposites for their effective use in different areas [24-36]. Keeping this idea in mind in the present investigation, we have reported synthesis of silver nanoparticles (AgNPs) with the help of Clerodendron infortunatum broth and immobilization of the colloidal silver onto cotton fabric in order to introduce an innovative method for the antibacterial finishing of cotton fabrics to check the menace of different bacterial infections caused by Staphylococcus aureus. The prepared AgNPs have been characterized using the standard techniques like: X-ray diffraction, scanning transmission electron and microscopy and UV- visible spectroscopy. An effort has been also been made to understand the possible involved mechanism for the biosynthesis of AgNPs in the light of existing facts.

Experimental

Synthesis of silver nanoparticles using clerodendron infortunatu

Prior to the experiment, C. infortunatum leaf was rinsed thoroughly by de-ionized water. Leaf extract was prepared by taking 25 g of leaf thoroughly washed, dried, cut into fine pieces, mixed with 100 ml of 50 % ethanol in a 250 ml Erlenmeyer flask and mixture was boiled for 15 min before decanting till the colour changes to from clear transparent to light yellow-green. The leaf mass was pressed by wrapping in serene cloth and 50 ml extract was collected under laminar flow. It was doubled in volume by adding 50 % ethanol and was treated as source extract. For reduction of silver ions, 5 ml of C. infortunatum leaf extract was added drop-wise into 25 ml of 0.25 molar aqueous solution of AgNO₃ with constant stirring at 50-60 °C on steam bath. As soon as, C. infortunatum extract was mixed in aqueous solution of silver ion, it starts to change colour from deep straw to yellowish brown and finally black due to excitation of surface plasmon resonance which indicates the formation of silver nanoparticles. The synthesized nanoparticles by C. infortunatum leaf extract were centrifuged at 5500 rpm for 15 min and subsequently redispersed in de-ionized water to get rid of any uncoordinated biological molecules.

Loading of silver nanoparticles cotton fabrics

At first, cotton fabrics were washed dried and autoclaved. Experiments were performed on samples with maximum dimension of 30 cm \times 15 cm. Cotton fabrics were padded with AgNPs solutions at concentration of 100 ppm; which was achieved through diluting the original solution of 2000 ppm AgNPs with distilled water. For the successive treatment of fabrics with colloidal silver, the solution was agitated continuously. All samples were immersed in such colloid bath for 1 min then squeezed to 100 % wet pick up with laboratory padder at constant pressure. Samples were dried at 60 °C for 3 min, followed by curing at 80 °C for 2 min. The antibacterial efficacy was evaluated quantitatively for: (1) untreated fabrics and (2) treated with AgNPs solution [**37**].

Characterizations

The formation of AgNPs was checked by X-ray diffraction (XRD) technique. The XRD spectrum was taken with an X-ray diffractometer (XPERT-PRO, PW3050/60) at room temperature, using CuK_{α} radiation $\lambda = 1.5406$ Å over a range of Bragg angles 25° to 90°. TEM micrograph of AgNPs was obtained using a high resolution Bruker transmission electron microscope operated at an accelerated voltage of 200 keV. The specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under Infrared lamp. The scanning electron micrograph (SEM) and energy dispersive X-ray (EDX) pattern of

AgNPs loaded cotton fabric were taken using a computer controlled scanning electron microscope (JEOL-JSM840A). The absorption spectra of the sample were measured by a computer controlled UV-visible spectrophotometer (Hitachi U-2800, Japan).

Antibacterial activity testing

The bacterial suspension was spread on nutrient agar in petri plate to create confluent lawn of bacterial growth. The wells of 6 mm were prepared by borer. Exactly of the same diameter three cotton pieces were carefully cut and were soaked in 10, 15 and 20 µl of Ag sol (prepared by the above method) respectively and were placed in the well. The well without silver nanoparticles was treated as control. These plates were incubated to 24 h at 35 °C. The lowest concentration at which the Petri plate did not show any visible growth after microscopic evaluations was considered as minimum inhibitory concentration (MIC). The susceptibility of test organisms was determined after 24 h by measuring zone of inhibition around each well to nearest mm. The results of antimicrobial activity were compared with control experiment. In control, no zone of inhibition was observed; indicating that the activity is due to bio-inspired silver nanoparticles. All antimicrobial parameter have been studied in triplicate and the best visible result has been reported.



Fig. 1. Indexed XRD pattern of silver nanoparticles at room temperature and the line profile (Top) indicates the peak positions according to PCPDF No. #89-3722. Inset: TEM micrograph of silver nanoparticles synthesized using *Clerodendron infortunatum* leaf extract.

Results and discussion

The crystalline nature of AgNPs was confirmed from XRD analysis. **Fig. 1** shows the indexed XRD profile of AgNPs, at room temperature. The peaks of the XRD-pattern were indexed and cell parameters were determined using experimental 2θ -values of peaks. The three peaks of XRD were assigned to the diffraction (111), (200), (220), (311) and (222) planes of face-centered cubic (*fcc*) silver having the lattice parameter a = 4.0779 Å which is in agreement with the standard literature (ICDD no. #89-3722). The presence of broad peaks, indicate that either particles are of very small crystallite size or semi-crystalline in nature. A little difference of 0.0071 Å between the cell parameter of bulk and nanoparticles has been observed. Consequently, a lowering in unit cell volumes from 68.19 Å³ (for bulk Ag) to 67.81 Å³ (for AgNPs) has been noticed which could be due to the nanosizing effect. An estimate of the size of the nanoparticles was made from the line broadening of the (111) reflection using the Debye-Scherrer formula: $P_{111} =$ $0.89\lambda / \beta_{1/2} \cos\theta$, where $\beta_{1/2} =$ full width at half maximum. The average particle size was estimated to be of the order of 18 nm. Inset of **Fig. 1** illustrates a typical TEM image of AgNPs synthesized after reduction of AgNO₃ with *C. infortunatum* leaf extract. The micrographs clearly show individual nanoparticles which are almost spherical in shape. The sizes of particles are found to be in the range of 2 to 6 nm.



Fig. 2. EDS pattern of AgNPs and SEM image of cotton fabric (inset) adhered with AgNPs prepared using *Clerodendron infortunatum* extract.

In order to verify the elemental composition of the AgNPs soaked cotton fabric, X-ray energy dispersive spectroscopy (EDS) study was carried out. The EDS peak (**Fig. 2**) corresponding to silver confirms the purity and formation of AgNPs and supports our initial hypothesis that the plant leaf extract of *C. infortunatum* is able to produce silver nanoparticles. The small peaks of oxygen and carbon in EDS comes from the cotton fabric. Further, inset of inset of **Fig. 2** depicts simply SEM image of AgNPs soaked cotton fabric while inset of **Fig. 2** illustrates the magnified SEM image of the same sample which clearly shows the bounded AgNPs onto the fabric.



Fig. 3. UV-vis spectrum of AgNPs synthesized using *Clerodendron infortunatum* leaf extract. The inset shows AgNPs as deposit and above which is colloids of AgNPs in *Clerodendron infortunatum* leaf extract medium.

UV-vis spectroscopy is an important technique to ascertain the formation and stability of nanoparticles in aqueous solution. Fig. 3 shows the UV-vis spectrum recorded for AgNPs. It is well known that colloid of AgNPs exhibits lovely straw-yellow color which arises due to excitation of surface plasmon vibrations. The surface plasmon resonance was observed in the range of visible region at 414 nm. Also, the plasmon bands are broadened with an absorption tail in the longer wavelengths, which could be due to the size distribution of the particles. This observation supports the distribution of particle sizes observed in TEM image (inset Fig. 1). Besides, the biosynthesized AgNPs was laid aside at room temperature in the laboratory and inspected after three months. There was obviously no observed aggregation in the solution, and its absorption value showed almost the same. This simply suggested that the AgNPs synthesized by C. infortunatum bears very good stability which might be due to encapsulation by the different proteins present in the leaf parenchyma.



Fig. 4. Photographic image of (a) control experiment and (b) bacterial inhibition zones against *Staphylococcus aureus* (MRSA) created by the silver nanoparticles soaked cotton fabric.

Fig. 4 shows photographic image of bacterial inhibition zones against Staphylococcus aureus, produced by AgNPs prepared using C. infortunatum. This results in a good antibacterial activity with an average diameter of the inhibition zones of 5 mm, which clearly indicated the antibacterial efficacy of AgNPs onto the cotton fabrics. Photographic images of bacterial inhibition zones against S. aureus, produced by the silver nanoparticles (sol) prepared with different concentrations of silver nanoparticles. The average diameter of the bacterial inhibition zone was correlated to antibacterial activity of the silver nanoparticles, *i.e.*, the larger the clear area around the well, the higher the inhibitory efficiency. The nanoparticles, prepared at low AgNO₃ molar ratios (0.5, 1 and 2), showed a good antibacterial activity with an average diameter of 2.5 mm of inhibition zone. However, the silver nanoparticles, prepared at high AgNO₃ molar ratios (5, 10 and 20), did not produce any inhibition zones.

The Plant *Clerodendron infortunatum* shows an appreciably high wealth of metabolites of both the type (primary as well as secondary). They have been suitably screened, isolated, characterized and studied for the biological activities **[38]**. Earlier too, the wealth of this widely available medicinal plant was explored phytochemically and was found to contain apigenin, acacetin, cabruvin, quercetin, scutellarein-7-glucuronide, hispidulin, 7-hydroxy-4',3,5 trimethoxy flavones and a new

flavones glycoside, characterized as the methyl ester of acacetin-7-o-glucuronide in the light of UV-vis, IR, NMR and Mass spectroscopic studies. Among steroids the compounds detected were β -sitosterol, clerosterol (24 α - and 24β epimers) and acetyl derivatives in light of UV-vis, IR, NMR and Mass spectroscopic studies. Among terpenes the major compounds being detected were clerodin in the light of UV-vis, IR, NMR and Mass spectroscopic studies [39-44]. Along with this, altogether four organic acids were also detected in Clerodendron infortunatum and they were tartaric acid DL, adipic acid and benzoic acid/p-toluic acid [38]. Recently, the phytochemical analysis of the plant extracts further confirmed the presence of alkaloids, steroids, terpenoids, phenolics, flavonoids, tannins and saponins and these compounds are known to exhibit the physiological activities and medicinal properties of plants [45]. The basic physicochemical properties of the phenol functional group is quite versatile and that involves hydrophilicity through H-bond dipole-dipole interactions, metal chelation, hydrogen atom transfer, single electron transfer and finally hydrophobicity through π -stacking and vander Waals interactions (Fig. 5) [46]. In addition, it is known that tartaric acid (H₂Tart) is energetically labile molecule. It undergoes interesting pattern of optical isomerism. Recently, mean value of heat of combustion for tartaric acid was calculated to be 1124.5 kJ/mol [47]. The value of heat of dissociation was calculated to be 27.17 kJ/mol for the temperature above 50 °C [48]. It clearly indicates that this energy is probably sufficient to accomplish a nano-transformation. Further, this process involves complexation of metal ion $(Ag^{2+} in this case)$ by polyfunctional carboxyl acids such as tartaric acid having one hydroxyl group. Upon heating, water evaporates resulting in chelation. Therefore, the effect of such biochemical ambiance could have made the reaction to occur more easily. So, altogether Tannins, Organic acids, along with other metabolites like Flavonoids are oxido-reductively agile and may effectively contribute towards nanomaterial (AgNPs) synthesis. The schematics for the biological synthesis of silver nanoparticles using Clerodendron infortunatum has been illustrated in Fig. 6.



Fig. 5. Physico-chemical properties of phenol functional group.

Soaking of silver sol prepared with the help of *Clerodendron infortunatum* causing the inhibition of gram positive bacteria like *Staphylococcus aureus* is a positive development in the sense that it may lead to adding a new face to textile industries. Further due to the presence of unused metabolites the inhibition of bacterial growth is

attenuated. Steroids are reported to possess antimicrobial activity. Moreover, Tannins have astringent properties; it hastens the healing of wounds and inflamed mucous membranes. Also, the characteristics of saponins include the precipitation and coagulation of red blood cells [49], while antimicrobial properties of the different secondary metabolites are very well established and recently hydrolysable tannins have also been reported to show potential antibacterial effects against *Helicobacter pylori* [50].



Fig. 6. Schematics for the biosynthesis of AgNPs using *Clerodendron infortunatum* leaf extract.

By incorporating nanoscale silver into textiles, the manufacturers can make materials that use a small amount of silver to kill the microbes present on the surface of the clothing material, thus can be treated with silver nanoparticles to help prevent spoilage rising from microbial growth in damp areas. The enhancement in activity shown by AgNPs derived from *C. infortunatum* leaves is due to the synergistic effect of silver and conglomerate of secondary metabolites. Currently, we are assaying other medicinal plants for their capability for nanotransformation and industrial and/or biomedical applications.

Conclusion

Present investigation has two fold advantages in the sense that in one hand suggests effective utilization of a medicinally promising weed in synthesizing antimicrobial nanoparticles like silver and its applications in textile industries on the other. Also, it is a green, high yield, fast and low cost approach. Reduction of silver nanoparticles accomplished due to phytochemicals like phenolics, tannins, organic acids, etc.

Author contributions

Conceived the plan: akj, kp; Performed the expeirments: akj; Data analysis: kp; Wrote the paper: akj, kp. Authors have no competing financial interests.

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