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Biological Synthesis of Silver Nanoparticles from *Cochlospermum Religiosum* and their Antibacterial Efficacy

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Abstract
The synthesis of ecofriendly nanoparticles is evergreen branch of nanoscience for biomedical application. In the present study biosynthesis of silver nanoparticles and its activity on bacterial pathogens were carried out. SNPs were rapidly synthesized using leaf extract of medicinal plant *Cochlospermum religiosum* and the formation of nanoparticles was observed within two minutes. The results recorded from UV-Vis spectrum and AFM analysis supports the biosynthesis and characterization of silver nanoparticles. The UV-Vis spectrum has the absorbance peak at 260 nm and the spherical shaped SNPs showed variation in size ranging from 40 to 100 nm. Further the synthesized SNPs showed effective inhibitory activity against pathogens *Bacillus, E.coli, Pseudomonas, Klebsiella* and *Staphylcoccus.* They are highly toxic to *E.coli* and *Staphylococcus*. Moderately toxic to *Bacillus, Pseudomonas* and *Klebsiella.*

Key words:
Antibacterial activity, *Cochlospermum religiosum*, phytosynthesis, silver nanoparticles

INTRODUCTION

Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. They are in great demand in the developed world for primary healthcare because of their efficacy, safety, and lesser side effects [1]. India has a very long safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy, Naturopathy [2]. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world [3].

Cochlospermum religiosum is a very beneficial tree and is used from very ancient times in India for curing a lot of ailments. Gum of *Cochlospermum religiosum* is used for stomachic, sedative, gonorrhea, syphilis and asthma. Paste of stem bark is applied over the bone fractured areas [4].The herb vendors sell the bark of *Cochlospermum religiosum* as a remedy to diabetes. It is used in combination with kalimirch [5]. Powder of bark is used with water during jaundice [6].

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. Nanotechnology is the understanding and control of matter generally in the 1-100 nm dimension range. The application of nanotechnology to medicine, known as nanomedicine, concerns the use of precisely engineered materials at this length scale to develop novel therapeutic and diagnostic modalities [7].

The design, synthesis and characterization of biologically synthesized nanomaterials have become an area of significant interest [8]. Nanotechnology has the potential to revolutionize veterinary medicine, animal health and other areas of animal production [9]. The convergence of nanotechnology and biomedical sciences opens the possibility for a wide variety of biological research topics and medical uses at the molecular and cellular level [10].

The use of nanotechnology in the field of medicine could revolutionize the way we detect and treat damage to the human body and disease in the future, and many techniques only imagined a few years ago are making remarkable progress towards becoming realities. Toxicity studies on pathogen opens a door for nanoparticle applications in medicine. Biological synthesis of metal is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phyto-toxic effects [11]. Currently, the increase of bacterial resistance to antibacterial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Hence the SNPs synthesized from the leaf of *Cochlospermum religiosum* may be used against the bacterial agents.

MATERIALS AND METHODS

Biogenesis of silver nanoparticles

The leaves of *Cochlospermum religiosum* were collected from Tirumala hills and were shade dried for fifteen days. The dried leaves were ground to a fine powder. One mM silver nitrate was added to the plant extract to make a final solution of 200 ml. and centrifuged at 18000 rpm for 25 minutes. The collected pellets were stored at -4˚C. The supernatants were heated at 50˚C to 95˚C. A change in the colour of the solution was observed during heating. The reduction of pure Ag^{2+} ions were monitored measuring the UV-Vis spectrum of the reaction media at five hours after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Visible spectrophotometer. Thin films of the samples were prepared on a carbon coated cupper grid and AFM analysis of the sample was carried out.

Antibacterial efficacy analysis

Pure cultures of *Bacillus, E.coli, Pseudomonas, Klebsiella* and *Staphylococcus* were procured from the Department of Microbiology of Sri Venkateswara University. The experiments of antibacterial analysis were carried out in the Department of Microbiology of Sri Venkateswara University. The sensitivity testing of the plant extract was determined by using disc diffusion method [12]. Eighteen hours old five bacterial broth cultures were used as inoculums after adjusting their population to cfu ml^{-1} using 0.9% (w/v) sterile saline by the method as described by Forbes *et al.* [13] 0.5 ml of standard inoculums of bacterial species were pipetted separately in to sterile petriplate

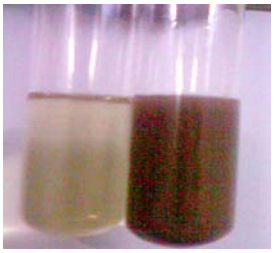
contained 20 ml. of melted agar medium in each plate and mixed well by gently swirling on the table top. The seeded plates were allowed to solidify. Sterile paper discs previously soaked in 10µg ml^{-1} concentration of known plant extract with silver nanoparticles were carefully placed on the labeled seeded plates. After 24 hours inhibition zone formation was noted. The experiments were repeated thrice and mean values of one diameter were presented.

RESULTS AND DISCUSSION

The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The extract was pale yellow in colour before addition of $Ag(No_3)_2$ and this was changed to brownish colour suggesting the rapid formation of silver nanoparticles (Fig.1b). The time duration of change in colour was 2 minutes. The change of colour indicates the biosynthesis of silver nanoparticles. Brown colour in aqueous solution is due to the Surface Plasmon Resonance Phenomenon. The synthesis of silver nanoparticles had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of silver nanoparticles synthesized from leaf extract of *Cochlospermum religiosum* has absorbance peak at 260 nm (Fig.1c).

Silver nanoparticles synthesized by using leaf extract of *Clerodendrum inerm* showed absorbance in between 200-400 nm [14], *Euphorbia hirta* showed absorbance peak at 380 nm [15] and of *Svensonia hyderabadensis* showed absorbance peak at 390 nm [16]. In many other cases like silver nanoparticles synthesized by using leaf extracts of *Eucalyptus hybrid* [17], *Acalypha indica* [18], *Nelumbo nucifera*[19], *Solanum torvum* [20], *Helianthus annus* [21] and *Cassia auriculata* [22] the absorbance peaks were between 400 and 450 nm. When compared with these plants silver nanoparticles synthesized from leaf extract of *Cochlospermum religiosium* were active at relatively lower wavelength.

Fig 1(a)



(1) (2)

Fig 1(b)

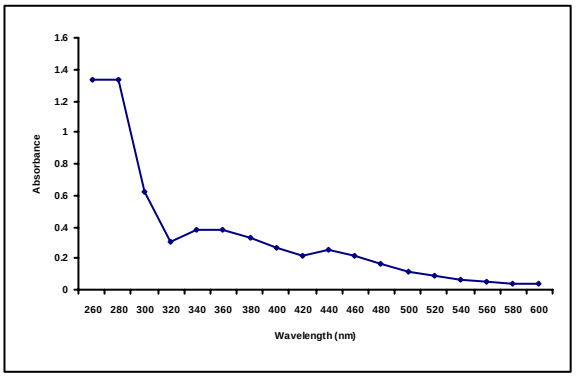


Fig 1 (c)

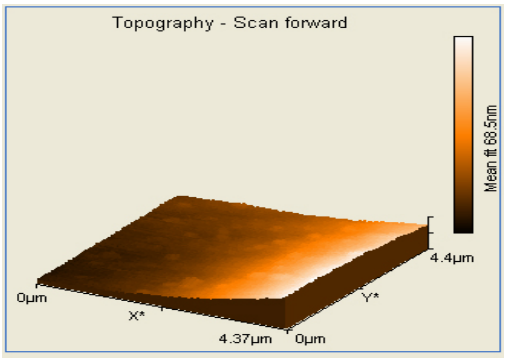


Fig 1(d)

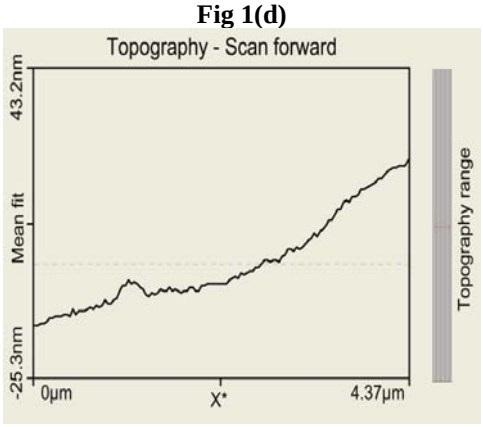


Fig 1(e)

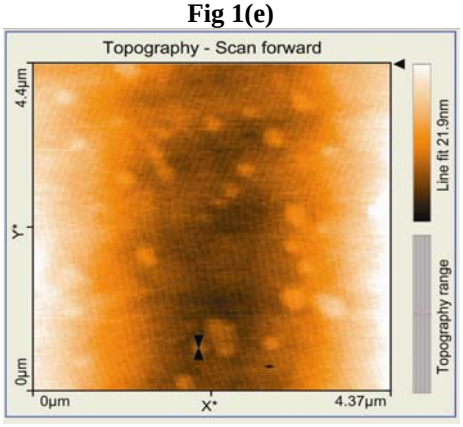


Fig 1(f)

Fig.1: (a) Natural habitat, (b) The colour change of leaf extracts of *Cochlospermum religiosum* (1) blank extract without silver nitrate (2) stem extract with 1 mM silver nitrate; (c) UV-VIS spectroscopy of synthesized silver nanoparticles; (d) Three dimensional structure, (e) Graph and (f) Size of SNPs

The AFM analysis of leaf extract of *Cochlospermum religiosum* showed spherical shaped silver nanoparticles formed with diameter ranging from 40 to 100 nm. The 3D image shows the silver nanoparticles of highest 68.77 nm (Fig.1 d, e, f). The AFM analysis of leaf extract of *Citrulus colocynthus* showed the size of silver nanoparticles as 31 nm [23], of *Syzygium cumini* as 30 nm [24] and of *Citrulus limon* as 50 nm [25]. The particle size of silver nanoparticles synthesized from the leaf extract of *Glycine max* was from 25to 100 nm [26], that of *Moringa* olifera was from 5 to 80nm [27]. So the size of the nanoparticles varies with the plant. By altering the pH, strength of elements, plant sources, incubation temperature of the nanoparticle synthesis reaction mixture and the

synthesis methods, it is possible to create a wide range of different nanoparticles. Nanoparticles of various sizes and properties may be obtained by further tapping the plant bioresources of diverse type in wild environment [28].

Antibacterial efficacy of silver nanoparticles

The biogenesis of silver nanoparticles using medicinal plants was found to be highly toxic against different pathogenic bacteria of selected species. The tested concentrations of plant extract are 10 µg ml^{-1}. The silver nanoparticles synthesized from the leaf extract of *Cochlospermum religiosum* showed effective inhibitory activity against *Bacillus, E.coli, Pseudomonas, Klebsiella*, and *Staphylococcus*. They are highly toxic to *E.coli* and *Staphylococcus*, moderately toxic to *Bacillus*, *Pseudomonas*, and *Klebsiella* (Table.1 & Fig.2).

Table -1:
Inhibition zones of SNPs synthesized from the leaves of *Cochlospermum religiosum* against bacterial species

Name of Bacteria	*Leaf extract (in mm)*	*Silver nanoparticles (in mm)*	*Silver nitrate (in mm)*
Klebsiella	03	11	10
Pseudomonas	04	12	11
Bacillus	05	15	13
Staphylococcus	03	20	15
E. coli	03	20	19

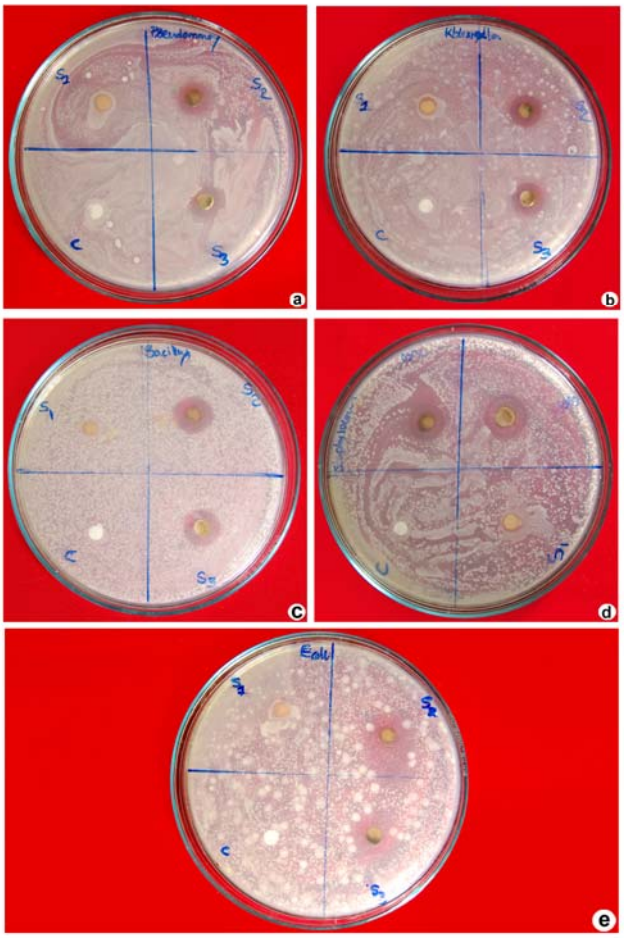


Fig.2: Antibacterial activity of leaf SNPs of *Cochlospermum religiosum* (a) *Pseudomonas*, (b) *Klebsiella*, (c) *Bacillus*, (d) *Staphylococcus (e) E. coli*

Similar results were obtained in the case of *Rhizophora apiculata* which showed inhibition against *E.coli, Klebsiella, Proteus, Salmonella, Staphylococcus, Bacillus* and *Pseudomonas* [29]. Inhibition against *E.coli* and *Staphylococcus* was observed in the case of *Mentha piperita* [30] , *Cacumen platycladi* [31] and *Cleome viscosa* [32] . *Acalypha indica* showed high toxicity to *E.coli* [18] and *Solanum torvum* showed high toxicity to *Pseudomonas* and *Staphylococcus* [20] .The silver nanoparticles of *Boswellia ovalifoliolata* showed inhibition against *E.coli, Klebsiella*, and *Bacillus*, where as the silver nanoparticlesof *Shorea tumbuggaia* inhibited the growth of *E.coli, Pseudomanas* and *Proteus* species [33]. Silver nanoparticles synthesized from Mulberry leaf show good antibacterial activity. Such AgNPs produced by the environmentally friendly method have the potential for use in antibacterial and medical applications [34] .

The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well-known [35]. Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles [35]. Morones *et al*. [36] demonstrated using the Scanning Tunneling Electron Microscopy (STEM) and the X-ray Energy Dispersive Spectrometer (EDS), showed silver nanoparticles not only at the surface of cell membrane, but also inside the bacteria. This then suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria causing damage by interacting with phosphorus and sulphur-containing compounds such as DNA. Silver tends to have a high affinity to react with such compounds. One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multiresistant bacterial strains, and the development of new antimicrobial substances [35].

CONCLUSION

The present study included bioreduction of Ag^{2+} ions by the leaf extract of medicinal plant *Cochlospermum religiosum* and the antibacterial activity of silver nanoparticles of leaf extract. The study revealed that the plant is a good source for the synthesis of silver nanoparticles at fast rate. The aqueous silver ions were exposed to the extract and the synthesis of silver nanoparticles was confirmed by the brown colour formation within 2 minutes. The antibacterial efficacy against different species of bacteria confirmed that the silver nanoparticles of the leaf extract of *Cochlospermum religiosum* are capable of rendering antibacterial efficacy and strengthen the medicinal value of the plant. The phyto-synthesis of silver nanoparticles is simple and convenient to handle, most advantageous and also eco-friendly.

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